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1 **Sweet and nonsweet taste discrimination of nectarines using visible and near**  
2 **infrared spectroscopy**

3  
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26 **ABSTRACT**

27 The feasibility of using visible and near infrared spectroscopy technology combined  
28 with multivariate analysis for discriminate cv. 'Big Top' and cv. 'Diamond Ray'  
29 nectarines, varieties that resulted very difficult to differentiate visually in the  
30 production line but showed important differences in taste affecting the acceptance by  
31 final consumers, have been studied. The relationship between the diffuse reflectance  
32 spectra and both nectarine varieties was established. Five hundred nectarine  
33 samples (250 of each variety) were used for the study. Spectra tests were performed  
34 by using a spectrometer capable of measuring in two different spectral ranges (600–  
35 1100 nm and 900–1700 nm). The spectra were used to develop two accurate  
36 classification models. The methods were based on linear discriminate analysis (LDA)  
37 and partial least squares discriminate analysis (PLS-DA). The results showed that  
38 PLS-DA model was achieved 100 % classification accuracy while LDA ones also  
39 demonstrated satisfying result (84 %). These results concluded that visible and near  
40 infrared spectroscopy (VIS-NIR) can yield an accurate classification for nectarine  
41 varieties with similar appearance but different taste, to get a correct identification in  
42 the sales lines and generate better acceptance by final consumers.

43

44 **Keywords:** nectarine, sweet taste, nonsweet taste, VIS-NIR spectroscopy,  
45 discrimination, chemometrics

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50

## 51 **1. INTRODUCTION**

52 Nectarine and peach fruit [*Prunus persica* (L.) Batch] are the second most important  
53 fruit crop in the European Union (EU) after apple (Iglesias & Echeverría, 2009).  
54 Recently, there has been an important innovation in the field of fruit varieties  
55 searching for improvements in colour and size, consistency of pulp, texture, taste and  
56 flavour. The new varieties obtained show an attractive range of colours, tastes and  
57 forms as well as extend the maturity timetable, which have generated an excellent  
58 acceptance by consumers in both national and international markets (Iglesias, 2013;  
59 Iglesias & Casals, 2014).

60 The most appreciated attributes in fruit consumers have been described to be taste,  
61 food safety (absence of pesticides), easiness of consumption and cost (Wandel &  
62 Bugge, 1997; Radman, 2005; Dragsted, 2008). Regarding taste, generally, except in  
63 some countries like Germany or England where there is preference for nonsweet  
64 tastes (Cembalo *et al.*, 2009), the consumer prefers sweet and balanced tastes. In  
65 fact, the introduction in the market of 'Big Top' nectarine variety supposed a  
66 remarkable innovation for its sweet taste (<6 g L<sup>-1</sup> malic acid) and excellent  
67 consistency, being widely accepted by national and international markets.

68 Recently, new varieties of nectarines completing the collection period from late May  
69 to late September have been introduced in the market. This varietal range is  
70 complemented by new or existing varieties showing similar appearance, but a  
71 balanced or nonsweet taste (> 6 g L<sup>-1</sup> of malic acid) as occurs in the case of  
72 'Diamond Ray' variety. In nectarine fruit, it is essential to differentiate the varieties  
73 from the processing line, which would allow the consumer to choose the ones that  
74 best adapt with their preferences.

75 The application of VIS-NIR technology to the analysis of fruit has allowed the  
76 prediction of chemical composition, notably sugar content (Li *et al.*, 2013; Reita *et al.*,  
77 2008), and textural parameters (Lee *et al.*, 2012; Sánchez *et al.*, 2011), as well as  
78 the identification of varieties (Li *et al.*, 2016; Guo *et al.*, 2016) and the measurement  
79 of quality-related parameters (Pérez-Marín *et al.*, 2011). This technique is relatively  
80 rapid, simple, cost-effective, non-destructive, and environmentally friendly. Its  
81 application in combination with chemometrics has been successfully used in the non-  
82 destructive discrimination between varieties of agricultural products such as peach  
83 (Guo *et al.*, 2016), bayberry (Li *et al.*, 2007), orange (Suphamitmongkol *et al.*, 2013),  
84 and pummelos (Li *et al.*, 2016).

85 This study aimed to evaluate the ability of VIS-NIR spectroscopy to discriminate  
86 between two varieties of nectarine (cv. 'Big Top' and cv. 'Diamond Ray') that because  
87 its similar colour and appearance resulted very difficult to differentiate visually in the  
88 production line but showed important differences in taste affecting the acceptance by  
89 final consumers. Two supervised methods (LDA and PLS-DA) were used for this  
90 purpose.

91

## 92 **2. MATERIALS AND METHODS**

### 93 **2.1. Experimental procedure**

94 A total of 500 nectarines with commercial maturity with uniform size and absence of  
95 any external damage were harvested in a commercial orchard of Lérida, Spain, and  
96 stored at 0.1 °C with 87 % relative humidity to prevent the evolution of maturity during  
97 the experiment and to extend their shelf-life (Gorny *et al.*, 1998). Half of the total  
98 samples belonged to the variety 'Big Top' and the other half to variety 'Diamond Ray'.  
99 These varieties were selected because they are grown at the same period and have

100 similar evolution and physical appearance, although they differ critically in some of  
101 their organoleptic properties.

