FIRST STEPS TO PREDICTING PULP COLOUR IN WHOLE MELONS USING NEAR-INFRARED REFLECTANCE SPECTROSCOPY

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Abstract

NIRS technology was used for the non-destructive measurement of melon-pulp colour (a*, b*, C* and h*), one of the main indicators of ripeness and quality. A total of 432 Cantaloupe and Galia melons were used in the construction of calibration models, testing various spectral signal pretreatments and both linear and non-linear regression algorithms. The coefficient of determination (r^2) and the standard error of cross-validation (SECV) obtained for parameters a* (0.96, 2.16), b* (0.85, 3.25), C* (0.82, 3.76) and h* (0.96, 3.64) in intact fruit confirmed the *a priori* viability of NIRS technology with MPLS regression for measuring melon ripeness and quality. Moreover, the application of a LOCAL algorithm improved the ability of models to predict all the internal-colour quality parameters studied. These results suggest that NIRS technology is a promising tool for monitoring ripening in melons and thus for establishing the optimal harvesting time.

Keywords: near-infrared spectroscopy, melon, internal colour, MPLS regression, LOCAL algorithm.

1. Introduction

Harvesting melons at the ideal stage is especially critical to their storage life and eating quality. Although sweetness is the key attribute affecting eating-quality, other properties such as aroma, flesh colour and texture—depending on the fruit concerned—are also indispensable indicators of overall quality (Vallone et al., 2013).

The quality of muskmelons (*Cucumis melo)* at harvest is traditionally estimated on the basis of a number of subjective external features, chief among which are background colour, net development, and stem abscission (Simandjuntak, Barrett, & Wrolstad, 1996; Portela & Cantwell, 1998; Cantwell & Kasmire, 2002).

Cantaloupes may be harvested when the fruit begins to separate from the stem, when the external colour beneath the netting begins to change from green to yellow-green (bearing in mind that skin colour typically transitions from grey to dull green when immature, deep uniform green at maturity, and light yellow at full ripeness), and when the net is well developed with a waxy covering (Cantwell, 1996). To ensure excellent eating quality in melons, it is critical to harvest them at a sufficiently advanced stage when the sugars have already accumulated in the fruit, since postharvest changes in sugar concentrations are small (Pratt, Goeschl, & Martin, 1977; Lester & Shellie, 1992). Similarly, although skin colour may change after harvest, pulp colour changes very little, so that harvesting at the appropriate stage of maturity is crucial to good internal visual quality (Cantwell, 1996).

Honeydew melons are harvested by maturity, which is very difficult to judge since the abscission zone, a valuable harvest criterion for Cantaloupes, does not form until the fruit is overripe (Pratt, Goeschl, & Martin, 1977). Maturity classes are grouped predominantly by changes in ‘ground colour' from greenish to cream with yellow accents. Cantwell (1996) notes that Honeydew melons may be considered mature but unripe when the external colour is white with a greenish aspect, the peel is slightly fuzzy, there is no aroma, when the melon splits when cut, and when the pulp is crisp. They may be classed as mature and ripening when the external colour is white with traces of green, the peel is not fuzzy but slightly waxy, the aroma changes from slight to noticeable, the melon splits when cut and the flesh is crisp. The characteristics of ripe Honeydews are as follows: ground colour is creamy white with yellow accents, peel is clearly waxy, the characteristic aroma is noticeable and the blossom-end yields slightly to pressure. Pratt, Goeschl, & Martin, (1977) report that ripening in Honeydew melons is associated with increased respiration and ethylene production rates, aroma development and softening.

The Galia melon is a hybrid originating from a Cantaloupe-Honeydew cross, larger than a Cantaloupe, and with deep green flesh. Ripeness is measured not by softness at the stem but rather by colour and fragrance (Escribano & Lazaro, 2012).

