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Prediction of 'Nules Clementine' mandarin susceptibility to rind breakdown disorder using Vis/NIR spectroscopy.

ABSTRACT

The use of diffuse reflectance visible and near infrared (Vis/NIR) spectroscopy was explored as a non-destructive technique to predict 'Nules Clementine' mandarin fruit susceptibility to rind breakdown disorder (RBD) by detecting rind physico-chemical properties of individual intact fruit from different canopy positions. Vis/NIR spectra were obtained using a LabSpec® spectrometer. Reference physico-chemical data of the fruit were obtained after 8 weeks of storage at 8°C using conventional methods and included RBD, H°, colour index, fruit mass loss, rind dry matter, sugar (sucrose, glucose, fructose, total sugars), phenolic acid concentrations. Principal component analysis (PCA) was applied to analyse spectral data to identify clusters in the PCA score plots and outliers. Partial least squares regression (PLSR) was applied to spectral data after PCA to develop prediction models for each quality attribute. The spectra were subjected to a test set validation by randomly dividing the data into calibration (60%) and validation (40%) sets. PLS-discriminant analysis (PLS-DA) models were developed to sort fruit based on canopy position and RBD susceptibility. Fruit position within the canopy had a significant influence on rind biochemical properties. Outside fruit had higher rind sugar, phenolic acids and dry matter content and lower RBD index than inside fruit. The data distribution in the PCA and PLS-DA models displayed four clusters that could easily be identified. These clusters allowed distinction between fruit from different preharvest treatments. NIR calibration and validation results demonstrated that sugars, dry matter,

colour index and mass loss were predicted with significant accuracy. The good correlation between spectral information and sugar content demonstrated the potential of Vis/NIR as a non-destructive tool to predict fruit susceptibility to RBD.

Keywords: Non-destructive technique · Vis/near infrared spectroscopy· Rind breakdown disorder (RBD) · Citrus · 'Nules Clementine' Mandarin · Canopy position.

1. Introduction

South Africa produces approximately 100 000 tons of Clementine mandarins per annum, making it the third largest producer and exporter of Clementine mandarins in the world after Spain and Morocco (Barry and Rabe, 2004). The development of various types of physiological disorders limits the postharvest storage capability and causes commercial losses. A lack of understanding the physiological mechanism underlying these disorders affects both supply and profits. The challenge is significant regarding rind breakdown disorder (RBD) of 'Nules Clementine' mandarins (*Citrus reticulate* Blanco.) that do not manifest during harvest grading and postharvest treatments but develop about 3 to 5 weeks after harvest.

RBD is initially manifested on the equatorial plane as small, irregular, slightly sunken and colourless patches of about 3 to 6 mm in diameter scattered about the flavedo (the outer-most, pigmented part of citrus rind) of the fruit (Cronje et al., 2011). These sunken areas, occurring directly above and among the oil glands of the flavedo, coalesce

47 producing larger affected areas, turning redish-brown to dark-brown, become dry and
48 necrotic in the severe stages of the disorder with extended storage period (Cronje, 2007,
49 2009). According to Agustí et al. (2001) browning of affected rind surface appears to be
50 the result of oxidative processes.
51
52 Intensive research has been conducted towards determining factors triggering RBD. As a
53 result, it has been established by several research groups that different microclimates are
54 influencing sensitivity of fruit to RBD and similar rind disorders such as rind pitting on
55 oranges (Alférez and Zacarìas, 2001) and grapefruit (Alférez and Burns, 2004) and peteca
56 spots on lemons (Wild, 1991). In a study conducted in Spain (northern hemisphere),
57 Almela et al. (1992) reported that these fluctuations could also be observed among fruits
58 from the same tree and the incidence being by exposure of individual fruit to the sun.
59 These investigators further showed that fruit oriented to the north-west (NW) in canopy
60 were most affected by the disorder. In a later study conducted in the same country, Agustí
61 et al. (2001) corroborated that fruit positioned in the NW face of the tree to be more
62 prone to develop the rind pitting disorder and this was reported to be consistent over five
63 seasons. Similar observations were reported in 'Fortune' (Duarte and Guardiola, 1995)
64 and 'Encore' (Chikaizumi, 2000) mandarin fruit, where it was maintained that the
65 disorder affects mainly the exposed fruit from the north-west quadrant of the tree.
66
67 In a study conducted in South Africa (southern hemisphere), fruit position, and therefore
68 exposure to high (outside) or low (inside) light levels in the canopy, affect the flavedo
69 concentration of carbohydrates during fruit development (Cronje, 2009; Cronje et al.

2011). The latter authors reported the flavedo from fruit borne on the outside of the canopy to have significantly higher sucrose, glucose and fructose content than the fruit borne inside the canopy. Interestingly, the results obtained by this group of investigators revealed a correlation between fruit position, rind sugar content and ultimately development of RBD. The incidence of rind breakdown was higher on inside fruit compared with the outside fruit and this was consistent from season to season and could be attributed to their exclusion from adequate sunlight during their fruit development. In addition, fruit borne inside the canopy had lower chlorophyll and carotenoid contents, and therefore poorer rind colour (Khumalo, 2006), and lower carbohydrates rendered them susceptible to the disorder (Cronje, 2009). Although intensive research aimed at appreciating RBD has been conducted, the disorder still occurs frequently and unpredictably, reducing the quality of the fruit (Almela et al., 1992; Cronje, 2005).

There is therefore a need to develop an objective, fast and non-destructive assessment that can be used to determine/predict citrus fruit susceptibility to rind disorders accurately. Non-visible information, such as that provided by near infrared (NIR) region of the spectrum can improve the inspection by detecting rind biochemical profile and hence the detection of non-visible physiological disorders (Blasco et al., 2007). Most current non-destructive quality measurement using NIR spectroscopy (NIRS) has been developed to assess fresh fruit according to their internal quality attributes (Butz et al., 2005). Very limited research work has been conducted to develop a technology that can assess, predict and monitor the physiological disorders and rind physiological disorders of citrus fruit in particular. Nevertheless, NIRS has been used successfully to detect

