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Nondestructive Determination of Total Soluble Solid Content and Firmness in Plums Using Near-Infrared Reflectance Spectroscopy

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The fruit industry requires rapid, economical, and nondestructive methods for classifying fruit by internal quality, which can be built into the processing line. Total soluble solid content and firmness are the two indicators of plum internal quality that most affect consumer acceptance. These parameters are routinely evaluated using methods which involve destruction of the fruit; as a result, only control batches can be analyzed. The development of nondestructive analytical methods would enable the quality control of individual fruits. Near-IR spectroscopy (NIRS) was used to assess total soluble solid content (SSC, °Brix) and firmness (N) in intact plums. A total of 720 plums (*Prunus salicina* L. cv. 'African Pride', 'Black Diamond', 'Fortune', 'Laetitia', 'Larry Anne', 'Late Royal', 'Prime Time', 'Sapphire', and 'Songold') were used to obtain calibration models based on reference data and near-IR spectral data. Standard errors of cross-validation (SECV) and coefficients of determination for cross-validation (r^2) were (0.77 °Brix; 0.83) for total soluble solids content and (2.54 N; 0.52) for firmness. Results suggest that NIRS technology enables fruit to be classified in terms of total soluble solid content and firmness, thus allowing increased sampling of each production batch and ensuring a given quality with greater precision and accuracy.

KEYWORDS: near-IR; spectroscopy; plum; quality; nondestructive test; soluble solid content; firmness

INTRODUCTION

Postharvest quality in stone fruits is ultimately defined in terms of consumer acceptance and includes appearance, texture, and flavor; nutritional value; and safety (*1, 2*). For plums, soluble solid content (SSC) and firmness are two of the most important internal quality parameters.

Kader and Mitchell (*3*) report that SSC increases with ripening but that the use of SSC alone as a ripeness index is limited by variation among varieties, production area, and season. Nevertheless, Crisosto (*4*) suggests that SSC can be considered a good quality index.

Firmness is a key quality parameter in plums, since it is directly related to fruit ripeness, and is often a good indicator of shelf life (*5, 6*). Fruit firmness has major economic implications, soft fruits being more susceptible to bruising (*2*).

Conventional quality sensors used for fruit inspection and classification are often time-consuming (between 15 and 30 min to analyze several parameters) and generally destructive; thus, they cannot be applied to online analysis, being only applied to small groups of samples. There is currently considerable interest in the development and application of nondestructive methods for the quality control of individual fruits in the processing plant, enabling determination of both chemical and physical characteristics, as well the shelf life of the product. The plum industry requires quality-analysis methods that can be built into the processing line, allowing decisions to be taken in real time. If the industry can sort the plums rapidly and nondestructively, more uniformly ripe fruit can be marketed and oversoft fruit discarded.

Near-IR spectroscopy (NIRS) technology provides a nondestructive analytical method offering swift, accurate, and fast measurement. A single instrument can be used for a wide range of products and parameters instantaneously. A single spectral analysis of each fruit, collected in around 1 min, is needed to have a complete analytical profile. However, although this technology has considerable potential and offers various advantages for quality analysis, its widespread use in the fruit industry is hindered by a number of constraints inherent in this type of product, chief among which are the following: high moisture content, which hampers the capture of spectral information relevant for other attributes of interest; considerable differences between individual fruits (e.g., sugar gradients);

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substantial variation in product size and shape, resulting in inconsistent optical geometry; and the perishable nature of the product (*7*).

Previous research has demonstrated the potential of this technology for the quantitative and qualitative characterization of peaches (*8–12*), nectarines (*12*), cherries and apricots (*13, 14*), plums (*15*), and other stone fruits (*16, 17*). However, most of these studies were performed with instruments measuring in a narrow region of the near-infrared, generally between 800 and 1100 nm, which probably limits the development of applications providing sufficient predictive capacity for determining more complex quality parameters. Moreover, many of the instruments used work in the transmittance mode, which hinders their incorporation in processing lines and also requires greater light intensity, which might damage fruit by overheating (*18*). This is particularly true of thin-skinned fruits such as plums, for which the reflectance mode is more suitable, since light penetration is not markedly impeded by skin thickness, as would be the case in thick-rind fruits (*19*).