Growers and consumers generally estimate melon quality in terms of aroma, softness to the touch and surface colour (Lester, 2006). However, while these are important for establishing product quality and optimal harvesting time, there are other key criteria which cannot be assessed externally, and require non-destructive methods in order to avoid damage to the fruit. Pulp colour is one such criterion: as Cantwell (1996) has noted, fall and winter Cantaloupe melons may be ripe on the inside but have a green peel colour. Cantwell and Portela (1998) highlight the link between pulp colour and surface defects such as sunburned areas and large ground spots (poorly netted areas where melons touch the ground), reporting that average pulp chroma (orange colour) values are highest in good-quality pieces, intermediate in ground-spot pieces, and lowest in pieces from sunburned areas.

Growers and the industry would clearly benefit from fast, precise and, above all, non-destructive techniques. NIRS technology not only meets these requirements, but also offers a number of other advantages which make it ideal for meeting current demands in terms of control and traceability: low cost per sample analysed; little or no need for sample preparation; ability to analyse a wide range of products and parameters; a high degree of reproducibility and repeatability; and reduced interference from colour of fruit samples. NIRS can be built into in-line processes, and – since no reagents are required – produces no waste.

NIR spectroscopy has been used successfully to predict colour in animal products such as fresh breast muscle (Abeni & Bergoglio, 2001), deboned chicken breast (Liu, Lyon, Windham, Lyon, & Savage, 2004), and beef (Andrés et al., 2008; Prieto, Andrés, Giráldez, Mantecón, & Lavín, 2008; Prieto et al., 2009; Cecchinato, De Marchi, Penasa, Albera, & Bittante, 2011), as well as external colour in mandarins and oranges (Sánchez, De la Haba, Serrano, & Pérez-Marín, 2012; Sánchez, De la Haba, & Pérez-Marín, 2013).

Even though the prediction of internal colour is a key factor in establishing optimal harvesting time, no published research appears yet to have addressed this criterion.

The overall aim of this study was to evaluate the ability of NIR technology to predict internal colour in melons, a quality parameter strongly influencing consumer acceptance or rejection of the product.

2. Material and methods

2.1. Fruit samples

A total of 432 melons – N = 220 Cantaloupe (*Cucumis melo* L. var. reticulates Naud., Vulcano cultivar) and N = 212 Galia (*Cucumis melo* L. var. reticulates Naud., Siglo, Deneb, Esmeralda and Solarking cultivars) – were harvested in glasshouses belonging to the Provincial Fruit and Vegetable Harvesters' and Exporters' Association in Almeria, Spain.

On arrival at the laboratory, fruit was promptly placed in cold storage, at 5ºC and 95% relative humidity. Prior to each measurement, fruit samples were left in order to allow the near-surface temperature to stabilize at the laboratory temperature of 20ºC.

2.2. Reference data

Internal colour was analysed on the pulp surface using a Minolta Chroma Meter CR-400 (Minolta Co. Ltd., Osaka, Japan). Two consecutive readings were taken in the equatorial region of the fruit; readings were averaged for each sample. Colour was expressed as CIELAB (a*, b*, C*, h*) colour space, where a* and b* define red-greenness and blue-yellowness, respectively (CIE, 2004). Chroma (C*) and hue angle (h*) were calculated as (a*^2 + b*^2)^(1/2) and tan^{-1}(b*/a*), respectively. Illuminant C and 2º standard observer measurements were made in all cases.

2.3. NIR analysis

NIRS analysis was performed using a Perten DA-7000, Flexi-Mode diode array spectrometer (Perten Instruments North America, Inc., Springfield IL, USA), operating between 400-1700 nm with a 5 nm scanning interval.

Fruits were scanned using the instrument in the standard upright position. Samples were irradiated from below by the light source. The distance of measurement between the sample and the instrument was 120 mm, with a large, circular surface viewing area (diameter 127 mm). The horizontal distance between the light source and the detectors was 80 mm.