93 surface bruising in apple (Geeola et al., 1994), surface defects in peach (Miller and
94 Delwiche, 1991), storage disorders in kiwifruit (Clark et al., 2004) and drying internal
95 disorder in Tangerine citrus (Peiris et al., 1998). Recently, Teerachaichayut and co-
96 workers (2011) successfully used NIR spectroscopy to non-destructively predict pericarp
97 hardening disorder in magosteen fruit. The trend has constantly shifted towards
98 developing reliable and cost effective technologies to non-destructively screen fruit
99 physiological disorders. Recently, Zheng et al. (2010) used NIR in the reflectance mode
100 to predict oleocellosis sensitivity in citrus fruit. A review by Magwaza et al. (2011)
101 discusses the recent developments and application of Vis/NIR spectroscopy to non-
102 destructively evaluate internal and external fruit quality.
103
104 In summary, the knowledge of biochemical changes in the rind of citrus fruit that could
105 be used to precisely predict fruit rind condition and therefore susceptibility to rind
106 disorders is limited. However, previous research by Cronje et al. (2011) indicated that
107 fruit position within the canopy affects rind biochemical profile, particularly
108 carbohydrate concentration and hence susceptibility of fruit to RBD. This study aims to
109 explore the use of diffuse reflectance Vis/NIR spectroscopy in the wavelength range of
110 350-2500 nm as a non-destructive tool to predict susceptibility to RBD by detecting rind
111 physico-chemical properties of individual intact fruit from different canopy positions.
112
113 **2. Material and methods**
114

115 *2.1. Site, fruit sampling and postharvest handling*

116

117 A total of 15 'Nules Clementine' mandarin trees in an orchard at Stellenbosch University
118 experimental farm, Western Cape Province, South Africa (33°53'4.56"S, 18°37'36.84"E)
119 were identified and marked based on their health and fruit setting ability. On each tree,
120 200 fruit from sun-exposed and 200 from shaded canopy positions were randomly
121 selected and tagged. To increase our success of having fruit with RBD, a method of
122 enhancing the disorder demonstrated by Cronje (2009) was adopted. Briefly, during
123 January 2011 (after physiological drop about four months until commercial maturity),
124 half of the selected fruit from each position was covered with brown paper bags without
125 removing or covering subtending leaves. The study consisted of four preharvest
126 treatments, viz., outside, outside bagged, inside and inside bagged.

127

128 Upon reaching commercial maturity, on 16 May, individual fruit were harvested
129 according to industry practice, coded according to treatment and canopy position and
130 underwent all commercial postharvest practices, including drenching (Thiabenzole, 500
131 mg/L; Imazalil, 500 mg/L and 2,4-dichlorophenoxyacetic acid, 125 mg/L) and waxing
132 (polyethylene citrus wax, Citrushine®, Johannesburg, South Africa). After which they
133 were brought to the postharvest evaluation laboratory, sorted to remove any defective
134 fruit and weighed. A total of 80 blemish free fruit (20 fruit from each treatment) were
135 selected to provide fruit samples for non-destructive and destructive measurements. After
136 phytosanitary inspection and certification, these fruit were separately packed in boxes

137 marked, sent at room temperature via a courier service to Cranfield University (CU) in
138 the United Kingdom, where postharvest storage took place.
139
140 *2.2. Spectral acquisition*
141
142 Vis/NIR spectra were obtained upon arrival at CU using a method described by Kuang
143 and Mouazen (2011). Spectral acquisition from intact fruit samples was carried out using
144 a mobile fibre-optic Vis/NIR spectrophotometer (350-2500nm) (LabSpec2500(r) Near
145 Infrared Analyzer, Analytical Spectral Devices Inc., USA) in diffuse reflectance mode
146 equipped with one Si array (350-1000nm) and two Peltier cooled InGaAs detectors
147 (1000-1800 nm and 1800-2500 nm). The sampling interval of the instrument was 1 nm.
148 However, the spectral resolution was 3 nm at 700 nm and 10 nm at 1400 nm and 2100
149 nm. A high intensity probe with an in-built light source was used. A quartz-halogen bulb
150 of 3000 Kelvin light source and a detection fibre are gathered in the high intensity probe
151 enclosing a 35° angle.
152
153 Prior to scanning the fruit samples, and periodically at intervals of 30min, white reference
154 measurements were taken. Fruit samples were placed in direct contact with the high
155 intensity probe. Reflectance spectral data was acquired from 8 position of the fruit; 4
156 from equatorial spots of the fruit and 2 from the stem-end and 2 from the stylar-end of the
157 fruit, averaged and used for spectral pre-processing and multivariate analysis.
158

2.3. *Physico-chemical measurements to obtain reference values*

2.3.1. *Storage conditions, RBD rating, weight and rind colour*

After scanning, fruits were stored in a cold room with delivery air temperature of 8°C, a temperature which is known to cause the highest degree of RBD incidence (Khumalo, 2006). During cold storage, fruit were scored weekly, for the incidence of RBD for the duration of 8 weeks. RBD was scored on a subjective scale from 0 = no breakdown to 3 = severe breakdown. RBD was then expressed as RBD index (RBDI), estimated as previously described (Alférez et al., 2003) and calculated according to the following formula previously reported for chilling injury and peel pitting by Lafuente et al. (1997) and Lafuente and Sala (2002):

$$RBDI = \frac{\sum \{RBD(0-3) \times \text{No.of fruit in each class}}{\text{Total number of fruit}} \quad (1)$$

Fruit were weighed weekly using a calibrated balance (Mettler Toledo, ML3002E / 01, Switzerland). Rind colour components were measured in L*a*b* colour space using Minolta CR-400 colourimeter (Chroma Meter CR-400, Konica Minolta Sensing Inc., Japan) after calibration using standard white tile (CR-A43; Y = 93.1, x = 0.3138; y = 0.3203). From L*, a* and b* colour parameters, colour index (CI) was calculated according to Jimenez-Cuesta et al. (1981) using the following formula:

$$CI = \frac{1000 \times a}{L \times b} \quad (2)$$

2.3.2. *Sample preparation*

After 8 weeks in storage, fruit samples were destructed where rind was peeled from the rest of the fruit. The pulp was juiced and the juice used for fresh TSS analysis. TSS was measured with a digital hand-held refractometer (Palette, PR-32α, Brix 0.0-32.0, Atago, Co. LTD, Japan) using 1 mL of freshly squeezed juice and expressed as °Brix. The rind from each sample was snap frozen in liquid nitrogen, separated into two portions, of which one portion was stored at -40°C and the other at -80°C freezer until further analysis.

Fresh frozen rind samples were freeze-dried in Edwards Modulyo freeze drier (W. Sussex, UK) for 7 days at 0.015 kPA and -55°C. Lyophilized samples were weighed and water content was calculated from freeze dried samples and expressed as a percentage of fresh weight. Samples were then ground using pestle and mortar into fine powder and returned into the freezer prior to being used for sugar and phenolic acids determination by HPLC.

