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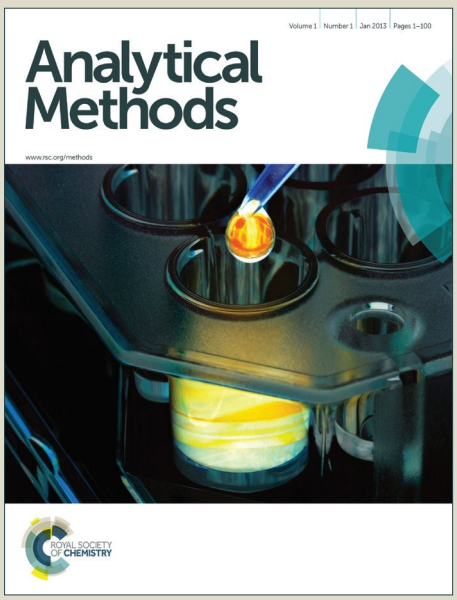

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Classification of jaboticaba fruit at three maturity stages using NIRS and LDA

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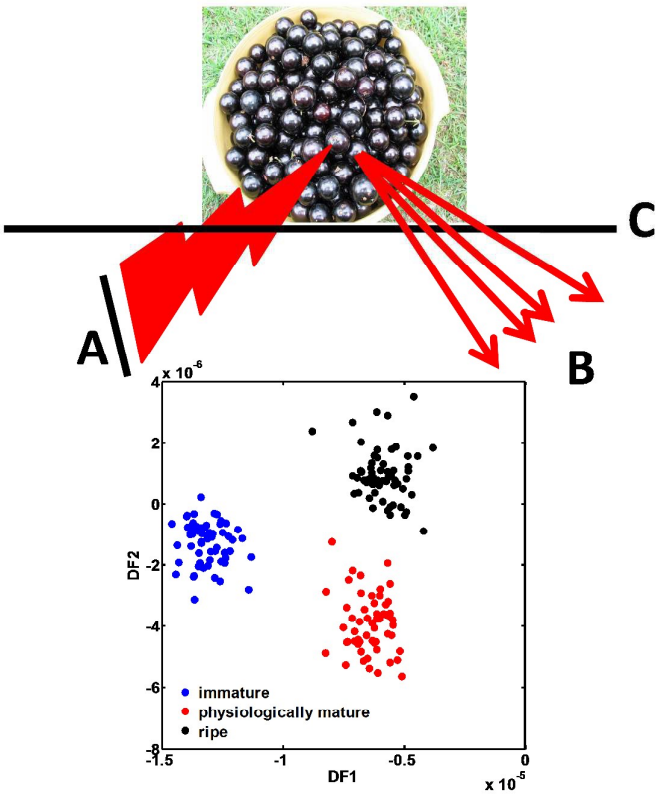
ToC



Abstract: This study proposes a rapid and non-destructive method of jaboticaba [*Myrciaria cauliflora (Mart.)* O. Berg] fruit classification at three maturity stages based on skin colour (immature - fruit completely green, physiologically mature - fruit turning from green to purple, ripe - fruit completely purple) using Near-Infrared Reflectance Spectroscopy (NIRS) combined with principal component analysis–linear discriminant analysis (PCA–LDA), and variable selection techniques employing a successive projection algorithm (SPA–LDA) or genetic algorithm (GA–LDA). One hundred eighty jaboticaba fruit samples in three maturity stages were used and the multivariate classification accuracy results were tested based on sensitivity, specificity, positive (or precision) and negative predictive values, Youden index, positive and negative likelihood ratios. The immature stage the classification models PCA-LDA, GA-LDA and SPA-LDA achieved sensitivity of 100% in the validation set. The results obtained in this study suggest that the proposed method is a promising alternative for assessing jaboticaba fruit maturity, opening the possibility for automation in packing houses.

Keywords: NIRS; PCA-LDA; SPA-LDA; GA-LDA; Validation.