Each fruit was placed centrally upon the fruit holder, with the stem-stylar axis horizontal. Three separate spectral measurements were made, after rotating the sample through 120º each time. The three spectra were averaged to provide a mean spectrum for each intact fruit.

2.4. Spectral repeatability

All chemometric calculations were performed using WinISI software package version 1.50 (Infrasoft International, Port Matilda, PA, USA). Spectrum quality was evaluated using the Root Mean Squared (RMS) statistic (Shenk & Westerhaus, 1995a, 1996). This statistic indicates the similarity between different spectra of a single sample, in this case between the three spectra collected per sample. An admissible limit for spectrum quality and repeatability was determined following the procedure described by Martínez, Garrido, De Pedro, & Sánchez (1998) to calculate the standard deviation (STD) limit from the RMS statistic and obtain an RMS cut-off value.

2.5. Population structuring and detection of spectral outliers prior to calibration

Principal Component Analysis (PCA) was performed on a set of N = 432 samples in order to decompose and compress the data matrix. After PCA, the centre of the spectral population was determined in order to detect outlier samples. The Mahalanobis distance (GH) was calculated between each sample and the centre; samples with a GH value greater than 3 were considered outliers (Shenk & Westerhaus, 1995a). As spectral pre-treatments, the Standard Normal Variate (SNV) plus Detrending (DT) (Barnes, Dhanoa, & Lister, 1989) procedure was used to remove the multiplicative interferences of scatter, and one derivative mathematical treatment was performed: window-wise filtering (1,5,5,1) where the first digit is the order of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in a running average or smoothing and the fourth is the second smoothing (Shenk & Westerhaus, 1995b; ISI, 2000).

2.6. Construction and validation of prediction models by MPLS regression

Once spectral outliers (9 of the original 432 samples) had been removed, a set consisting of 423 samples of the two different melons (Cantaloupe and Galia) was used to construct calibration models. The set was divided into two: a calibration set containing about 75% of the samples (N = 320 samples) and a test set containing the remaining 25% (N = 103 samples) (Table 1). These samples were selected following the method proposed by Shenk and Westerhaus (1991) using the Center algorithm included in the WinISI software to calculate the Global Mahalanobis distance (GH). Samples were ordered based on the Mahalanobis distance to the centre of the population, and three of every four were selected to form part of the calibration set.

Modified Partial Least Squares (MPLS) regression (Shenk & Westerhaus, 1995a) was tested for the prediction of colour (a*, b*, C*, h*) in melons in the 535-1650 nm range. Signal noise at the beginning (400-535 nm) and end (1650-1700 nm) of the spectral range was eliminated. To prevent over-fitting, six cross-validation groups were used.

For each analytical parameter, various mathematical treatments were evaluated for scatter correction, including the Standard Normal Variate (SNV) and Detrending (DT) methods (Barnes, Dhanoa, & Lister, 1989). Furthermore, four derivate mathematical treatments were tested in the development of NIRS calibrations: 1,5,5,1; 2,5,5,1; 1,10,5,1 and 2,10,5,1 (Shenk & Westerhaus, 1995b).

The statistics used to select the best equations were: standard error of calibration (SEC), coefficient of determination of calibration (R^2), standard error of cross-validation (SECV), coefficient of determination for cross-validation (r^2), RPD or ratio of the standard deviation of the original data (SD) to SECV, and the coefficient of variation (CV) or ratio of the SECV to the mean value of the reference data for the calibration set. These latter two statistics enable SECV to be standardized, facilitating the comparison of the results obtained with sets of different means (Williams, 2001).

The best models obtained for the calibration set, as selected by statistical criteria, were subjected to evaluation using samples not involved in the calibration procedure. A test set composed of 103 samples, not used previously in the model, was evaluated. Models were evaluated following the protocol outlined by Windham, Mertens, & Barton (1989).