2.3.3. *Extraction and HPLC quantification of non-structural sugars*

Sugar was extracted from 150 mg of fruit rind powder using 62.5% (v/v) aqueous methanol as described elsewhere by Terry et al. (2007). Following extraction,

204 concentration of fructose, glucose and sucrose was determined using an Agilent 1200
205 series HPLC binary pump system (Agilent, Berks., UK), equipped with an Agilent
206 refractive index detector (RID) G1362A, based on the method described by Crespo et al.
207 (2010). Briefly, sample extracts was diluted (1:10), and injected into a Rezex RCM
208 monosaccharide Ca^{+} (8%) column of 300 mm x 7.8 mm diameter (Phenomenex,
209 Torrance, CA) with a Carbo-Ca^{2+} guard column of 4 mm x 3 mm diameter
210 (Phenomenex). Temperature of the column was set at 80°C using a G1316A
211 thermostarted column compartment. The mobile phase used was HPLC-grade water at a
212 flow rate of 0.6 ml/min (Giné Bordonaba and Terry, 2008). The presence and abundance
213 of the selected sugars was calculated by comparison of peak area with peak of known
214 standards using ChemStation Rev. B.02.01.
215
216 *2.3.4. Extraction and HPLC quantification of phenolic compounds*
217
218 Phenolic acids were extracted and quantified using a method described elsewhere by
219 (Magwaza et al., 2012). Briefly, a 150 mg of freeze dried rind powder was dissolved into
220 3 mL 70:29.5:0.5 (methanol:H_2O:HCL). Samples were extracted at 35°C in water bath
221 for 30 minutes, agitated for 30s every 5 minutes and the flocculate filtered through a 0.2
222 µm syringe filter. Phenolic acid concentrations were determined using the HPLC system
223 equipped with an Agilent DAD G1315B/G1365G photodiode array with multiple
224 wavelength detector.
225

226 *2.3.5. Determination of antioxidant capacity*

227

228 Antioxidant capacity was measured on freeze-dried samples following the method by
229 Crespo et al. (2010). The absorbance of prepared sample solutions was measured
230 spectrophotometrically at 517 nm using a Camspec M501 UV/vis spectrometer.
231 Basically, the antioxidant capacity determination with 2,2-diphenyl-1-picrylnhydrazyl
232 (DPPH) is based on the properties of DPPH, which its radical form has an absorption
233 band at 517 nm and disappears upon reduction by an antiradical compound.

234

235 *2.4. Data analysis*

236

237 *2.4.1. Statistical analysis*

238

239 Statistical analyses carried out using SPSS 10.0 for Windows (SPSS Inc. Chicago, USA).
240 Data was subjected to analysis of variance (ANOVA). Least significant difference values
241 (LSD; P=0.05) were calculated for mean separation (Landahl et al., 2009).

242

243 2.4.2. NIRS analysis, calibration development and validation

244

245 Before analysis, the reflectance spectra in Indico format (Indico Pro 5.6 software,
246 Analytical Spectral Devices Inc., USA) were transformed to absorbance (log (1/R).
247 Calculations of the average of 8 spectra obtained from each fruit, pre-processing and

248 calibration methods were executed using the Unscrambler chemometric software (The
249 Unscrambler Version 9.2, Camo Process, SA, Trondheim, Norway).
250
251 Several pre-processing methods including, smoothing using moving average and
252 Savitzky-Golay methods, full multiple scatter correction (MSC), Savitzky-Golay first
253 derivative and second derivative, minimum and maximum normalisation and vector
254 normalisation (SVN) were tested to correct light scatter and reduce the changes of light
255 path length. After pre-processing trials, the optimal model performance was obtained
256 using the Savitzky-Golay second derivative with the polynomial order of 5 and MSC.
257 Savitzky-Golay second derivative was used to correct light scattering properties while
258 MSC was used to correct for additive, multiplicative effects of the spectra, and pathlength
259 variations (Leonardi and Burns, 1999; Gómez et al., 2006).
260
261 In order to determine effective wavelength, discriminate fruit from four canopy positions
262 using Vis/NIR and to detect outliers, PCA was performed using full cross validation.
263 Partial least squares regression (PLSR) was applied to spectral data to develop prediction
264 models for each quality attribute. A PLS variant known as partial least squares
265 discriminant analysis (PLS-DA) was also used in order to classify fruit from different
266 canopy positions according to the spectra. A method by Chen et al. (2011) was used in
267 the application of PLS-DA. Briefly, fruit from each of the canopy positions in the
268 calibration set was assigned a dummy variable as a reference value (outside = 1, outside
269 bagged = 2, inside = 3 and inside bagged = 4). In addition, due to discrete nature of RBD
270 scores, samples were assigned a binary dummy variable as a reference value, which was

271 an arbitrary number whether the sample belongs to a particular position or not. RBD
272 affected fruit were set as reference data one, while unaffected fruit were assigned to 1
273 (Teerachaichayut et al., 2011).
274
275 To develop PLS models, the dataset was randomly separated into two subsets, 60% for
276 calibration and 40% for test set validation. The regression statistics of developed models
277 was described by the value of the root mean square error of calibration (RMSEC), root
278 mean square error of validation or prediction (RMSEP), the Perason correlation
279 correlation coefficients (R) between predicted and observed reference values, number of
280 latent variables (LVs), and the residual predictive deviation (RPD), described by
281 Williams and Sobering (1996) as the ratio of the standard deviation of the reference data
282 for the validation set to the RMSEP. The ideal model should have higher R and RPD
283 values as well as lower RMSEC and RMSEP values. The optimal number of LVs was
284 determined as the minimum number of LVs corresponding to the first lowest value of the
285 RMSEC or RMSEP from the plot of the RMSEC or RMSEP for increasing number of
286 LVs (Davey et al. 2009). The stability of the calibration model was tested by
287 interchanging validation and calibration data sets and checking that the differences in the
288 regression statistics obtained were small (Alvarez-Guerra et al., 2010).
289
290 **3. Results and discussion**
291

292 *3.1.Rind breakdown disorder and biochemical profile of fruit from different canopy*
293 *positions.*
294
295 Symptoms of rind breakdown disorder were visible on affected fruit after five weeks of
296 continuous storage at 8°C. RBD was significantly affected by preharvest manipulation of
297 sunlight exposure (Table 1). Outside fruit had the lowest susceptibility to develop the
298 disorder compared to other preharvest treatments. Fruit position within the canopy on its
299 own did not show a significant difference on fruit susceptibility to RBD. However,
300 exclusion of sunlight by bagging fruit resulted in increased fruit susceptibility but only
301 showed a significant difference on fruit located inside the canopy. These findings are
302 consistent with those observed by Almela et al. (1992). In their study, these authors
303 established that the sensitivity of fruit to development of rind spots related to RBD in
304 'Fortune' mandarins was influenced by different microclimates. Similar to observations
305 reported by Cronje et al. (2011), fruit position within the canopy affected rind colour
306 index (CI). Fruit borne on the outside of the tree canopy had the highest CI and hence
307 were more orange while shaded fruit had pale, yellow rinds.
308
309 Foregoing research suggests that rind water status is a factor prevailing in the
310 susceptibility of citrus fruit to rind physiological disorders (Cohen et al., 1994; Alférez
311 and Burns (2004). In this study, fruit from the bagged treatments, both inside and outside
312 of the canopy, were characterized by high postharvest weight loss, and this was
313 essentially due to water loss by transpiration, as this account for 90% of total weight loss
314 (Ben-Yehoshua, 1969). Water loss from the fruit results from a water pressure gradient