102 On arrival at the laboratory, fruit were cleaned, individually numbered, and randomly  
103 divided each variety into five sets of 50 fruit. The VIS-NIR spectra of the fruit in each  
104 set were collected and their physicochemical properties were analysed by standard  
105 destructive methods.

106

## 107 **2.2. Visible and near infrared spectra acquisition**

108 Diffuse VIS-NIR reflectance spectra of intact nectarines were collected using a  
109 multichannel spectrometer platform (AvaSpecAS-5216 USB2-DT, Avantes BV, The  
110 Netherlands) equipped with two detectors. The first detector (AvaSpec-ULS2048  
111 StarLine, Avantes BV, The Netherlands) included a 2048-pixel charge-coupled  
112 device (CCD) sensor (SONY ILX554, SONY Corp., Japan), 50  $\mu\text{m}$  entrance slit and a  
113 600 lines  $\text{mm}^{-1}$  diffraction grating covering the visible and near infrared range from  
114 600 nm to 1100 nm (VNIR) with a spectral FWHM (full width at half maximum)  
115 resolution of 1.15 nm and a spectral sampling interval of 0.255 nm. The second  
116 detector (AvaSpec-NIR256-1.7 NIRLine, Avantes BV, The Netherlands) was  
117 equipped with a 256 pixel non-cooled InGaAs (Indium Gallium Arsenide) sensor  
118 (Hamamatsu 92xx, Hamamatsu Photonics K.K., Japan), 100  $\mu\text{m}$  entrance slit and a  
119 200 lines  $\text{mm}^{-1}$  diffraction grating covering the near infrared range from 900 nm to  
120 1700 nm (NIR) with a spectral FWHM resolution of 12 nm and a spectral sampling  
121 interval of 3.535 nm.

122 The measurements were done using a bi-directional fibre-optic reflectance probe  
123 (FCR-7IR200-2-45-ME, Avantes BV, The Netherlands). The probe is configured with  
124 an illumination leg which connects to a stabilised 10 W tungsten halogen light source

125 (AvaLight-HAL-S, Avantes B0V, The Netherlands) and the other leg of the fibre-optic  
126 probe was connected to both detectors for simultaneous measurement. A personal  
127 computer equipped with a customised software developed at IVIA using the interface  
128 packages for Windows provided by the spectrometer maker (Avantes BV, The  
129 Netherlands) was used for controlling both detectors and acquiring the spectra. The  
130 integration times were adjusted for each spectrophotometer using a 99 % reflective  
131 white reference (WS-2, Avantes BV, The Netherlands), so that the maximum  
132 reflectance value over each wavelength range was around 90 % of saturation  
133 (Lorente *et al.*, 2015). The dark spectrum was obtained by turning off the light source  
134 and completely covering the tip of the reflectance probe. The integration time was set  
135 to 120 ms for the VNIR detector and 550 ms for the NIR detector due the different  
136 features of both detectors. For both detectors, each spectrum was obtained as the  
137 average of five scans to reduce the thermal noise of the detector (Nicolaï *et al.*,  
138 2007). The average reflectance measurements of each sample (S) were then  
139 converted into relative reflectance values (R) with respect to the white reference  
140 using dark reflectance values (D) and the reflectance values of the white reference  
141 (W), as shown in Eq. (1):

142

$$143 \quad R = \frac{S - D}{W - D} \quad (1)$$

144

145 Prior to spectral measurements, the temperature of the nectarines was stabilised at a  
146 room temperature of  $22 \pm 1$  °C. All the measurements were performed by placing the  
147 surface of the fruit on the equipment. Measurements were taken at two points on  
148 each side of fruit and mean values of the spectra were used for the analysis.

149

### 150 **2.3. Determination of the quality attributes**

151 Destructive methods were performed immediately after the spectral acquisition to  
152 determine quality attributes for reference values. The external and the flesh colour  
153 were measured using a spectrophotometer (CM-700d, Minolta Co., Tokyo, Japan)  
154 between 400 and 700 nm every 10 nm. The colour was evaluated using the L\*, a\*  
155 and b\* system proposed by the International Commission on Illumination (CIE). L\*a\*b\*  
156 were determined from the reflectance spectra, considering standard illuminant D65  
157 and standard observer 10°. L\* refers to the luminosity or lightness component, a\*  
158 (intensity of red (+) and green (-)) and b\* (intensity of yellow (+) and blue (-)) are the  
159 chromaticity coordinates. The total colour difference ( $\Delta E$ ) between the 'Big Top'  
160 samples and the 'Diamond Ray' samples was calculated by Eq. (2).

161

$$162 \quad \Delta E = \sqrt{(L^*_{BT} - L^*_{DR})^2 + (a^*_{BT} - a^*_{DR})^2 + (b^*_{BT} - b^*_{DR})^2} \quad (2)$$

163

164 where subscript 'BT' refers to the colour reading of 'Big Top' samples and 'DR' refers  
165 to the colour reading of 'Diamond Ray' samples.

166 Nectarine firmness was measured using a Universal Testing Machine  
167 (TextureAnalyser-XT2, Stable MicroSystems, Haslemere, England) to perform  
168 puncture tests using a 6 mm diameter cylindrical probe (P/15ANAMEsignature) to a  
169 relative deformation of 30 % at a speed of 1 mm s<sup>-1</sup>. Two measurements were  
170 performed for each fruit on opposite sides along the equator. The fracture strength  
171 ( $F_{max}$ ) was analysed for all samples expressing the maximum force, in Newtons,  
172 applied to break up the sample.