2.7. Construction of prediction models using the LOCAL algorithm

For each parameter, an optimization design for the LOCAL algorithm was set up by varying the number of calibration samples (*k*) from 40 to 100 in steps of 20 and the number of terms (*l*) from 10 to 14 in steps of 2. This yielded a factorial design of 4 x 3 = 12 runs. Finally, the number of PLS factors discarded was set to the first four.

As in MPLS calibrations, other factors needed to be optimized, including signal pretreatments (light scatter correction and derivatives) and the spectral region used. During LOCAL equation development, the spectral region and signal pretreatments indicated in Section 2.6 were used.

The effect of the different settings on the performance of LOCAL was evaluated by comparing the standard error of prediction (SEP), the coefficient of regression for external validation (r^2), the bias, and the bias-corrected standard error of prediction SEP(c). Furthermore, the accuracy of prediction of LOCAL was compared to the SEP, r^2 and bias of MPLS prediction.

3. Results and discussion

3.1. Spectral repeatability

Optimization of spectrum quality and repeatability is crucial in order to develop robust and accurate models. Statistical methods such as a defined RMS cut-off limit can be useful for this purpose. The RMS cut-off was calculated as indicated in section 2.4. The mean STD for the samples analysed was 62,497 µlog(1/R), representing an RMS cut-off of 71,441 µlog(1/R). Any sample whose triplicated screening scans yielded an RMS above this value was eliminated and repeated until values fell below that limit, thus ensuring a high degree of spectrum repeatability.

No reference to the calculated RMS cut-off value for intact melons has been found in the literature.

The mean spectrum of the three replicates of each sample was used for further analysis.

3.2. Spectral features

Typical log (1/R) spectra for intact Cantaloupe and Galia melons, obtained on the Perten DA-7000 instrument, are shown in Fig. 1. The main absorption peaks coincided for both melon varieties at 680 nm, 970 nm, 1190-1210 nm and 1440 nm.

In the visible region of the spectrum, absorbance spectra measured on Cantaloupe and Galia melons were similar in shape, with peaks occurring at positions corresponding to known chlorophyll absorption bands: strong absorption by chlorophyll a was evident at 680 nm, with a shoulder at 630 nm due to absorption by chlorophyll b (McGlone, Jordan, & Martinsen, 2002; McGlone, Martinsen, Clark, & Jordan, 2005). Stchur, Cleveland, Zhou, & Michel (2002) report a strong inverse correlation between the presence of this band and fruit sugar content. In addition, red pigments (carotenoids and anthocyanins) have a typical absorption band in the 490 to 550 nm region of the visible spectrum (Strayer, 1995).

In the near infrared region, aqueous hydroxyl functional groups were detected at 760, 840, 970 and 1440 nm, as is usually the case for fruit, and particularly for melons, which are 90% water (Williams, 2001; McGlone, Martinsen, Clark, & Jordan, 2005). Williams (2001) reports a sugar-related absorption band at around 1200 nm.

3.3. Calibration development

Cross-validation statistics for the best models obtained for the prediction of internal colour (a*, b*, C* and h*) in intact Cantaloupe and Galia melons using the MPLS algorithm are shown in Table 2.

For colour parameter a*, the MPLS method yielded the best calibrations using D1 log (1/R). Vis-NIR spectroscopy models displayed remarkable predictive ability for this parameter (r^2 = 0.96, SECV = 2.16, RPD = 5.30); Shenk & Westerhaus (1996) suggest than an r^2 value greater than 0.9 indicates excellent quantitative information. The RPD (5.30) value demonstrated the robustness and power of the calibration models obtained for a*.

Non-destructive prediction of a* in melon pulp is highly valuable, since this parameter is linked to pulp carotene—and particularly β-carotene—content (Reid, Lee, Pratt, & Chichester, 1970). During ripening, the pulp attains the maximum orange colour typical of the Cantaloupe melon; a* is thus a good indicator of maturity in melons (Simandjuntak, Barrett, & Wrolstad, 1996). Conversely, declining a* values are associated with loss of the typical orange colour hue during storage (Beaulieu, 2005).