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1. Introduction

Jaboticaba is a small tree, native to the central-south region of Brazil. Among the *Myrciaria* genus the most important species are the *Myrciaria cauliflora* (DC) Berg (cv. Açú) and the *Myrciaria jaboticaba* (Vell) Berg (cv. Sabará) which produce adequate fruit for both industry and fresh consumption [1].

The jaboticaba bloom season occurs after intense vegetative growth at the end of winter and beginning of spring, with blossoms emerging from the trunks and branches. In Brazil, *Myrciaria cauliflora (Mart.)* O. Berg (jaboticaba 'Açú') fruit season varies according to the production region, but it generally happens from September to January in São Paulo State [1,2]. The fruit development follows a simple sigmoid growth pattern which is marked by a slow initial growth up to 12 days after blossom (DAB), and after 35 DAB the growth rate is accelerated by a rapid cell volume expansion due to high water absorption. The growth rate stabilizes at 57 DAB while the chlorophyll present in the skin degrades and anthocyanin levels increase. Fruit development takes approximately 60 days with fruit reaching a final weight of around 5 g [3].

In general, fruit maturity can be determined by using various quality attributes such as size, weight, colour, sugar content, acidity, ratio of soluble solids content and titratable acidity (SSC/AT), aroma and days after blossom[4]. However, jaboticaba maturity is commonly determined by colour, as soluble sugar and acidity may greatly vary according to climate conditions [5]. The colour modifications related to jaboticaba fruit maturity is correlated to the sharp increase in chlorophyll levels 30 DAB, reaching its maximum content around 50 DAB. Next, chlorophyll levels decline and coincide with flavonoids synthesis, mainly anthocyanins, that increase during maturation and are responsible for the purple colour of the jaboticaba fruit [3]. The main anthocyanins in

jaboticaba 'Açú' fruit are cyanidin 3-glucoside and delphinidin 3-glucoside [6]. On the other hand, pheonidin 3-glucoside and its glycone is the most prominent in 'Sabará' jaboticaba (*Myrciaria jaboticaba*) [7]. Based on the changes in anthocyanins and chlorophyll levels, the jaboticaba fruit ripening appears to begin 55 DAB [3]. At this point (55 DAB), the concentration of sugar is at its maximum (400 g kg^{-1}) with soluble solids content (SSC) reaching 18.6°Brix [8]. The lowest concentration of organic acids is also reported at 0.4% of citric acid (Corrêa, 2006). According to Teixeira et al. (2011), jaboticaba is a non-climacteric fruit and does not ripen after harvest; the fruit should be harvested when its appearance and quality are ideal for consumption. In this regard, jaboticaba should be harvest when the fruit is fully developed and has a purple color, as immature fruit are acidic, they do not ripen, and their flavor will not improve after harvest. A tool based on a rapid, non-destructive internal quality measurement method that could aid in the identification of immature jaboticabas would be useful as a worker training aid for pickers to reduce the harvest of immature fruit, thus eliminating the shipping and handling costs of marketing quality fruit, and would improve quality and consumer satisfaction.

Within this scope, one of the most promising directions for the development of innovative solutions is the use of spectroscopic methods. Near-infrared reflectance spectroscopy (NIRS) associated with multivariate techniques have proven to be useful for measuring some quality parameters of jaboticaba fruit, such as soluble solids [9] and total anthocyanin content [10], and there are no references regarding the application of NIRS on the determination of jaboticaba fruit's maturity stages. The present study investigates the use of NIRS and chemometric techniques such as principal component analysis–linear discriminant analysis (PCA–LDA) [11], successive projection algorithm

(SPA–LDA) [12] and genetic algorithm (GA–LDA) [13,14] for the discrimination of intact jaboticaba fruit without any prior metabolite extraction. As an alternative, variable selection methods can be used to identify specific spectral variables that convey useful information for the analytical problem at hand. To our knowledge, there is no reported use of NIRS for qualitative analysis in jaboticaba fruit (maturity stages), without the need for metabolite extraction/purification.