In recent years, NIRS instruments have undergone radical changes; they are much more versatile in terms of the infrared region in which measurements can be made, more portable, and better adapted to hostile working areas (e.g., high temperatures and vibrations); low-cost instruments have also started to appear in the market (*19, 20*). However, before this technology can be successfully transferred to the fruit industry and, especially, implemented on a large scale, further research is required into the interaction of NIRS radiation from different regions of the near-infrared with intact fruits, with a view to optimizing instruments depending on the type of fruit and the parameters to be measured.

The present study sought to determine whether a NIRS diode array instrument with a broad spectral range (400–1700 nm) could provide suitable calibrations for the prediction of SSC and firmness in intact plums. Particular emphasis was placed on evaluation of the spectral range for optimal calibrations.

MATERIALS AND METHODS

Fruit. Plums (*Prunus salicina* L.) cv. 'African Pride', 'Black Diamond', 'Fortune', 'Laetitia', 'Larry Anne', 'Late Royal', 'Prime Time', 'Sapphire', and 'Songold', grown in Seville (Spain), were harvested between June and September 2006. A total of 720 individual fruits were selected.

On arrival at the laboratory, fruit was promptly placed in cold storage at 0 °C and 95% relative humidity (RH). Prior to each measurement, the fruit sample was left at room temperature to allow the near-surface fruit temperature to rise to, and stabilize at, the laboratory temperature of 20 °C.

Three different calibration sets, as a function of the varieties used and the numbers of samples available for each variety, were used to obtain NIRS prediction models. First, the possibility of developing separate models for each variety was evaluated using the two sets for which most samples were available: C1 (250 plums, cv. 'Fortune') and C2 (183 plums, cv. 'Late Royal' for SSC, and 143 plums, cv. 'Late Royal' for firmness).

Since only a small number of samples of the remaining varieties were available for developing single-variety calibrations, the possibility of using a multivariety set was evaluated. The third set (C3) therefore comprised all the available samples (720 plums for SSC and 680 for firmness), of nine varieties ('African Pride', 14 samples; 'Black Diamond', 26 samples; 'Fortune', 250 samples; 'Laetitia', 66 samples; 'Larry Anne', 53 samples; 'Late Royal', 183 samples for SSC and 143 for firmness; 'Prime Time', 28 samples; 'Sapphire', 26 samples; and 'Songold', 74 samples).

Spectrum Collection. NIR spectra of intact fruit were measured using a Perten DA-7000 diode-array vis + near-IR spectrophotometer (Perten Instruments North America, Inc.). The instrument has two measuring positions for spectrum capture (up-view and down-view): here, the down-view was used: i.e., light was focused on the fruit from above, as it rotated on the sample dish.

For each fruit, two reflectance spectra were captured at equidistant positions around the equator. The spectrophotometer scanned at 5 nm, across a range encompassing the entire visible (400–780 nm) and the near-IR (780–1700 nm) wavelengths.

Reference Data Analysis. Soluble solid content (SSC) and firmness (compression test) were determined using traditional destructive tests. Soluble solid content (in °Brix) was measured as the mean of two refractometer readings taken for plum juice squeezed from longitudinal slides, using a temperature-compensated digital refractometer (model ATC-1, Atago Co., Tokyo, Japan). Fruit firmness measurements were made with a hand-held penetrometer (Effegi, Italy) using a 8 mm diameter plunger. A disk of skin measuring around 2 cm in diameter was removed using a stainless steel vegetable peeler. Two measurements were made, one on each of the opposite cheeks, midway between the stem-end and the calyx-end; measurements were averaged to give a mean penetrometer firmness. The maximum depth of penetration was 8 mm, and the rate of penetration was subjectively controlled by hand at about 4 mm s^{-1} (2 s to maximum depth).