315 prevailing between the fruit rind, which is close to saturation with water, and the less
316 saturated outer atmosphere (Ben-Yehoshua *et al*., 1994; Macnish *et al*., 1997).
317
318 Fruit position within the canopy also had a significant influence on rind biochemical
319 properties. Results in Table 1 showed that outside fruit had higher TSS, dry matter
320 content, glucose and total phenolic acid concentrations compared to shaded samples. The
321 effect of canopy position on fruit quality was documented by (Barry et al., 2000**)** who
322 reported that fruit from the south western top part of the canopy had significantly higher
323 soluble solids contents, lower titratable acid content and higher ratio than fruit borne in
324 the north east bottom position. In this study, bagged fruit from outer portion of the
325 canopy had significantly higher glucose concentration of 92.03 mg/g DW compared to
326 inside fruit, which had 66.92 mg/g DW glucose concentration. On the contrary, sucrose,
327 and total carbohydrate of bagged fruit from inside the canopy were significantly higher in
328 relation to other three positions. It was therefore noteworthy that these fruit also had
329 highest susceptibility to RBD.
330
331 In addition to carbohydrate contents, phenolic acid concentration and antioxidant
332 capacity were affected by fruit exposure to sunlight. Unbagged samples from the inside
333 and outside position had relatively higher phenolic acid content of 39.29 and 37.65 mg/g
334 DW when compared to outside bagged and inside bagged samples which had 33.48 and
335 34.68 mg/g DW, respectively. Moreover, the antioxidant activity of inside bagged
336 samples, i.e. fruit with the highest RBD, was much higher than those with low RBD
337 disorder. It has reported previously that the presence antioxidant species or the lack

thereof could be implicated to the development of various postharvest disorders including non-chilling peel pitting in 'Navelate' oranges (Cajuste and Lafuente, 2007). The results presented above are in accord with the notion that manipulating light levels around an individual fruit reduces rind condition and susceptibility to the disorder. Pearson correlation analysis between physico-chemical properties and RBD development had very low correlation coefficients which serve to prove the complexity of factors involved in the development of this disorder. Furthermore, the lack of a specific threshold values below or above which all fruit become affected and above or below which all fruit stay healthy suggests that several preharvest and postharvest factors also play a role.

3.2.Vis/NIR spectroscopy

3.2.1. Distribution of prediction and validation reference data

In this study, reference data set was partitioned into the calibration (60% of n) and validation (40% of n) set. Table 2 shows the distributional statistics for reference datasets used in calibration and test validation. The reference measurements of all parameters in calibration and validation were fairly normally distributed round the means. Although the selection method for calibration and validation was random, validation data set was scrutinised to ensure that the validation data sets were confined within a boundary of the calibration set. The interpretation of calibration results depends greatly on the precision of the determined reference data and enough variation in both calibration and validation data (Lu et al. 2006). As is apparent in the range and CV% values of the data presented,

361 the calibration and validation values of the sample quality parameters cover a large range,
362 which is helpful for developing calibration models for NIR spectroscopy (Clément et al.
363 2008). For instance, the mean concentration of sucrose values used for calibration and
364 validation were 101.40 and 78.81 mg/g DW with standard deviation of 43.27 and 34.15
365 mg/g DW, respectively. The range of total sugars in the calibration set was from 121.91
366 to 511.11 mg/g DW and the range of validation set was from 141.91 to 492.28 mg/g DW
367 with corresponding CV % of 29.38 and 36.31%, respectively.
368
369 *3.2.2. Spectrum description*
370
371 The absorbance spectra presented in Figure 1(a) portrays the typical spectra obtained
372 from intact "Nules Clementine" mandarins subjected to different preharvest treatments.
373 Each line represents the average spectra from 20 fruit in each preharvest treatment.
374 Spectral features were similar to those obtained by Gómez and co-workers (2006). Strong
375 absorption bands around 670, 740, 980, 1200, 1450, 1780 and 1930 nm were observed.
376 Absorption at these wavebands were, respectively, due to red absorbing pigments,
377 particularly chlorophyll (Clément et al. 2008), third overtone of O-H stretching, second
378 overtone of H-O-H stretching modes of water, second and first overtones of C-H
379 stretching as well as the third overtone of O-H, C-H and C-H_2 deformation associated
380 with sugar solution reported by Kawano et al. (1993) and Golic et al. (2003). It should be
381 noted that the average spectra of inside bagged fruit (with highest RBD) had a distinctly
382 stronger absorbance in the waveband between 600 and 900 nm. This was similar to
383 results obtained by Zheng et al. (2010) for prediction of olleocelosis disorder, where large

384 variations in absorbance spectra were observed among fruit with different sensitivities in
385 the same waveband. Since the intensities of reflectance vary with concentrations of
386 biochemical constituents of the sample (Williams and Norris, 2001) this band may
387 possibly be related to fruit sensitivity to rind physiological disorders such as oleocellosis
388 and RBD.
389
390 3.2.3. *Pre-processing methods*
391
392 The spectra of solid samples such as fruit are influenced by physical properties such as
393 shape, size, path length, etc. (Leonardi and Burns 1999), which create noise and
394 determine light scattering properties. As a common practice in NIRS, obtained spectra
395 was subjected to several pre-processing methods and the suitable pre-processing methods
396 were selected. Results presented in Table 3 show that Savitzky-Golay second derivative
397 with the fifth order polynomial (Figure 1(b)) provided the best results for the PCA
398 classification and PLS model for predicting RBD. MSC (Figure 1(c)) gave best results for
399 PLS prediction of physico-chemical properties such as h°, CI, weight loss, dry matter
400 content, and carbohydrate concentrations as well PLS-DA classification by preharvest
401 treatments.
402
403 *3.2.4. Vis/NIR- based PCA and PLS classification models*
404
405 Individual spectra from 8 positions within the fruit and the average spectra were tested to
406 develop PCA and PLD-DA classification models. Average spectra showed better models
407 than individual spectra; and thus subsequent analyses were based on average spectra.

PCA was performed on Vis/NIR spectra to compare spectral characteristics of fruit from different preharvest treatments. The PCA applied to the spectra using Savitzky Golay second derivative pre-processing revealed better grouping of the samples than other tested pre-processing methods. The data distribution in the PCA score plot presented in Figure 2 displayed four clusters that could easily be identified. These clusters allowed distinction between fruit from different preharvest treatments. The first two principal components (PC) accounted for 68.0 % of the total variability, PC1 explains the 53.0 % of the variance and PC2 explained 15.0 % of the variance. The effective wavelength band for this classification was from 350 to 1200 nm with a strong absorption at 670 nm influenced by chlorophyll and three at 740, 980, 1200 nm corresponding to water (O-H) functional groups. From this, it could be concluded that a combination of colour and moisture content of the rind play an important role in discriminating from different positions of the canopy.