173 Immediately after firmness measurements, juice samples were extracted to estimate  
174 the total soluble solids content (TSS) and titratable acidity (TA). The TSS was



175 determined by refractometry (%) with a digital refractometer (set RFM330+, VWR  
176 International Eurolab S.L Barcelona, Spain) at 20 °C with a sensitivity of ± 0.1 %.  
177 Samples were analysed by triplicate and average values were calculated. The  
178 analysis of the TA was performed with an automatic titrator (CRISON, pH-burette 24,  
179 Barcelona, Spain) with 0.5 N NaOH until a pH of 8.1 (UNE34211:1981), using 15 g of  
180 crushed nectarine which was diluted in 60 mL of distilled water. The TA was  
181 determined based on the percentage of citric acid that was calculated using Eq. (3).

182

$$183 \quad TA \left[ \frac{g \text{ citric acid}}{100 g \text{ of sample}} \right] = \frac{\left( \frac{A \times B \times C}{D} \right) \times 100}{E} \quad (3)$$

184

185 where A is the volume of NaOH consumed in the titration (in L), B is the normality of  
186 NaOH (0.5 N), C is the molecular weight of citric acid (192.1 g mol<sup>-1</sup>), D is the weight  
187 of the sample (15 g) and E is the valence of citric acid (E = 3).

188

#### 189 **2.4. Spectral pre-processing**

190 The spectral data were organized in a matrix, where the rows represent the number  
191 of samples (#N = 500 samples) and the columns represent the variables (X-variables  
192 and Y-variables). The X-variables, or predictors, were the spectral signals from two  
193 detectors. The Y-variables, or responses, were the categorical 'dummy' variables  
194 created by assigning different values to the different classes to be discriminated. In  
195 the case of PLS-DA the Y-variable was a vector with ones (for the cv. 'Big Top') and  
196 zeros (for the cv. 'Diamond Ray'). However, for LDA the number of samples in the  
197 training set must be larger than the number of variables included in the model (Kozak  
198 & Scaman, 2008; Sádecká *et al.*, 2016), so requiring a variable selection or variable

199 reduction. This was done using the Principal component analysis (PCA) scores as  
200 input data; since linear combinations of the original variables called principal  
201 components (PCs) are uncorrelated (Rodríguez-Campos *et al.*, 2011).

202 The raw spectral were transformed to apparent absorbance ( $\log(1/R)$ ) values for  
203 linearising the correlation with the concentration of the constituents (Hernández *et al.*,  
204 2006; Shao *et al.*, 2007; Liu *et al.*, 2009) using The Unscrambler X software package  
205 (CAMO, Norway). Then, the raw spectra belonging to the two detectors were  
206 normalised (Bakeev, 2010) dividing each variable by its standard deviation  
207 (Bouveresse *et al.*, 1996). This way, the spectral intensities are rescaled to a  
208 common range, allowing the comparison of spectra acquired using two detectors with  
209 different resolution. In addition, different pre-processing techniques were examined to  
210 decrease the scattering effect (Santos *et al.*, 2013), to remove the high frequency  
211 noise and improve the signal the ratio signal-noise. Savitzky-Golay smoothing with a  
212 gap of three data points (Carr *et al.*, 2005) combined with extended multiplicative  
213 scatter correction (EMSC) (Martens *et al.*, 2003; Bruun *et al.*, 2007) were considered  
214 the best results for the VIS-NIR spectra, and those two pre-processing and second  
215 derivate with Gap-Segment (2.3) were the best results for the NIR spectra (Cortés *et*  
216 *al.*, 2016).

217

## 218 **2.5. Multivariate data analysis of spectral data**

219 Principal component analysis (PCA) (Naes *et al.*, 2004), partial least squares  
220 discriminant analysis (PLS-DA) and linear discriminant analysis (LDA) were used in  
221 this work through the Unscrambler X software package. PCA was selected as the  
222 method for outlier detection (through the analysis of Hotelling's  $T^2$  and squared  
223 residual statistics) and identification of most important nectarine VIS-NIR spectral

224 features. PLS-DA and LDA were used to classify the nectarines in terms of variety.  
225 These discriminant analyses seek to correlate spectral variations (X) with defined  
226 classes (Y), attempting to maximise the covariance between the two types of  
227 variables.

228 A training set was used selecting randomly 80 % of the studied samples to develop a  
229 calibration model. Each calibration model was internally validated using the leave-  
230 one-out cross validation technique (Huang *et al.*, 2008). In order to correct the  
231 relative influences of the different instrumental responses on model, a  
232 standardization technique was used, where the weight of each X-variable was the  
233 standard deviation of the variable (Bouveresse *et al.*, 1996). An independent test set  
234 composed of 20 % of remaining samples was used for the evaluation and  
235 comparison of the classification models (Soares *et al.*, 2013).