No references have been found in the literature to the measurement of a* in the pulp of intact melons using NIRS technology. However, Sánchez, De la Haba, Serrano, & Pérez-Marín (2012) used a diode-array instrument (Corona 45 VIS/NIR, spectral range: 380-1700 nm) and a hand-held MEMS device (Phazir 2400, spectral range: 1600-2400 nm) to measure external colour in intact oranges, reporting results poorer than those obtained here (RPD = 1.92 CV = 3.78% for the Corona 45 VIS/NIR; RPD =1.49, CV = 4.76% for the Phazir 2400).

Performance statistics inferior to those recorded here were also obtained by Sánchez, De la Haba, & Pérez-Marín (2013) when using the MEMS spectrophotometer for the on-tree measurement of external colour in mandarins (RPD = 2.04, CV = 33.30%).

The calibration model displaying the greatest predictive capacity for the b* colour parameter (r^2 = 0.85; SECV = 3.25; RPD = 2.61) was obtained using D1 log (1/R); quantification was good, according to the Shenk & Westerhaus (1996) classification.

There are no published reports on the measurement of b* in intact melon using NIRS technology, only Sánchez, De la Haba, & Pérez-Marín (2013) reported RPD value of 1.43 and CV value of 4.22% for the prediction of this parameter in intact mandarins using a MEMS instrument in the spectral range 1600-2400 nm. However, this parameter is linked to the behaviour of photosynthetic pigments such as chlorophyll and carotenoids during melon ripening, and may thus act as an indicator of ripeness and thus of optimal harvesting time (Martínez-Madrid, Martínez, Pretel, Serrano, & Romojaro, 1999); non-destructive measurement of b* is therefore of considerable value.

As Table 2 shows, good predictive ability (r^2 = 0.82; SECV = 3.76; RPD = 2.33) was recorded for the measurement of C* (Shenk & Westerhaus, 1996).

Values for C*, like those of a* and b*, increase significantly during ripening, due to higher carotenoid levels, and thus also provide a useful indicator of fruit ripeness (Sánchez, De la Haba, & Pérez-Marín, 2013).

The predictive capacity of the best model for the h* colour parameter may be considered excellent (r^2 = 0.96, SECV = 3.64, RPD = 5.22) in terms of the recommendations made by Shenk & Westerhaus (1996).

Hue angle increases with maturity in melons, indicating a change from light to darker orange in Cantaloupe and a decline in greenness in Galia (Simandjuntak, Barrett, & Wrolstad, 1996).

Although NIRS technology appears not to have been used hitherto for measuring C* and h* in melons, Sánchez, De la Haba, & Pérez-Marín (2013) used NIRS to measure these colour parameters in on-tree mandarins during ripening, obtaining models whose predictive capacity was inferior to that recorded here both for C* (RPD = 1.68, CV = 7.38%) and for h* (RPD = 1.31, CV = 9.03%).

3.4. Comparison of internal colour prediction in melons using the LOCAL algorithm versus MPLS regression

The LOCAL algorithm was also used to predict internal quality-related parameters, and results for prediction of the 103-sample external validation set were compared with those obtained using MPLS regression.

SEP, SEP (c), bias and r^2 values obtained with the best mathematical treatment for each parameter in the 12 runs (3 values for l and 4 for k) are shown in Table 3. The table also shows the combination of k and l yielding the lowest SEP for each parameter (k = 60 and l = 10 for a*; k = 40 and l = 14 for b*; k = 40 and l = 12 for C*, k = 40 and l = 10 for h*).

For predicting the external validation set, the LOCAL algorithm used only 40 samples to predict b*, C* and h*, and 60 samples for a*; rather than using all 320 samples in the calibration set (as was the case for MPLS regression), only those samples whose spectra were considered representative of the calibration set were used.