Spectral data was further subjected to discriminant analysis by assigning fruit from each canopy positions to a dummy variable (1, 2, 3 and 4 for outside, outside bagged, inside bagged and inside, respectively. Figure 3 depicts performance of the PLS-DA model to classify fruit based on their origin within the tree canopy using full spectral range (350-2500 nm) and MSC spectral pre-processing. The prediction accuracy determined using 24 test set for validation was high (r = 0.971, RMSEP = 0.304).

3.2.5. Vis/NIR- based PLS prediction models

Table 3 shows summary statistics for calibration and validation results for the prediction of different physico-chemical properties with PLS models. After testing different wavelength ranges based on observed peaks and information provided in the literature, wavelength bands that gave the lowest RMSEC and RMSEP were selected to develop calibration models for each physico-chemical property. As would be expected, models for colour parameters (h° and CI) were developed using visible range (350-700 nm) of the spectrum, whereas RBD model was developed with the region between 350-1000 nm. This was in accordance with the range used by Zheng et al. (2010) to develop models for predicting susceptibility of citrus fruit to oleocellosis, another rind physiological disorder.

Prediction models for sucrose, fructose, glucose, total sugars, TSS, dry matter and water loss were developed using wavelength range between 900 to 1800 nm. According to the absorption bands of common foods constituents provided by Williams and Norris (2001), all these biochemical components have absorption bands in this spectral region. Results obtained in this study are similar to previous studies suggesting that the range from 350-1800 nm is suitable for predicting colour parameters (Sun et al., 2009), dry matter (Guthrie et al., 2005a, b), sucrose, fructose, glucose (Tewari et al., 2008) and sugar content (Liu et al., 2010).

Models for predicting quality parameters such as colour index, h°, DM and water loss showed significantly high performance, with predictive *R* values ranging from 0.91 to 0.98 and RPD ranging from 2.34 to 4.13 for water loss and CI, respectively (Figure 5). NIR calibration and validation results demonstrated that sugars, dry matter, colour index

454 and mass loss were predicted with significant accuracy. The prediction performance for
455 sugars (sucrose, fructose, glucose, and total sugars) was high with *R* of 0.88, 0.94, 0.95,
456 and 0.95 and corresponding RMSEP values of 24.36, 11.41, 11.58, 31.04 mg/g DW,
457 respectively (Figure 6). Although the accuracy is slightly lower, these results were
458 comparable to those reported in the literature by Tewari et al. (2008) for the prediction of
459 sucrose, fructose and glucose of grapefruit.
460
461 As suggested by Davey et al. (2009) and Saeys et al. (2005), although the correlation
462 between NIR predicted and reference values is high, it is also very critical to verify the
463 accuracy of the model by referring to the RPD values. These authors stated that RPD
464 values below 1.5 are considered unusable, those between 1.5 and 2.0 are suitable for
465 rough prediction, those between 2.0 and 2.5 are suitable for quantitative predictions,
466 while RPD values between 2.5 and above 3.0 are respectively considered good and
467 excellent prediction models. The low RPD values, 0.30 and 1.40 for RBD and sucrose,
468 respectively, clearly indicate poor accuracy of these models. The poor accuracy of these
469 models could be attributed to high variation of these parameters in both calibration and
470 test set data. Furthermore, the low predictability of sucrose could possibly results due to
471 the difference in molecular weight of sucrose (MW=342.30 g/mol) compared to fructose
472 and glucose (MW=180.16) (Golic et al., 2003). This difference in molecular weight is
473 such that there are 1.89 times fewer number of sucrose molecules than glucose and
474 fructose in the same weight of sample. Therefore, the intensity of the bands associated
475 with hydrogen bonding is smaller in sucrose than in glucose and fructose.
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The complexity of biological factors involved in the development of RBD complicated the development of an acceptable model for predicting the disorder. However, the high ability of Vis/NIR to classify fruit based on their origin within the canopy using PLS-DA could have online practical interest for online application. Another reason for the poor prediction model for RBD is that calibration and test set also contained a large proportion of samples in which RBD didn't develop. The inability to generate the disorder due to unfavourable conditions to develop the disorder under this type of study occurs. To correct this problem in future studies, it will be necessary to increase sample size for both calibration and validation sets. This is to ensure that the distribution of the disorder is wide, normal around the mean and that it represents distribution present in a harvested population of mandarin fruit (Davey et al. 2009).

4. Conclusions

In this study, the positional effects within the canopy and bagging were significant in altering rind biochemical properties. Outside unbagged fruit had higher rind sugar, phenolic acids and dry matter content and lower RBD index than inside and bagged fruit. The data distribution in the PCA and PLS-DA models displayed four clusters that could easily be identified. These clusters allowed distinction between fruit from different preharvest treatments. NIR calibration and validation results demonstrated that sugars, dry matter, colour index and mass loss were predicted with significant accuracy. The exploration of the statistics of the developed models revealed the high potential that Vis/NIR spectroscopy has to non-destructively detect rind biochemical profile and hence susceptibility to RBD. The good correlation between spectral information and

biochemical information demonstrated the potential of Vis/NIR as a non-destructive tool to predict fruit susceptibility to RBD.

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Table 1: Physico-chemical profile of fruit from different canopy positions. Different alphabets next to figures (mean and standard error of the mean) in the same row are significantly different.

Quality Parameter	Canopy position			
	Outside	Outside bagged	Inside	Inside bagged
h°	67.34±0.58ns	75.50±1.46ns	62.94±11.32ns	74.01±0.97ns
CI	6.12±0.19b	3.62±0.38a	3.65±0.57a	3.99±0.27a
Mass (g)	107.63±4.68b	104.89±4.28b	89.29±4.95a	107.61±6.38b
RBDI	0.00±0.00a	0.05±0.03a	0.04±0.04a	0.19±0.05b
WL (g)	9.51±0.42ab	13.02±1.76bc	7.76±0.58a	13.62±1.67c
TSS (°Brix)	13.71±0.24b	11.89±0.28a	12.11±0.27a	11.94±0.25a
DMC (%)	30.00±0.78b	26.98±0.51a	28.780.96a	26.99±0.79ab
Sucrose (mg/g DW)	95.31±6.07b	93.59±9.51b	63.30±8.65a	114.94±12.51b
Glucose (mg/g DW)	92.03±5.29b	85.44±8.94ab	66.92±6.97a	77.09±8.50ab
Fructose (mg/g DW)	126.48±5.19ab	130.21±9.76b	100.65±8.52a	122.26±9.71ab
Total Sugars (mg/g DW)	313.82±11.58b	309.23±25.05b	230.88±22.82a	314.29±27.70b
Total phenolic acids (mg/g DW)	39.29±0.86b	33.48±1.10a	37.65±0.85b	34.68±1.25a
DPPH (µmol trolox eq./g DW)	0.95±0.04b	1.23±0.18b	0.50±0.11a	1.36±0.17b

687 Table 2: Mean, standard deviation (SD), range and coefficient of variation (CV %) for

688 calibration (60% of n) and validation (40% of n) subsets of mandarin fruit.