The purpose of chemometric tools to extract discriminating variance from the spectral fingerprint related to the maturity stages of jaboticaba was to reduce the possibility of losing relevant information for the classification task, employing statistical variable selection algorithms (SPA and GA) instead of a priori considerations. The SPA-LDA and GA-LDA algorithms are aimed at selecting a subset of variables with small collinearity and suitable discriminating power for use in classification problems involving $C \geq 2$ different classes and achieves several advantages, such as removal of noise and non-linearity, as compared to using the full spectrum. Furthermore, the proposed method was thoroughly validated in accordance with International guidelines [15]. Classification quality features such as sensitivity, specificity, positive (or precision) and negative predictive values, Youden index, positive and negative likelihood ratios were described and calculated.

2. Material and methods

2.1. Plant material

A total of 180 jaboticabas [*Myrciaria cauliflora* (Mart.) O. Berg cv. Açú] were harvested in three maturity stages based on skin colour, being: i) immature (fruit completely green); ii) physiologically mature (fruit turning from green to purple); iii)

ripe (fruit completely purple). Fruit collections happened in Ribeirão Preto, São Paulo State, Brazil (21º12'42" S, 47º48'24" W and 546 m a.s.l.). After harvest, the fruit was immediately taken to the laboratory where it was kept at room temperature (~25°C) for 1 h until uniform temperature was achieved. The jaboticabas were individually analysed for colour (CIE) and NIR diffuse reflectance.

2.2. Fruit colour

Colour measurement was individually performed at two sites on the equatorial line of each intact jaboticaba fruit using a Minolta CR-400 colorimeter (Minolta Corp., Japan), which measures colour according to the CIE system (L*, a*, b*). In addition, derived parameters such as Hue angle (°h), arctan (b*/a*), and chromaticity (C*) ([(a*) x 2 + (b*) x 2] x 0.5) were calculated according to the method described by McGuire (1992). The descriptive statistics for mass and fruit colour of the jaboticaba fruit are presented in Table 1.

[Insert Table 1 here]

2.3. NIR spectra acquisition

For each jaboticaba fruit, two reflectance spectra (1000–2500 nm, resolution of 2 mm and 64 scans), collected on the same sites where colour was determined, with a 100N FT-NIR spectrometer (PerkinElmer, Shelton, USA) coupled to a Near Infrared Reflectance Accessories (NIRA) (PerkinElmer, PN L125403L). The spectra were acquired using the Spectrum software version 10.03.02 (PerkinElmer, Shelton, USA).

2.4. Chemometrics' procedure and software

The calculations were carried out using the MATLAB r2014a software (http://www.mathworks.com) with PLS-toolbox (Eigenvector Research, Inc., Wenatchee, WA, USA, version 7.8). The average spectra were pre-processed using Savitzky-Golay smoothing and derivative (Savitzky-Golay first derivative). The preprocessing will be selected based on which furnish the best classification model. Following spectral acquisition, the data were analyzed using multivariate techniques of principal component analysis (PCA) for preliminary data reduction and the output was processed using linear discriminant analysis (LDA) and variable selection techniques employing successive projection algorithm (SPA) or genetic algorithm (GA) in conjunction with LDA for selecting an appropriate subset of wavenumbers for classification purposes.

The classic Kennard–Stone (KS) uniform sampling algorithm (Kennard and Stone, 1969) was adopted to divide the available samples into training (70% - 126 samples), validation (15% - 27 samples) and prediction sets (15% - 27 samples) for construction and validation of the PCA-LDA, SPA-LDA and GA-LDA models. The training set was used to obtain model parameters (including variable selection for LDA), and the validation set was employed to choose the best number of the PCs for PCA model. The optimum number of variables for SPA–LDA and GA–LDA was used to select variables employing the G function as cost function. The mutation and reproduction probabilities were kept constant, 60% and 10%, respectively. The initial population was carried out during 40 generations with 80 chromosomes each.