236

## 237 **2.6. Model performance evaluation**

238 The classification of the nectarine samples according to variety was on the basis of  
239 the 0.5 cutoff value for PLS-DA model. For that reason, the 'Diamond Ray' samples  
240 were classified correctly, if the predicted value was less than zero, and the 'Big Top'  
241 samples were classified correctly, if the predicted value was greater than zero. The  
242 determination coefficient ( $R^2$ ), root mean square error (RMSE) and the number of  
243 latent variables (LV) were used to evaluate the accuracy of the PLS-DA calibration  
244 model to predict new samples. In the case of LDA, the criterion for selection of latent  
245 variables is the maximum differentiation between the categories and minimizes the  
246 variance within categories (Adams, 1995; Naes *et al.*, 2002). The method produces a  
247 number of orthogonal linear discriminant functions, equal to the number of categories

248 minus one, that allow the samples to be classified in one or another category (Naes  
 249 *et al.*, 2002; Otto, 1999).

### 250 3. RESULTS AND DISCUSSION

#### 251 3.1. Analysis of the quality attributes

252 Table 1 shows the minimum, maximum, mean and standard deviation of the  
 253 physicochemical characteristics (fracture strength, total soluble solids, tritatable  
 254 acidity, and flesh and external colour coordinates) analysed in both varieties of  
 255 nectarines samples (#N = 250 samples for each variety).

256  
 257 **Table 1.** Descriptive statistics for the physicochemical characteristics of nectarines  
 258 during the storage period

					Flesh colour			External colour		
		TSS	TA	F <sub>max</sub>	L* <sub>flesh</sub>	a* <sub>flesh</sub>	b* <sub>flesh</sub>	L* <sub>ext</sub>	a* <sub>ext</sub>	b* <sub>ext</sub>
		(%)	(g 100g <sup>-1</sup> )	(N)						
cv. 'Diamond Ray'	Max	17	1.07	57	76	23	35	59	38	28
	Min	8	0.36	5	52	-6	23	25	15	4
	Mean	12	0.65	33	67	2	31	37	28	14
	Sdev	2	0.12	10	4	4	2	7	4	5
cv. 'Big Top'	Max	22	0.57	53	75	4	36	63	34	31
	Min	7	0.20	6	60	-8	30	26	8	4
	Mean	13	0.37	35	68	-2	33	37	27	14
	Sdev	2	0.07	7	3	2	1	6	4	5

259

260 No differences were observed between both varieties in soluble solids, firmness and  
 261 flesh and external colour.

262 The TSS ranged from 8 to 17 % with an average value of  $12 \pm 2$  % for cv. 'Diamond  
 263 Ray' and from 7 to 22 % with an average value of  $13 \pm 2$  % for cv. 'Big Top'. In all

264 cases, the values were greater than 8 % which is the minimum established by the  
265 European Union to market peaches and nectarines (R-CE No. 1861/2004). Several  
266 authors have reported a linear relationship between TSS and consumer acceptance  
267 (Crisosto & Crisosto, 2005) being TSS below 10 % generally unacceptable to  
268 consumers (Clareton, 2000).

269 The firmness of 'Diamond Ray' samples ranged from 5 to 57 N with an average value  
270 of  $33 \pm 10$  N, and 'Big Top' samples ranged from 6 to 53 N with an average value of  
271  $35 \pm 7$  N. According to Crisosto (2002) and Valero *et al.* (2007), these firmness  
272 values are in the commercial range considered 'ready to buy'.

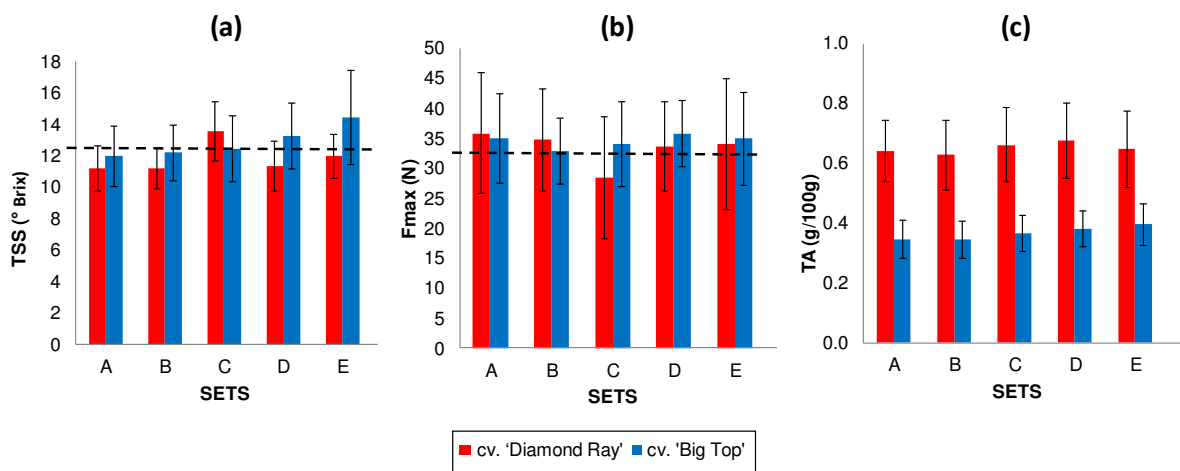
273 For flesh colour,  $L^*$ ,  $a^*$  and  $b^*$  ranged from 52 to 76, -6 to 23 and 23 to 35 for cv.  
274 'Diamond Ray' and from 60 to 75, -8 to 4 and 30 to 36 for cv. 'Big Top', with average  
275 values of  $67 \pm 4$ ,  $2 \pm 4$ ,  $31 \pm 2$  and  $68 \pm 3$ ,  $-2 \pm 2$ ,  $33 \pm 1$ , respectively. These values  
276 indicated that the flesh of both varieties has high luminosity and high intensity of  
277 yellow colour. For the external colour, the  $L^*$ ,  $a^*$  and  $b^*$  ranged from 25 to 59, 15 to  
278 38 and 4 to 28 for cv. 'Diamond Ray' and from 26 to 63, 8 to 34 and 4 to 31 for cv.  
279 'Big Top', with and average values of  $37 \pm 7$ ,  $28 \pm 4$ ,  $14 \pm 5$  and  $37 \pm 6$ ,  $27 \pm 4$ ,  $14 \pm$   
280 5, respectively. These values indicated that, externally both varieties have low  
281 luminosity and similar contribution of  $a^*$  and  $b^*$  in the colour of the samples,  
282 perceiving them with an orange tonality.