Chemometric Data Treatment. The WinISI software package ver. 1.50 (Infrasoft International, Port Matilda, PA) was used for the chemometric treatment of data (*21*). Before developing NIRS calibrations, the structure and spectral variability of the sample population was determined using the Center algorithm included in the WinISI software. This program performs an initial principal component analysis (PCA) to calculate the center of the population and the distance of samples (spectra) from that center in an *n*-dimensional space, using the Mahalanobis distance (GH); samples with a statistical value greater than 3 were considered outliers or anomalous spectra (*22, 23*).

The Center algorithm was applied in the five spectral regions (515–1400, 515–1650, 800–1100, 1100–1400, and 1100–1650 nm), subsequently used for obtaining calibrations; the Standard Normal Variate and Detrending methods were applied for scatter correction (*24*), together with the mathematical derivative treatment "1,5,5,1", where the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in a running average or smoothing, and the fourth is the second smoothing (*25*).

Calibrations were developed for predicting SSC and firmness in intact plums. The prediction equations were obtained using modified partial least squares (MPLS) as regression method (*23*). Partial least-squares (PLS) regression is similar to principal component regression (PCR) but uses both reference data (chemical, physical, etc.) and spectral information to identify the factors useful for fitting (*26*). MPLS is often more stable and accurate than the standard PLS algorithm. In MPLS, the near-IR residuals at each wavelength, obtained after each factor is calculated, are standardized (divided by the standard deviations of the residuals at a wavelength) before calculating the next factor. When developing MPLS equations, cross-validation is recommended to select the optimal number of factors and to

Table 1. Quality Indexes for Different Plum Varieties

		color		soluble solid content (°Brix)			firmness (N)		
cultivar	N^a	skin color	flesh color	mean	range	SD[b]	mean	range	SD[b]
African Pride	14	yellow	yellow	14.74	10.60–17.30	1.69	21.51	11.28–27.47	4.76
Black Diamond	26	dark violet	reddish	11.38	7.00–14.20	1.40	32.58	20.60–43.65	5.01
Fortune	250	bright red	amber yellow	14.96	11.10–19.10	1.04	36.53	10.30–49.05	5.74
Laetitia	66	dark red	amber yellow	12.69	9.40–16.30	1.26	30.27	21.58–48.56	5.26
Larry Anne	53	dark red	amber yellow	13.87	9.00–17.90	2.31	42.96	26.49–58.37	7.26
Late Royal	183/143[c]	dark red	amber yellow	14.85	6.75–20.10	2.11	32.69	22.56–44.15	4.14
Prime Time	28	red	yellow	14.34	12.00–18.80	1.55	28.24	19.62–37.77	4.23
Sapphire	26	red	reddish	11.48	10.30–12.70	0.70	22.96	8.83–33.35	6.98
Songold	74	yellow	yellow	14.96	12.00–18.60	1.28	31.65	23.05–53.46	4.96

[a] *N*, number of samples. [b] SD, standard deviation. [c] 183 samples for total soluble solid content and 143 samples for firmness.

avoid overfitting (*23*). For cross-validation, the calibration set was partitioned in four groups; then each group was validated using a calibration developed on the other samples; finally, validation errors were combined to obtain a SECV.

All multivariate regression equations were obtained using the Standard Normal Variate and Detrending methods for scatter correction (*24*). Moreover, four derivative mathematical treatments were tested in the development of NIRS calibrations: 1,5,5,1; 2,5,5,1; 1,10,5,1; and 2,10,5,1 (*25*).

In this work, for calibration development of both quality indexes, five spectral regions were tested: 515–1400, 515–1650, 1100–1400, 1100–1650, and 800–1100; the latter is the spectral region most commonly used in NIRS analysis of fruits (*19*). To eliminate spectral noise at the beginning and end of the complete spectral region, the regions between 400 and 515 nm and between 1650 and 1700 nm were discarded.

The statistics used to select the best equations were the standard error of calibration (SEC), the coefficient of determination (R^2), the standard error of cross-validation, and the coefficient of determination for cross-validation (r^2).