Validation is a crucial and mandatory step in the lifecycle of an analytical method. Receiver-operating characteristic (ROC) analysis for assessment of the quality classification performance is recommended standard practice for test evaluation studies and validation for non-binary tests [17]. In this study, measures of test accuracy such as

sensitivity (the confidence in a positive result for a sample of the label class is obtained), specificity (the confidence that a negative result for a sample of non-label class is obtained), Positive predictive value (PPV) (measures the proportion of correctly assigned positive examples and its value varies between 0 and 1), Negative predictive value (NPV) (measures the proportion of correctly assigned negative examples and its value varies between 0 and 1), Youden's index (YOU) (evaluates the classifier's ability to avoid failure), The likelihood ratios (LR+) (represents the ratio between the probability to predict an example as positive when it truly is positive, and the probability to predict an example as positive when it actually is not positive) (LR-) (represents the ratio between the probabilities to predict an example as negative when it is actually positive, and the probability to predict an example as negative when it truly is negative) were calculated as important quality standards in test evaluation. The quality metrics used in this study for evaluating the classification results can be calculated following the equations showed by Pérez-Castaño [18].

3. Results and discussion

The raw NIR spectra of intact jaboticaba 'Açú' fruit on the spectral region of 1000–2500 nm showed baseline offsets and bias due to the light scattering or concentration variation (Fig. 1). Visually, NIR spectra for the three maturation stages have no significant differences, though the main absorption peaks coincided for all three classes. For example, they showed the lowest molecular absorptivity in short wavelength region (Region 1:1000–1322 nm) and presented important contributions related to combination bands of the –OH functional group, symmetric and anti-symmetric stretching. In addition, this wavelength region is also related to C–H aromatic second overtones and C–H third overtones. Higher values in the first overtone–OH region (Region 2:1323–1600 nm) and still higher absorbance levels in the

combination region (Region 3:1601–2500 nm). As presented in the Fig. 1A, no discrimination among fruit at different maturity stages was possible.

[Insert Figure 1 here]

Taking into account the development of the method for discrimination, the data preprocessing strategy using Savitzky-Golay smoothing and derivative (Savitzky-Golay first derivative) was defined in performing each classification algorithm (PCA-LDA, SPA-LDA and GA-LDA). Fig. 1B shows the Savitzky-Golay smoothing and first derivative spectra of intact jaboticaba at three different maturity stages. Several absorption bands were observed at: 1150, 1200, 1344, 1380, 1396, 1432, 1900 nm and 2400 nm as shown in Fig. 1B. Most of these bands can be attributed to O–H absorbers. In order to achieve a predictive method with the goal of formulating a discrimination rule used to predict or allocate the maturity stages of unknown jaboticaba fruit into "immature," "physiologically mature" or "ripe" predefined classes and also to evaluate it as an exploratory tool to increase the understanding about the differences between classes.

The best PCA-LDA result was achieved by using four PCs, accounting for 99.0% of the variance, reaching most diagnostically significant ($p < 0.05$) for discriminating each maturity stage. The Fig. 2A shows the plot scores (DF1 × DF2) of the three maturity stages of fruit, viewing that there is overlapping among the three maturity stages with a minimal discrimination. For, SPA–LDA using 123 selected wavenumbers (Table 2), obtained by cost function G, achieved an improved segregation between classes (Fig. 2B) when compared with PCA–LDA. However, there was a slight overlap between "physiological mature" and "ripe" maturity stages. When NIR spectra

were employed to predict “immature,” “physiologically mature” or “ripe” jaboticaba fruit, it was observed that using GA–LDA associated variables (48 selected) gave better segregation than PCA–LDA and SPA–LDA together, as shown in Fig. 2C. As can be seen (in Fig. 2C) there is no overlapping between the three classes, which indicates that the NIR spectrum conveys appropriate information for fruit classification at three maturity stages using NIRS. This meant that the "physiologically mature" and "ripe" jaboticaba could be clearly separated from immature jaboticaba by the GA-LDA model.