283 The main differences between both varieties of nectarine was TA, being the  
284 'Diamond Ray' variety more acid than the 'Big Top' variety, or like Reig *et al.* (2013)  
285 defined them as nonsweet and sweet varieties respectively. All sets of the cv.  
286 'Diamond Ray' had an average value of  $0.65 \pm 0.1$  g 100 g<sup>-1</sup>, unlike the average value  
287 of sets of the cv. 'Big Top' was  $0.37 \pm 0.1$  g 100 g<sup>-1</sup>. These results are in accordance  
288 with the sensorial profile realized by Iglesias (2012). The study concluded that the

289 only difference between these two varieties is in the perception of acidity. Similarly,  
 290 Reig *et al.* (2013) and Liverani *et al.* (2002) compared sweet cultivars (as 'Big Top',  
 291 'Gardeta' and 'Luciana') with nonsweet cultivars (as 'Diamond Ray', 'Amiga' and  
 292 'Rose Diamond'), and determined that they differed mainly in TA value and the  
 293 perception of acidity, being the rest of physicochemical characteristics, similar among  
 294 the cultivars.

295 Figure 1 shows the TSS,  $F_{max}$  and TA of nectarines measured for the different sets of  
 296 analysis for each variety. As expected, no differences were observed among the  
 297 different sets and varieties in terms of TSS and  $F_{max}$ . Nevertheless, big differences  
 298 were observed in TA which confers nectarines a different perception of sourness.  
 299 The average values of TSS of each set was close to the average threshold of 12.4 %  
 300 (Figure 1, a) for both varieties. The average values of  $F_{max}$  of each set of both  
 301 varieties were maintained around threshold of 33.9 N (Figure 1, b). Regarding TA, in  
 302 Figure 1 (c) could observe the compositional difference between the two varieties,  
 303 with an average value of  $0.65 \text{ g } 100\text{g}^{-1}$  for cv. 'Diamond Ray' and of  $0.37 \text{ g } 100\text{g}^{-1}$  for  
 304 cv. 'Big Top'.