RESULTS AND DISCUSSION

Calibration Database Composition and Spectrum Description. Numbers of samples analyzed for each variety, pulp color, and skin color are shown in **Table 1**, together with mean, range, and standard deviation (SD) values for each parameter analyzed.

Typical log(1/*R*) and D_2 log(1/*R*) spectra for intact plums are shown in **Figure 1**. The effect of derivatives was most apparent with the second derivative of a spectrum, which was able to separate overlapping absorption bands, displaying more clearly certain characteristic absorbance peaks. In the visible region, two peaks were detected (570 and 660 nm), characteristic of yellow and red fruit color, respectively. In the near-infrared region, water peaks were recorded around 950 and 1400 nm; a characteristic band around 910 nm was influenced by absorption exerted by the third sugar-related overtone (*16*), while a weak absorption band around 1170 nm was also sugar-related. Plum spectra are noticeably different from those of other stone fruits, such as peach and nectarine, especially above 910–920 nm, plum flesh being more translucent, absorbing less light, and requiring a shorter integration time (*17*).

Average spectra for the nine plum varieties analyzed are shown in **Figure 2**. In the visible region, variations in absorption bands were attributable to differences in skin and flesh color between varieties; this was particularly true of bands at 569−590 and 625−740 nm.

In the near-IR region, the average spectra for the different varieties tested displayed a broadly similar pattern, with the exception of 'African Pride' and 'Late Royal'. Absorption values for 'African Pride' were lower, a finding attributable not only to the yellowier color of skin and flesh but also to the fact the mean firmness value for this variety (21.51 N) was lower than that of the rest (**Table 1**). The average spectrum for 'Late Royal' displayed areas of signal saturation above 1200 nm.

Population Structuring. The Center algorithm was applied to the all-sample set in order to determine the structure of the population and define the calibration sets. Samples marked with *H* values higher than 3 were considered outliers (*22*). In the five spectral regions selected (515–1400, 515–1650, 800–1100, 1100–1400, and 1100–1650 nm), all samples of 'African Pride' were classed as outliers. A detailed inspection of 'African Pride' samples showed that they all had yellow skin and flesh, unlike the other varieties, with the exception of 'Songold', which also had yellow skin and flesh. The reason why 'African Pride' samples were classed by the Center algorithm as outliers while 'Songold' samples were not may be due to the difference in the number of samples tested (14 for 'African Pride' vs 74 for 'Songold') The outlier classification may thus be due to a combination of the small number of 'African Pride' sample spectra as a proportion of the total population (720), color differences with respect to the other varieties, and firmness differences as indicated earlier.

The other samples classed as outliers displayed physical damage affecting a large proportion of the external surface. In all, and depending on the spectral range used, between 10 and 15% of anomalous samples were discarded from the calibration sets used for developing predictive models.

Calibration Development. In an effort to optimize calibration performance for the prediction of SSC and firmness in intact plums, three approaches for calibration development were explored: the first set (C1) comprised samples of the 'Fortune' variety, the second set (C2) comprised 'Late Royal', and the third (C3) comprised all the varieties used.

The characteristics of the three calibration sets (mean, range, standard deviation, and coefficient of variation for the two parameters studied) are shown in **Table 2**.

Set C1 displayed the least variation for SSC (CV value 6.95%, compared with 14.21 and 13.08% for sets C2 and C3, respectively), even though C2 contained a single variety ('Late Royal') while C3 contained all the varieties used. Set C3 displayed the greatest variability for firmness (CV = 20.99%, compared with 15.71 and 12.66%, respectively, for sets C1 and C2).