The results obtained using the LOCAL algorithm were better than those achieved with MPLS regression (Table 3), although both strategies yielded values for the coefficient of determination which were comfortably over the minimum of $r^2 \geq 0.60$ recommended by Windham, Mertens, & Barton (1989).

For a* prediction, the LOCAL algorithm improved the coefficient of determination by 2% and reduced the prediction error by 25%. The coefficient of determination for b* parameter was also improved by about 2% and the prediction error reduced by over 5%.

For C* and h*, the accuracy and precision of the predictions obtained using the LOCAL algorithm were greater than those obtained using MPLS regression (Table 3). Values for r^2 were improved by 2% and 3%, while prediction error was reduced by 4% and 38%, for C* and h*, respectively.

Use of the LOCAL algorithm thus yielded a slight improvement in r^2 values for all parameters, as well as minimizing the prediction error for NIRS models constructed to predict internal-colour parameters in melons.

3.5. Effective wavelengths for predicting colour-related parameters

The loading plots corresponding to the best models obtained for predicting maximum levels of colour parameters are shown in Fig. 2. These plots show the areas across the spectral range where variance has influenced computing of the model to a greater or lesser extent, and the direction of that influence (positive or negative).

For predicting a*, representation of the four latent variables (LV) used in constructing the calibration equation using MPLS regression shows that the areas of the spectrum exerting greatest weight on model fitting were 610 nm, 630 nm, 655 nm, 685 nm and 725 nm in the visible region, and the 950 nm and 1410 nm areas relating to the absorption of sugars and water (Fig. 2). The same areas exerted greatest weight for parameter b* (Fig. 2).

For chroma (C*), peaks and valleys were observed in similar areas: 610 nm, 630 nm, 655 nm, 685 nm, and 725 nm, in addition to 950 nm and 1410-1480 nm (Fig. 2).

For h*, the most significant wavelengths were 615 nm, 625 nm, 655 nm, 685 nm and 725 nm in the visible region, together with 950 nm, 1225 nm, 1415 nm and 1480 nm, areas related to sugar and water absorption; their influence was either positive or negative, depending on the latent variable in question (Fig. 2).

Thus, wavelengths 630, 680, 725, 950, and 1410 nm are likely to be the most sensitive for colour-related parameters in intact melons.

4 Conclusions

NIRS technology, using a diode array spectrometer, proved to be suitable for assessing internal colour-related parameters in the pulp of intact melons, allowing ripeness to be evaluated not only in terms of external visual appearance but also in terms of internal colour. This could lead to major changes in harvesting techniques for melons, by providing farmers with a precise and accurate indication of the fruit's internal quality, thus enabling selective harvesting. It must be highlighted that the results obtained here should be considered the first step in the fine-tuning of NIRS for monitoring the ripening process in melons. Over the coming years, recalibrations may be required in order to enhance the robustness of the models obtained.

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Table 1 - Range, mean, standard deviation (SD) and coefficient of variation (CV) for the parameters studied in calibration ($N_{Calibration}$ = 320) and validation ($N_{Validation}$ = 103) sets.

Parameter	Set	Range	Mean	SD	CV (%)
a*	Calibration	-19.56-18.91	1.99	11.55	580.40
	Validation	-14.56-17.17	2.32	11.62	500.86
b*	Calibration	12.98-50.67	33.36	8.52	25.54
	Validation	13.53-47.19	33.77	8.89	26.33
C*	Calibration	13.51-53.44	35.27	8.87	25.15
	Validation	13.99-49.78	35.68	9.24	25.90
h*	Calibration	66.11-125.11	90.81	19.06	20.99
	Validation	69.07-114.87	90.37	19.08	21.11

Table 2 - Calibration statistics for the best equations obtained for the prediction of internal pulp colour (a*, b*, C* and h*) in Cantaloupe and Galia melons.