Quality parameter	Calibration data set			Validation data set		
	Mean±SD	Range	CV%	Mean±SD	Range	CV%
h°	73.14±5.11	63.53-89.27	7.05%	72.28±6.27	64.51-85.71	8.54%
CI	4.52±1.47	1.25-7.49	32.59%	4.29±1.76	0.07-7.04	41.12%
RBDI	0.09±0.18	0.00-0.56	205.84%	0.05±0.10	0.00-0.33	187.75%
Mass (g)	107.90±22.87	67.40-153.60	21.20%	94.58±15.07	70.7-123.1	15.93%
WL (g)	11.33±5.21	4.51-28.53	45.99%	10.83±6.45	4.87-25.84	59.52%
DMC (%)	27.85±3.14	22.84-35.41	11.28%	28.56±3.29	23.54-35.71	11.50%
Sucrose (mg/g DW)	101.40±43.27	35.17-207.91	42.66%	78.81±34.15	42.85-144.30	43.34%
Glucose (mg/g DW)	83.87±30.11	22.19-152.1	35.91%	75.69±32.59	36.01-127.60	43.06%
Fructose (mg/g DW)	124.30±33.52	53.72-195.9	26.96%	114.70±37.38	59.38-182.48	32.62%
Total Sugars (mg/g DW)	309.60±90.97	121.91-511.11	29.38%	269.17±97.74	141.91-492.28	36.31%
PLS-DA dummy variables	2.34±1.07	1.00-4.00	45.79%	2.63±1.17	1.00-4.00	44.67%

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Table 3: An overview of statistics obtained during calibration and validation of models for individual quality parameters.

Quality parameter			Calibration model			Validation model			
	LV	Prepr	R	RMSEC	RPD	R	RMSEP	RPD	Info. Region (nm)
h°	3	MSC	0.97	1.31	3.91	0.97	1.66	3.78	350-700
CI	3	MSC	0.98	0.37	3.93	0.98	0.43	4.13	350-700
RBD (binary scores)	5	2nd der	0.77	0.27	0.67	0.70	0.34	0.30	350-1000
WL (g)	10	MSC	0.92	2.01	2.59	0.91	2.76	2.34	900-1700
DMC (%)	8	MSC	0.98	0.68	4.65	0.96	0.92	3.57	900-1700
Sucrose (mg/g DW)	10	MSC	0.96	12.38	3.50	0.88	24.36	1.40	900-1700
Glucose (mg/g DW)	10	MSC	0.95	9.18	3.28	0.94	11.41	2.86	900-1700
Fructose (mg/g DW)	14	MSC	0.98	6.33	5.30	0.95	11.58	3.23	900-1700
Total Sugars (mg/g DW)	10	MSC	0.94	30.42	2.99	0.95	31.04	3.15	900-1700
PLS-DA dummy variables	10	MSC	0.98	0.20	5.25	0.97	0.30	3.85	350-2500

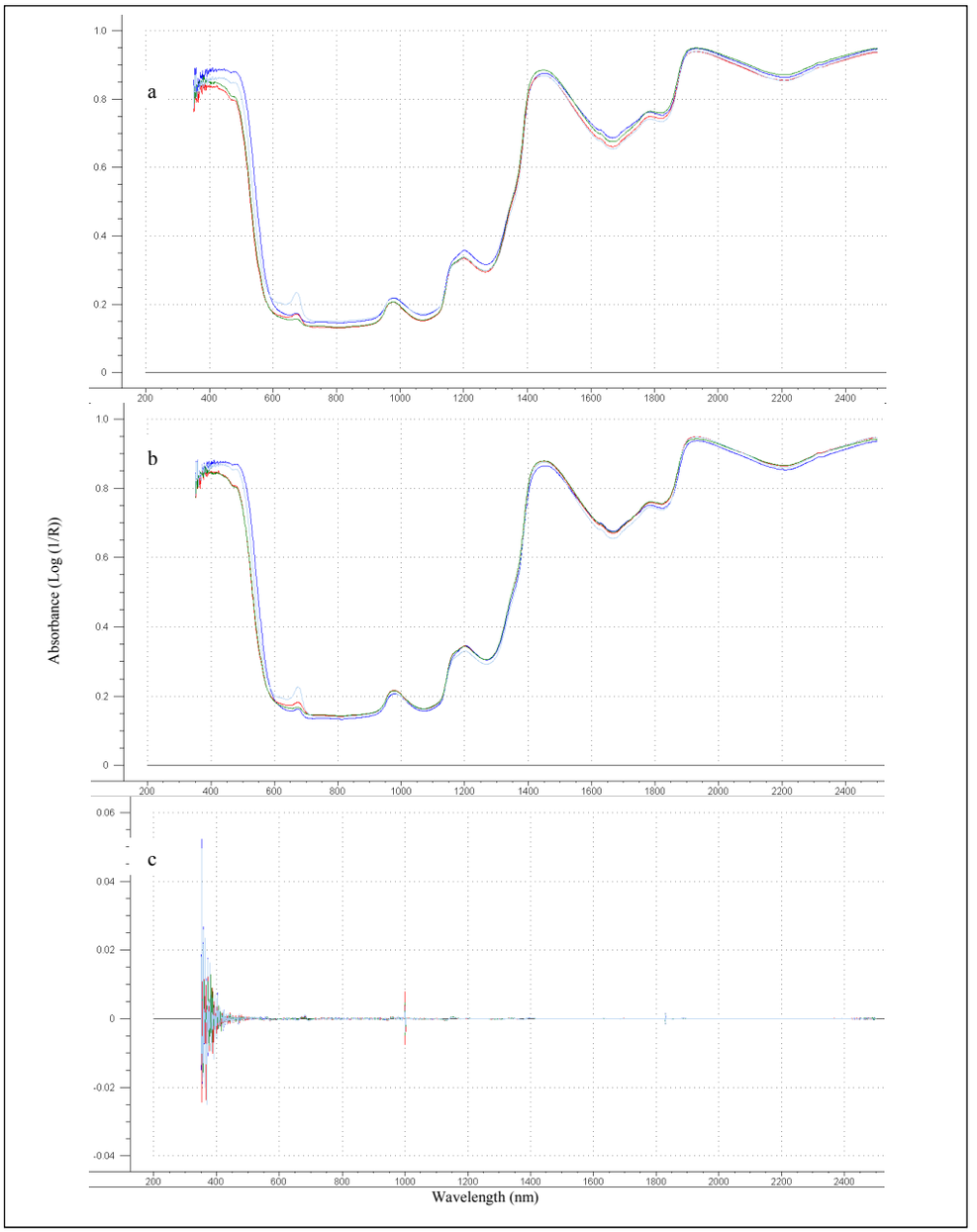

Figure 1: Typical average Vis/NIR spectra (350-2500 nm) of intact "Nules Clementine" mandarins obtained from fruit harvested from four different preharvest treatments; outside (*deep blue line*), outside bagged (*red line*), inside (*inside*), and inside bagged (*light blue line*). (**a**) Without pre-processing; (**b**) multiple scatter correction; (**c**) Savitsky-Golay first derivative of the spectra.