[Insert Figure 2 here]

[Insert Table 2 here]

The examination of the selected wavenumbers following SPA–LDA showed that the main physiological alterations discriminating “immature” vs. “physiologically mature” vs. “ripe” jaboticaba fruit were total sugars, organic acids, water, and to a lesser extent, carbohydrates[10]. Several selected wavelengths appear to be of particular interest, namely, the variables at1400, 1900 and 2300 nm, associated with O–H bonds of water, which means that changes caused by maturity stages would result in alteration of light scattering properties and affect absorption intensity of water or total sugar within jaboticaba fruit. Several selected wavenumbers (GA–LDA) appear to be of particular interest, namely, the variables at 1516 nm and 1827 nm, representing the carbohydrates.

However, the classifiers can now be arranged in decreasing order of performance as: GA–PCA>SPA–LDA>PCA–LDA. This ranking is easily established dealing with the main features related to the overall of test accuracy: sensitivity, specificity, positive, negative predictive values, Youden index, positive and negative likelihood ratios. Table 3 presents the overall classification reliability for the optimized

model (PCA–LDA, SPA–LDA and GA–LDA) of jaboticaba fruit at three maturity stages.

[Insert Table 3 here]

The results of sensitivity shown in Table 3, it is possible to verify that for the immature stage the classification models PCA-LDA, GA-LDA and SPA-LDA submit a score of 1 (100%), showing that the immature stage can be well classified by these multivariate methods. For physiological mature stage, the values achieved were (100%) for sensitivity using the PCA-LDA and the GA-LDA, and 0.77 (77%) using the SPA-LDA. The specificity for physiological mature stage was found to be 1(100%) with the PCA-LDA and 0.77 (77%) with GA-LDA and SPA-LDA. For the ripe stage, the sensitivity was 1 (100%) with PCA-LDA and the GA-LDA, and 0.88 (88%) with SPA-LDA. The specificity of the ripe stage was 1(100%), 0.55 (55%) and 0.88 (88%) using PCA-LDA, GA-LDA, and SPA-LDA, respectively.

Conclusion

The NIRS and supervised pattern recognition techniques for classification (PCA–LDA, SPA-LDA and GA-LDA) clearly demonstrate a rapid and non-destructive method for discriminating maturity stages of jaboticaba [*Myrciaria cauliflora* (Mart.)O. Berg cv. Açú] intact fruit, without any prior metabolite extraction. A classification method based on the modeling of NIR spectra with SPA-LDA and GA-LDA allowed for a successful discrimination of the maturity stages (immature, physiological mature and ripe) using 123 and 48 wavelengths, respectively. Finally, this method was thoroughly validated in accordance with accuracy tests, being considered sensitive, specific, accurate, and suitable for use as a promising alternative for assessing jaboticaba fruit maturity, opening the possibility for automation in packing houses. The

analyses are carried out quickly and the use of laborious procedures of chemical characterization is not required. Further investigation using spectra from additional varieties of jaboticaba should help to develop a more robust global model.

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Legends to Figures

Figure 1. Average NIR spectra of jaboticaba 'Açú' fruit examined: (A) raw and (B) first derivative.

Figure 2. The application of principal component analysis (PCA)–linear discriminant analysis (LDA) and variable selection techniques [successive projection algorithm (SPA) and genetic algorithm (GA)] to the segregation of three maturity stages. PCA–LDA results: (A) DF1 × DF2 plot calculated by PCA–LDA model from "immature" (blue) vs. "physiologically mature" (red) vs. "ripe" (black) maturity stages. SPA–LDA results: (B) (A) DF1 × DF2 plot calculated using the 123 selected wavelengths by SPA–LDA model from "immature" (blue) vs. "physiologically mature" (red) vs. "ripe" (black) maturity stages. GA–LDA results: (C) DF1 × DF2 plot calculated using the 48 selected wavelengths by GA–LDA model from "immature" (blue) vs. "physiologically mature" (red) vs. "ripe" (black) maturity stages.

Legends to Tables

Table 1. Average mass and colour of the jaboticaba fruit at three maturity stages.

Table 2. Variables for SPA–LDA and GA–LDA determined from the minimum cost function G used to achieve classification of "immature," "physiologically mature" and "ripe" jaboticaba fruit for a given validation data set.

Table 3. Values of quality performance features from three classification methods (PCA–LDA, SPA–LDA and GA–LDA) by NIR spectroscopy of jaboticaba fruit at different maturity stages (immature, physiological mature and ripe).