Parameter	Mathematical treatment	Mean	SD	Range	SEC	R^2	SECV	r^2	RPD	CV
a*	1,5,5,1	2.19	11.44	-14.26-18.66	1.96	0.97	2.16	0.96	5.30	98.63
b*	1,5,5,1	33.49	8.48	12.98-47.40	2.96	0.88	3.25	0.85	2.61	9.70
C*	1,5,5,1	35.35	8.76	13.51-50.77	3.40	0.85	3.76	0.82	2.33	10.63
h*	1,5,5,1	90.63	18.99	67.16-115.62	3.29	0.97	3.64	0.96	5.22	4.01

Table 3 - Validation statistics for the best models for predicting internal colour in Cantaloupe and Galia melons using MPLS and LOCAL algorithms.

Parameter	Regression method	Mathematic treatment	Factors	SEP	Bias	SEP (c)	r^2	Slope
a*	MPLS	1,5,5,1	15	2.45	-0.25	2.45	0.96	1.00
	LOCAL (*k* = 60)	1,5,5,1	10 (-4)	1.84	-0.01	1.85	0.98	1.02
b*	MPLS	1,5,5,1	14	3.33	0.46	3.31	0.86	0.96
	LOCAL (*k* = 40)	1,5,5,1	14 (-4)	3.12	-0.15	3.13	0.88	1.03
C*	MPLS	1,5,5,1	14	3.64	0.30	3.65	0.84	0.96
	LOCAL (*k* = 40)	1,5,5,1	12 (-4)	3.45	-0.14	3.47	0.86	1.03
h*	MPLS	1,5,5,1	14	3.74	0.30	3.74	0.96	0.97
	LOCAL (*k* = 40)	1,5,5,1	10 (-4)	2.31	0.30	2.30	0.99	1.01

Fig. 1 - Typical log (1/R) spectra for Cantaloupe and Galia melons.

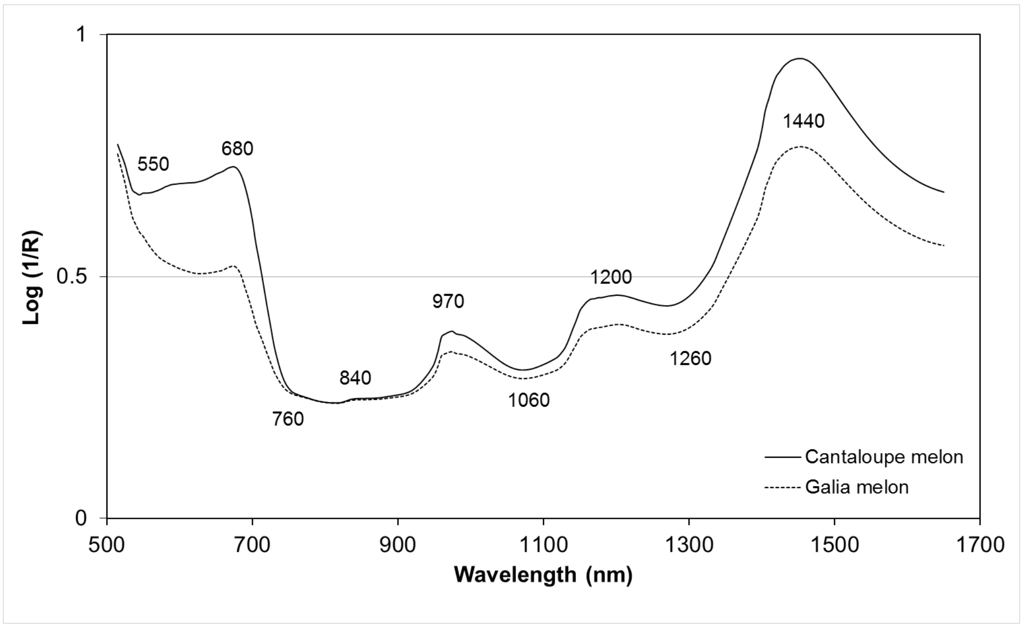

Fig. 2 - Loadings for internal colour related parameters of Cantaloupe and Galia melons.