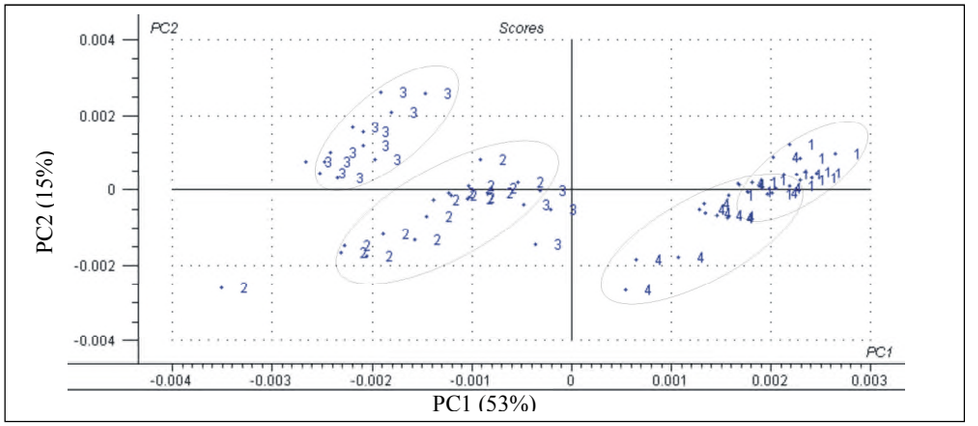

Figure 2: PCA plot for the two PC factors showing spectral ability to sort based on their origin within the tree canopy.

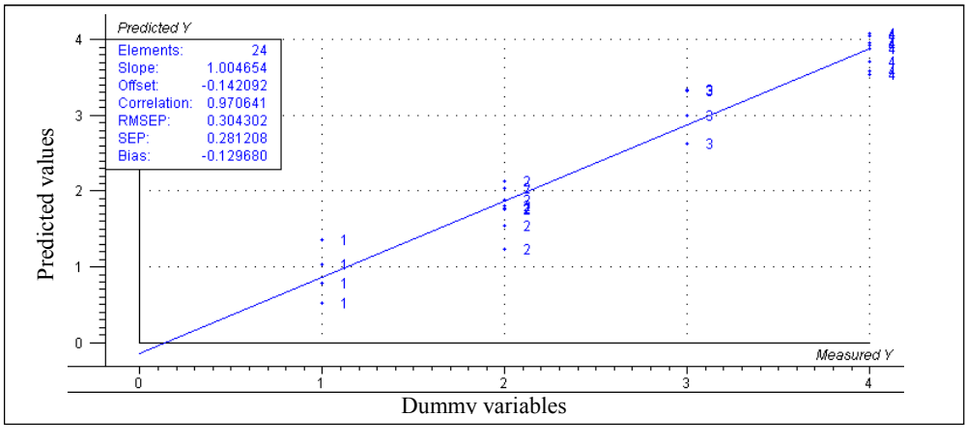

Figure 3: PLS-DA models showing Vis/NIR spectral ability to predict fruit origin within the tree canopy.

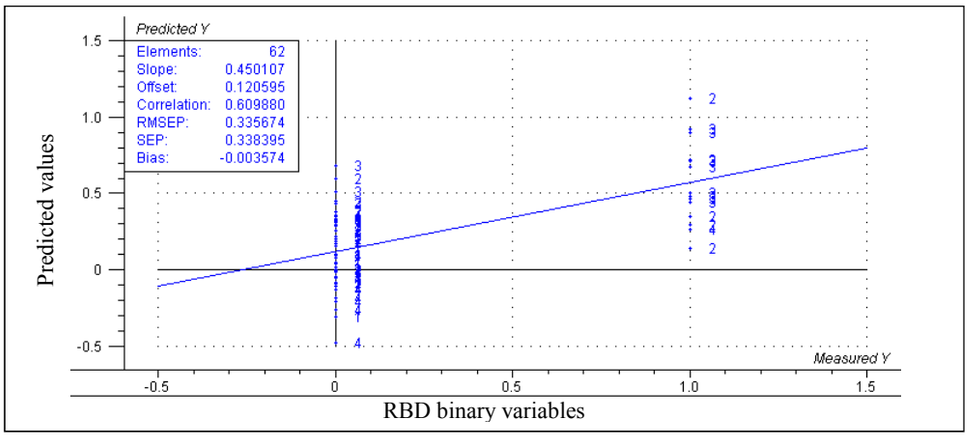

Figure 4: PLS scatter plot showing Vis/NIR model performance to classify fruit based on the occurrence of RBD.