The best results obtained for SSC and firmness, in each calibration set and spectral range used, are shown in **Tables 3** and **4**, respectively. There was no clear trend regarding which of the four derivation treatments tested provided the best results for the two applications. For SSC, it was found that when the two single-variety sets were used as training sets over the

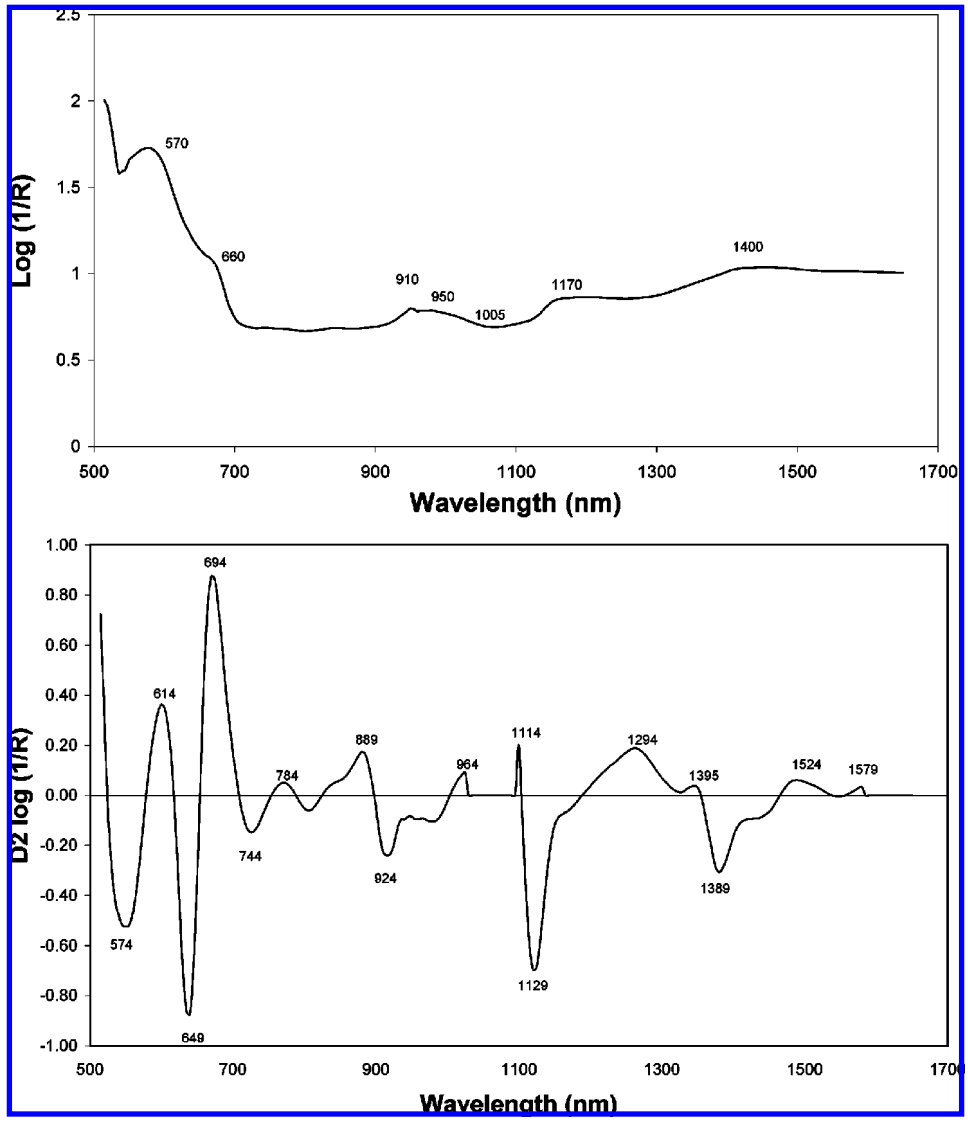

Figure 1. Typical average log(1/*R*) and D_2 log(1/*R*) spectra for intact plums.