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Figures

Figure 1

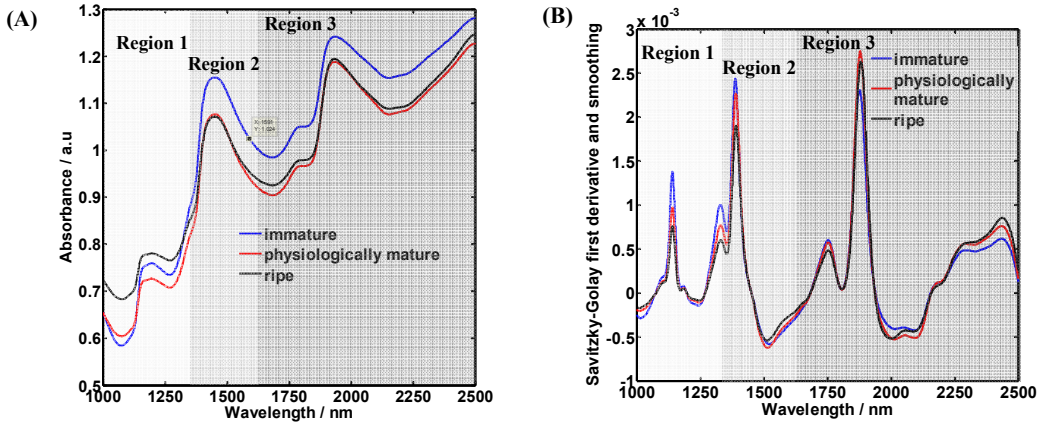

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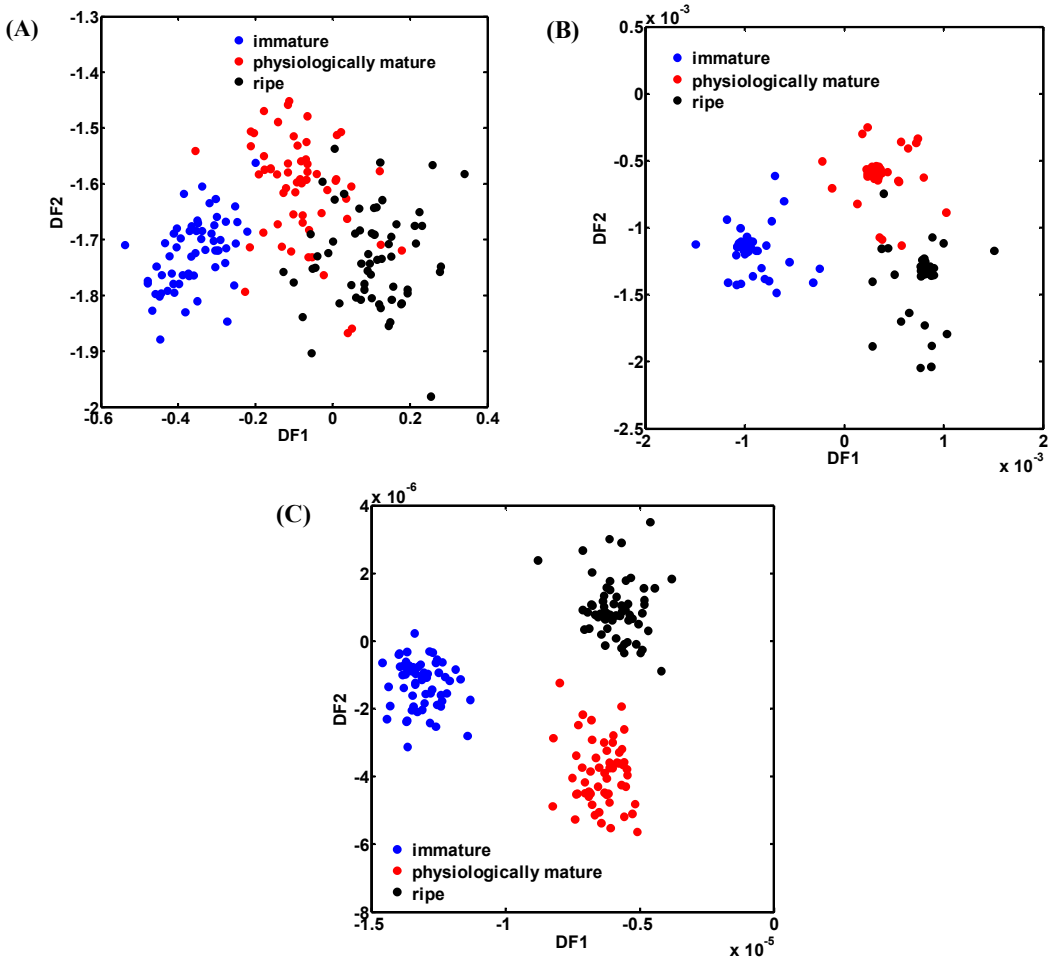
Figure 2