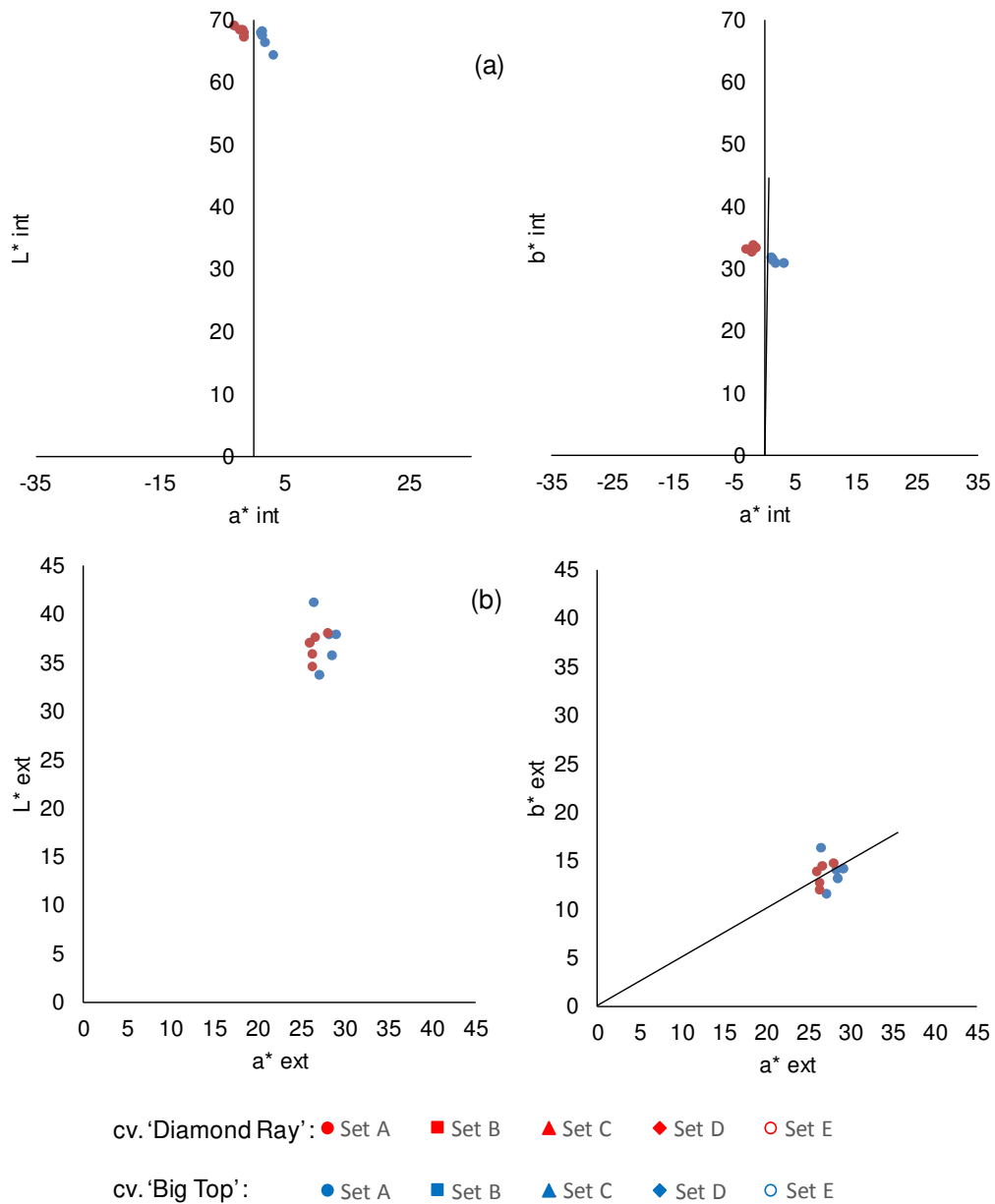
305



306

307 **Fig. 1.** Mean and standard deviation of a) TSS, b)  $F_{max}$  and c) TA of nectarines at  
 308 different sets of analysis. Discontinuous lines in the TSS plot (left) and mechanical  
 309 plot (right) indicate thresholds

310 Figure 2 shows the  $a^*-L^*$  and  $a^*-b^*$  colour planes of the flesh colour (Figure 2a) and  
 311 the external colour (Figure 2b) where the location of the five sets of the samples  
 312 analyzed can be seen. Isohue-line was plotted in  $a^*-b^*$  chromatic planes with the  
 313 mean value of the all samples,  $89.92^\circ$  and  $25.91^\circ$  for flesh colour and external colour,  
 314 respectively.



315

316 **Fig. 2.** Colour planes  $a^*-L^*$  and  $a^*-b^*$  of the flesh colour (a) and the external colour  
317 (b) of different sets of nectarines cv. 'Big Top' and cv. 'Diamond Ray' during the  
318 storage period.

319 Respect to the flesh colour, there was not differences in luminosity and chroma  
320 between sets and between varieties, whereas it can be observed slight differences in  
321 hue between varieties. Despite these differences, the overall perception of flesh  
322 colour would be very difficult to discriminate both varieties, especially during any  
323 industrial process where fruit must be inspected fast, as shown in the images of  
324 Figure 3 with examples of each of the sets analysed.

325



326

327 **Fig. 3.** Example of the internal appearance of both cultivars in each day of analysis.

328

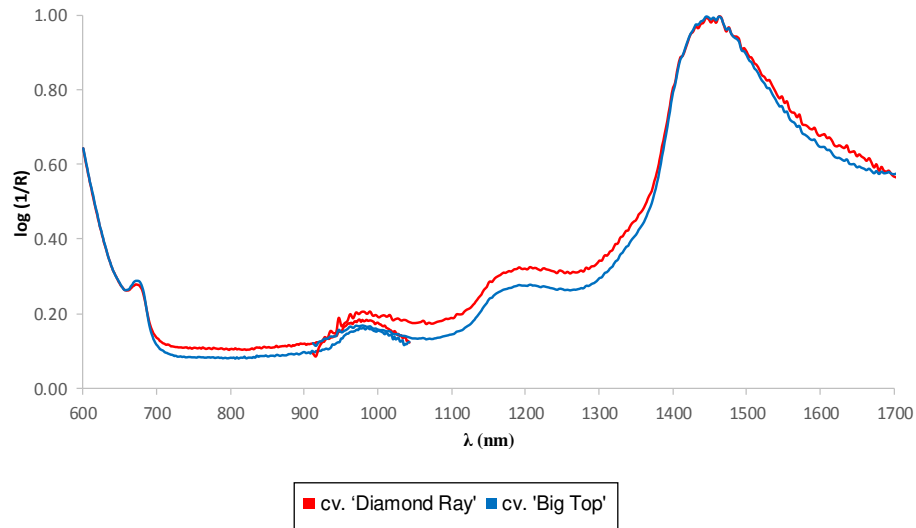
329 According to ISO 12647-2, colour differences ( $\Delta E$ ) lower than  $\pm 5$  units makes the  
330 human eye unable to discriminate two samples. In this case,  $\Delta E$  between both  
331 varieties measured with the colorimeter was  $\pm 4.5$ . Furthermore, differentiating  
332 nectarine varieties by the flesh colour require the destruction of the sample, and  
333 therefore this destructive analysis results in high costs and does not allow the whole  
334 production to be analysed (Torres *et al.*, 2013).