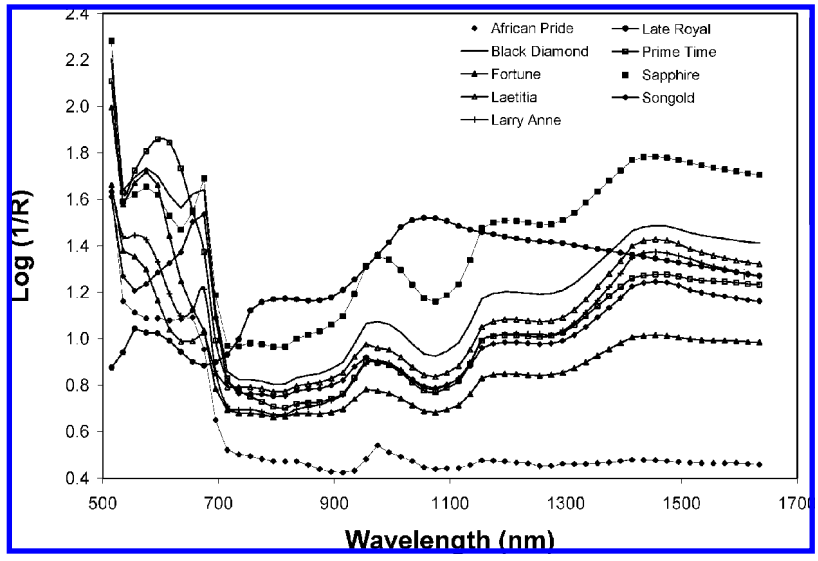

Figure 2. Typical average log(1/*R*) spectra for the different varieties of intact plums.

spectral ranges used, the second derivative was selected in most cases; however, when using the largest and most heterogeneous sample set (C3) for training purposes, first-derivative treatments provided the best results in three of the five spectral ranges tested. Similar trends were recorded for firmness: second-derivative treatments generally performed better for the single-variety sets, and first-derivative treatments, for the multivariety set.

Table 2. Mean, Range, Standard Deviation, and Coefficient of Variation for Different Calibration Sets

	parameters					
	soluble solid content (°Brix)			firmness (N)		
calibration set	C1	C2	C3	C1	C2	C3
N[a]	250	183	720	250	143	680
mean	14.96	14.85	14.37	36.53	32.69	33.96
range	11.10–19.10	6.75–20.10	6.75–20.10	10.30–49.05	22.56–44.15	8.83–58.37
SD[b]	1.04	2.11	1.88	5.74	4.14	7.13
CV[c] (%)	6.95	14.21	13.08	15.71	12.66	20.99

[a] N: Number of samples in calibration set. [b] SD: Standard deviation. [c] CV: Coefficient of variation.

Table 3. Calibration Statistics for the Equations Obtained Using Each Data Set and Spectral Range for the Prediction of Soluble Solid Content (°Brix) in Intact Plums

data set	spectral range	math treatment	mean[a]	SD[b]	SEC[c]	R^2 [d]	SECV[e]	r^2 [f]
C1	515–1400	2,5,5,1	14.98	0.90	0.49	0.75	0.54	0.64
C1	515–1650	2,5,5,1	14.96	0.89	0.40	0.80	0.48	0.71[g]
C1	800–1100	1,10,5,1	14.96	0.90	0.45	0.75	0.48	0.71[g]
C1	1100–1400	2,10,5,1	14.98	0.82	0.69	0.28	0.77	0.24
C1	1100–1650	1,5,5,1	14.98	0.84	0.57	0.54	0.61	0.47
C2	515–1400	2,5,5,1	15.31	1.87	0.67	0.87	0.77	0.83[g]
C2	515–1650	2,5,5,1	15.32	1.86	0.72	0.85	0.84	0.80
C2	800–1100	1,10,5,1	15.29	1.74	1.08	0.62	1.13	0.58
C2	1100–1400	2,5,5,1	15.38	1.70	1.45	0.27	1.51	0.23
C2	1100–1650	2,5,5,1	15.45	1.64	1.20	0.46	1.44	0.23
C3	515–1400	2,5,5,1	14.45	1.61	0.82	0.74	0.86	0.72[g]
C3	515–1650	1,5,5,1	14.49	1.64	0.89	0.71	0.94	0.67
C3	800–1100	2,5,5,1	14.47	1.59	0.94	0.65	0.96	0.63
C3	1100–1400	1,5,5,1	14.57	1.62	1.37	0.28	1.39	0.26
C3	1100–1650	1,5,5,1	14.54	1.68	1.38	0.33	1.42	0.29

[a] Mean, mean of the calibration set. [b] SD, standard deviation. [c] SEC, standard error of calibration. [d] R^2, coefficient of determination. [e] SECV, standard error of cross-validation. [f] r^2, coefficient of determination. [g] Best equation.