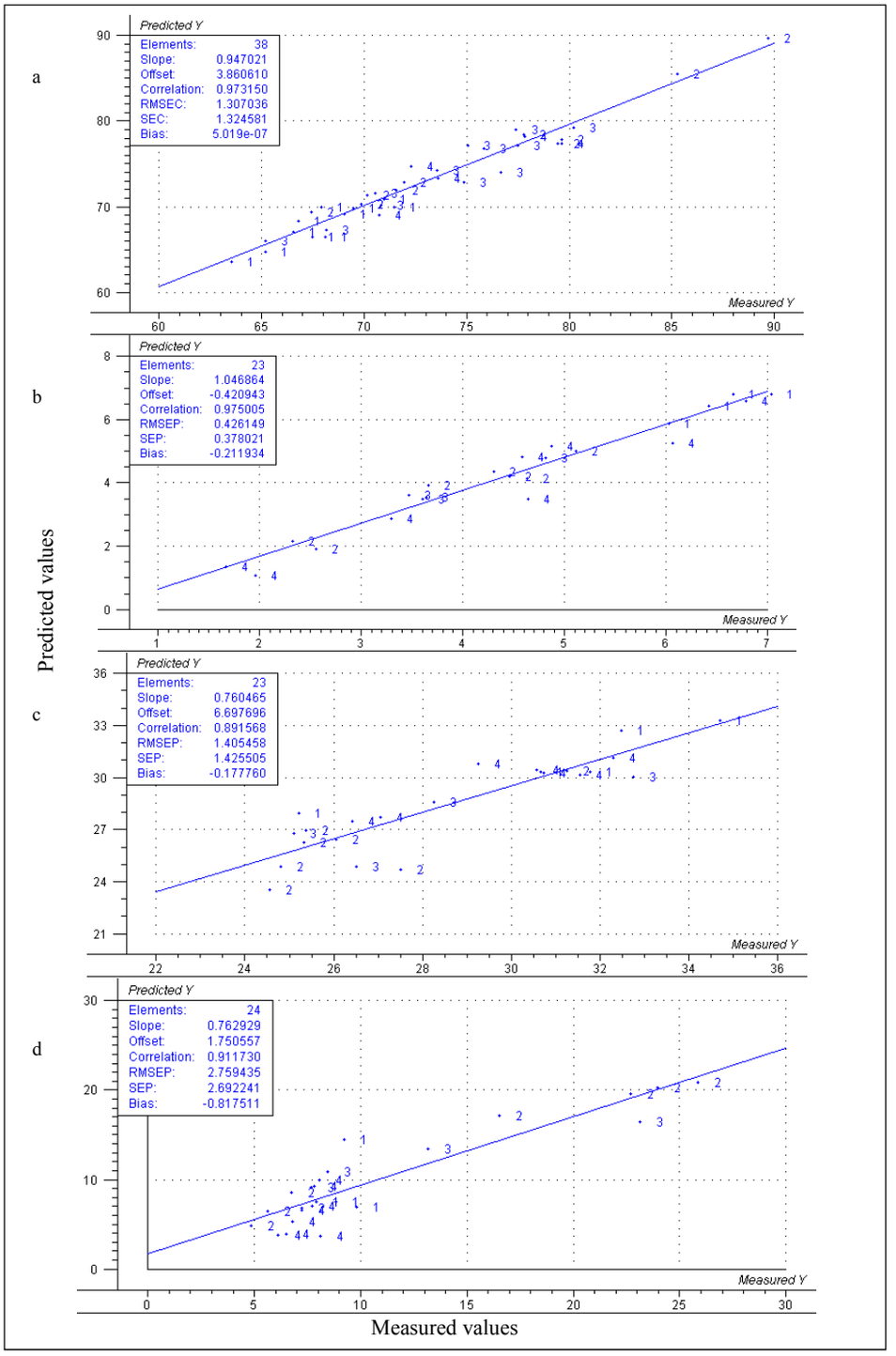

Figure 5: Scatter plots of Vis/NIR predicted versus measure values of hue angle (a), colour index (b), dry matter (%) (c), and weight/water loss (grams) (d).

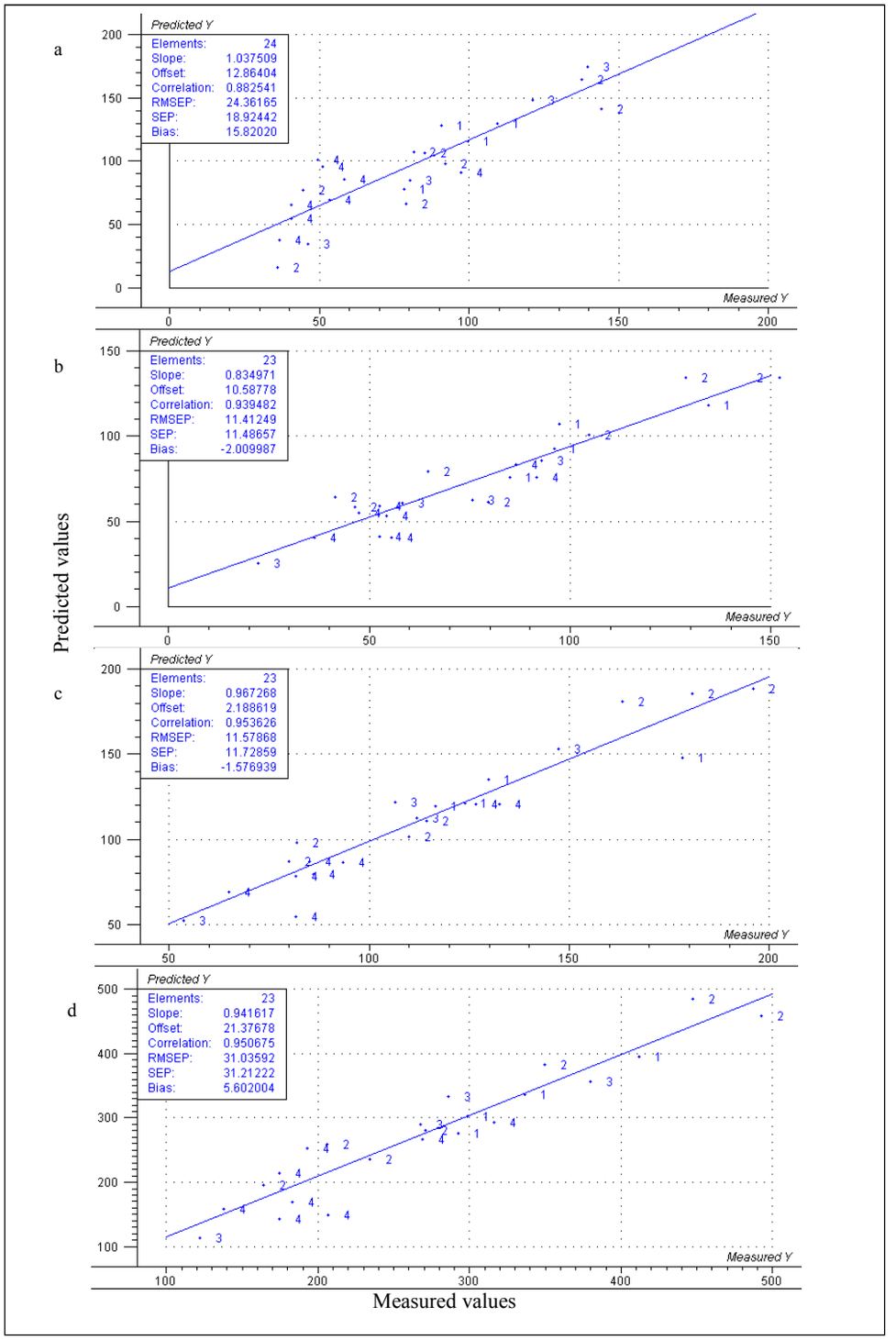

Figure 6: Scatter plots for Vis/NIR predicted versus measure values of sucrose (a), glucose (b), fructose (c), and total sugars (d). All results are expressed in mg/g DW.