335 Regarding the external colour of nectarines, no significant differences were found in  
336 the values of luminosity, chroma and hue for the sets and the varieties studied, with  
337 the average values of 37 for luminosity, 31.3 for chroma and 25.91° for hue, for both  
338 varieties. The  $\Delta E$  of external colour between varieties is  $\pm 1.5$ , and therefore barely  
339 perceptible. Then, this non-destructive analysis is not valid for varietal discrimination.  
340

### 341 **3.2. Visible and Near-infrared spectra of the two nectarine varieties**

342 Figure 4 represents the mean raw VNIR and NIR spectra for 'Diamond Ray' and 'Big  
343 Top' samples. The trend and absorbance bands of the spectral curves were similar.  
344 Previous studies have documented similar values (Pérez-Marín *et al.*, 2009; Pérez-  
345 Marín *et al.*, 2011; Martins *et al.*, 2016). The analysed varieties showed the same  
346 absorbance bands around 670 nm, 970 nm, 1160 nm and 1400 nm. Authors as  
347 Tijskens *et al.* (2007) confirmed that the absorption at 670 nm allowed evaluating the  
348 maturity of nectarine because is indicative of the presence of chlorophyll  
349 characteristic of green colour (Merzlyak *et al.*, 2003; Gómez *et al.*, 2006). The peak  
350 centred at 970 nm is present in the signal recorded by the two detectors. This peak  
351 and the present at 1450 nm related to pure water (Williams & Norris, 1987; McGlone  
352 & Kawano, 1998). A characteristic absorption band at around 1160 nm related to C-H  
353 stretching second overtone (Osborne *et al.*, 1993; Walsh *et al.*, 2004).  
354



355

356

**Fig. 4.** Raw VNIR and NIR spectra of for the two varieties of nectarines.

357

### 3.3. Varietal classification

358

Classification models were built based on supervised PLS-DA and LDA with the full  
 359 spectra. The best models were chosen with five and six LV for the PLS-DA and LDA  
 360 methods, respectively. The optimal number of LV was chosen according to the lowest  
 361 RMSECV by internal validation, i.e. 'leave-one-out', in combined analysis with the  
 362 cumulative variance in the X and Y blocks (Bachion de Santana *et al.*, 2016).

363

Table 2 shows the predictive ability for each validation set. We found that  
 364 classification accuracy of 100 % for both varieties by the PLS-DA method, and by the  
 365 LDA method were of 82 % and 86 % for variety 'Diamond Ray' and variety 'Big Top',  
 366 respectively.

367

368

**Table 2.** Varietal classification results for each method, presented as both as  
 369 percentage and absolute number of correctly classified samples in the validation  
 370 sets.

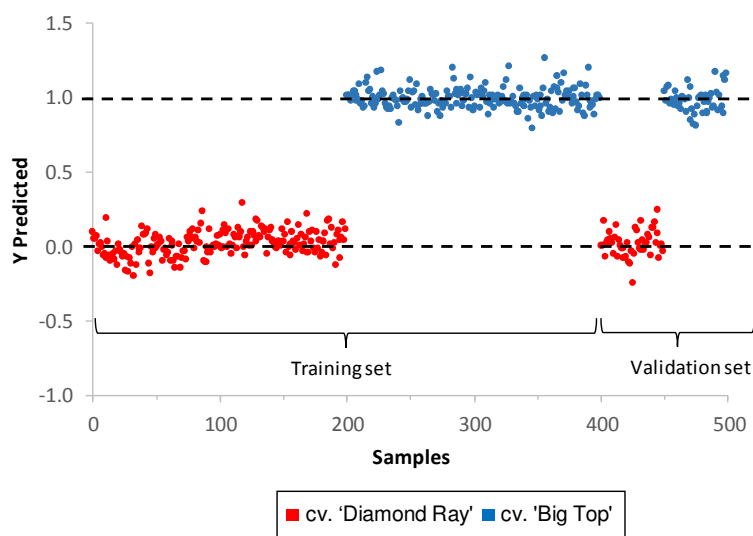
Methods	Classification accuracy		
	cv. 'Diamond Ray'	cv. 'Big Top'	Total samples

<b>PLS-DA</b>	100 %	100 %	100 %
	(50/50)	(50/50)	(100/100)
<b>LDA</b>	82 %	86 %	84 %
	(41/50)	(43/50)	(84/100)