Tables

Table 1

Maturity stages	Mass (g)	Fruit colour		
		Luminosity	Chromaticity	hue angle
Immature	11.49 a	45.44 a	124.31 a	21.09 a
Physiologically mature	6.36 b	45.50 a	177.88 b	5.75 b
Ripe	13.20 a	39.52 b	280.74 c	8.07 c

Averages followed by the same letter in the column are significantly different according to Tukey's test ($p < 0.05$).

Table 2

Chemometric analysis	Wavelengths (nm) selected							
SPA-LDA	1000	1003	1005	1008	1013	1016	1018	1026
	1028	1042	1068	1100	1127	1134	1146	1154
	1162	1173	1192	1206	1239	1254	1279	1298
	1314	1341	1368	1383	1391	1395	1401	1410
	1414	1422	1429	1437	1442	1447	1453	1465
	1476	1483	1497	1505	1520	1537	1554	1563
	1569	1576	1582	1600	1629	1650	1672	1689
	1704	1713	1731	1742	1749	1765	1784	1798
	1818	1832	1842	1867	1874	1881	1889	1898
	1914	1927	1946	1955	1964	1974	1986	2001
	2013	2030	2037	2046	2056	2064	2076	2085
	2103	2120	2134	2150	2167	2176	2185	2198
	2212	2224	2234	2242	2249	2260	2270	2283
	2294	2304	2316	2331	2340	2349	2362	2375
	2390	2403	2415	2430	2440	2450	2456	2462
	2473	2485	2495					
GA-LDA	1004	1020	1023	1029	1038	1055	1072	1093
	1112	1113	1134	1135	1137	1170	1211	1225
	1226	1231	1234	1237	1245	1256	1294	1380
	1381	1385	1469	1478	1481	1516	1533	1589
	1616	1618	1626	1693	1729	1806	1826	1827
	1876	1913	1914	2045	2206	2211	2212	2299

Table 3

Stage performance features	PCA-LDA	SPA-LDA	GA-LDA
Immature			
Sensitivity	1	1	1
Specificity	1	1	1
Positive predictive values (PPV)	1	1	1
Negative predictive values (NPV)	1	1	1
Youden index (YOU)	1	1	1
Positive likelihood ratios (LR+)	-	-	-
Negative likelihood ratios (LR–)	0	0	0
Physiological mature			
Sensitivity	1	0.77	1
Specificity	1	0.77	0.77
Positive predictive values (PPV)	1	0.77	0.81
Negative predictive values (NPV)	1	0.77	1
Youden index (YOU)	1	0.55	0.77
Positive likelihood ratios (LR+)	-	0.035	0.045
Negative likelihood ratios (LR–)	0	0.002	0
Ripe			
Sensitivity	1	0.88	1
Specificity	1	0.88	0.55
Positive predictive values (PPV)	1	1	0.69
Negative predictive values (NPV)	1	0.88	1
Youden index (YOU)	1	0.77	0.55
Positive likelihood ratios (LR+)	-	0.08	0.025
Negative likelihood ratios (LR–)	0	0.00125	0