371

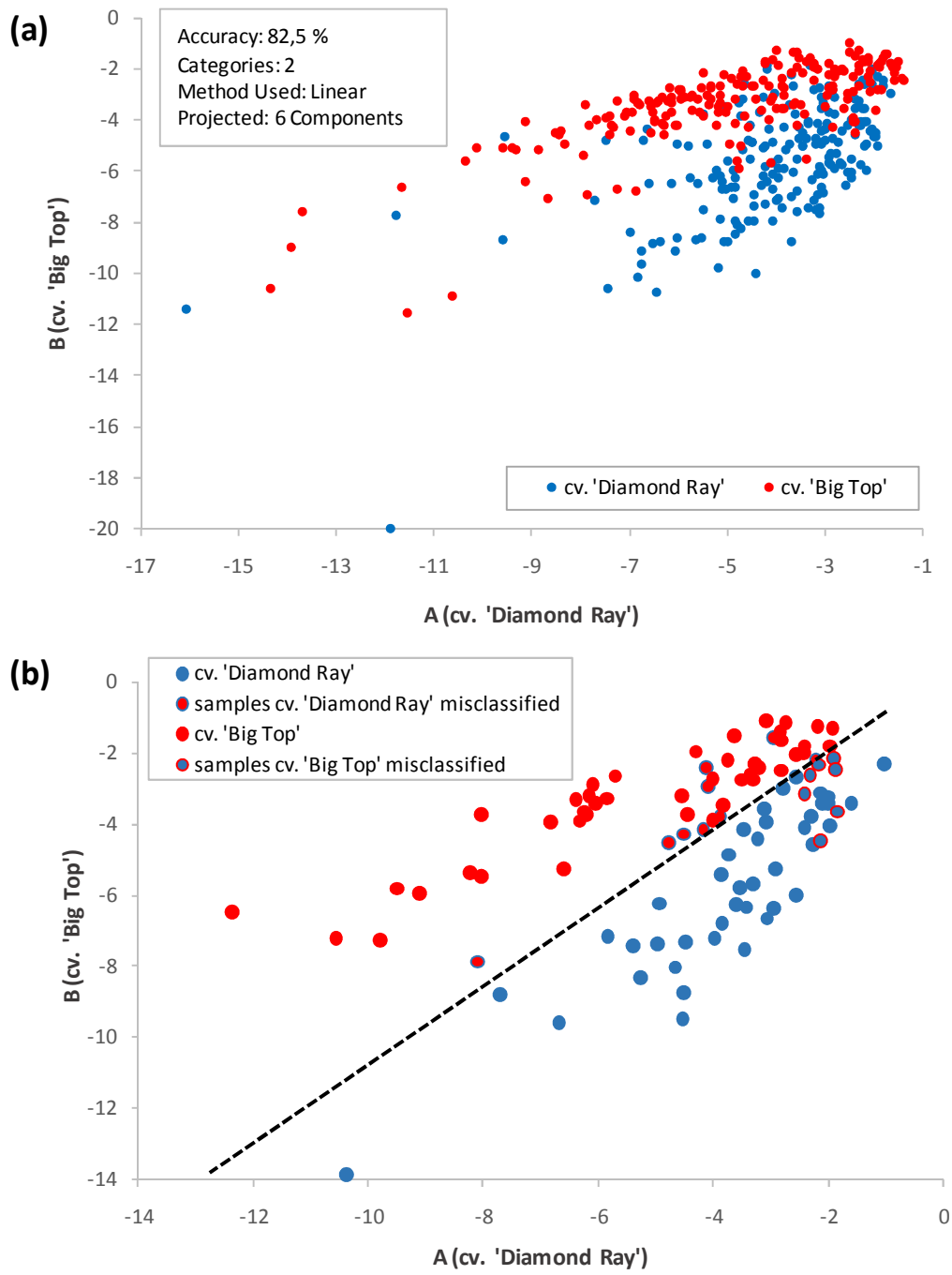
372 Figure 5 shows that all the training set and validation samples were correctly  
 373 classified by the PLS-DA model. In this situation, all 'Big Top' samples have  
 374 predicting values close to 1 thus classifying these as belonging to class '1', and  
 375 'Diamond Ray' samples have predicting values close to 0 and therefore classifying  
 376 these as belonging to class '0'. The values of the RMSE were 0.078 and 0.320 for  
 377 calibration and validation, respectively which exhibit a good agreement, indicating  
 378 that the calibration error is a good estimation of the standard error of prediction  
 379 observed in samples of the test set. Moreover, the test set gave similar result as the  
 380 calibration set, with  $R^2$  of 0.976 and 0.970 respectively, indicating a good  
 381 performance of the LDA model for varietal classification.

382



383





391

392 **Fig. 6.** Discrimination plot of the LDA results for (a) the training samples and (b) the  
 393 validation samples.

394

395 Several authors (Balabin *et al.*, 2010; Liu *et al.*, 2006; Sinelli *et al.*, 2007) have  
 396 reported that the PLS-DA method is more effectiveness than LDA. Concerning LDA,  
 397 this method suffers from several limitations as the number of variables cannot exceed

398 the number of samples (Roggo *et al.*, 2003) and it is not able to cope with highly col-  
399 linear data, which are quite common. To overcome some limitations, during the years  
400 other techniques, in particular PLS-DA, have been devised (Marini, 2010). Similarly,  
401 to us, Long *et al.* (2015) combined the NIRS with PLS-DA for the discrimination of  
402 transgenic rice and they achieved 100 % of the classification rate of validation test.  
403 Therefore, in general, the PLS-DA method can give a more robust recognition  
404 performance with high identification accuracy.

405

#### 406 **4. CONCLUSIONS**

407 In this work, classification models were developed in order to discriminate the two  
408 nectarine varieties (cv. 'Big Top' and cv. 'Diamond Ray') based on their VNIR and  
409 NIR spectral features. Two classification methods were evaluated on this data  
410 including PLS-DA and LDA. Both methods have satisfying classification accuracy 84  
411 % for LDA, and even 100 % accuracy is reachable by using PLS-DA from full  
412 spectra. The findings of this work suggest that it is possible to develop a non-  
413 destructive technique for discriminating varieties of nectarine with satisfying  
414 accuracy. It is essential to identify varieties with similar presentation in the sales line  
415 according to their sweet and nonsweet taste, to allow the consumer to choose the  
416 ones that best adapt with their preferences.

417

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424

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