Table 4. Calibration Statistics for the Equations Obtained Using Each Data Set and Spectral Range for the Prediction of Firmness (N) in Intact Plums

data set	spectral range	math treatment	mean[a]	SD[b]	SEC[c]	R^2 [d]	SECV[e]	r^2 [f]
C1	515–1400	2,5,5,1	37.06	5.31	4.05	0.42	4.37	0.33
C1	515–1650	2,5,5,1	36.92	5.20	3.48	0.55	4.01	0.41[g]
C1	800–1100	2,10,5,1	36.90	5.26	4.60	0.23	4.51	0.26
C1	1100–1400	*h*	*h*	*h*	*h*	*h*	*h*	*h*
C1	1100–1650	*h*	*h*	*h*	*h*	*h*	*h*	*h*
C2	515–1400	1,5,5,1	33.12	3.93	3.48	0.22	3.61	0.16
C2	515–1650	2,5,5,1	33.21	4.26	3.68	0.25	3.92	0.16
C2	800–1100	2,5,5,1	32.96	4.04	3.32	0.33	3.58	0.22
C2	1100–1400	1,10,5,1	33.19	3.71	2.64	0.49	2.74	0.46
C2	1100–1650	2,10,5,1	33.15	3.66	2.14	0.66	2.54	0.52[g]
C3	515–1400	1,10,5,1	34.47	5.75	4.33	0.43	4.44	0.40
C3	515–1650	1,5,5,1	34.33	5.75	3.95	0.53	4.16	0.48[g]
C3	800–1100	2,10,5,1	34.54	6.17	4.67	0.43	4.74	0.41
C3	1100–1400	1,10,5,1	34.42	6.15	4.92	0.36	5.02	0.34
C3	1100–1650	2,5,5,1	34.36	6.04	4.52	0.44	4.66	0.41

[a] Mean, mean of the calibration set. [b] SD, standard deviation. [c] SEC, standard error of calibration. [d] R^2, coefficient of determination. [e] SECV, standard error of cross-validation. [f] r^2, coefficient of determination. [g] Best equation. [h] No correlation obtained.

For set C1, the best prediction model for SSC was obtained at wavelength ranges of 515–1650 and 800–1100 nm, which yielded calibrations displaying identical predictive capacity (SECV = 0.48; r^2 = 0.71). For sets C2 and C3, the optimal spectral range for predicting SSC lay between 515 and 1400 nm (**Table 3**). This may be due to the signal saturation detected in spectra from C2 (also included in C3) above 1200 nm.

It was generally found, for all three sets, that when spectral regions with a lower-end cutoff were used, i.e. starting at 1100 nm, the predictive capacity of the models obtained was considerably reduced, confirming the existence below 1100 nm of key absorption peaks for determining fruit sugar content. Similar findings are reported by Abu-Khalaf and Bennedsen (*15*), who note the existence of three bands below 1100 nm (778–810, 902–935, and 960–990 nm) of major importance for near-IR determination of SSC in plums.

The equation displaying the greatest predictive capacity for SSC was obtained with set C2, using the second derivative treatment and the spectral range 515–1400 nm (SECV = 0.77 °Brix; r^2 = 0.83), that shows a high correlation level (91%) between the reference data and the near-IR-predicted values. Although this was a single-variety set, soluble solid content varied considerably. The statistical values obtained were better than those reported by Abu-Khalaf and Bennedsen (*15*), using a set comprising two plum varieties, analyzed in reflectance mode in the region of 700−1100 nm (SEP = 1.56 °Brix; r^2= 0.64), and than those recorded by Walsh et al. (*16*) working in transmittance mode between 734 and 931 nm (SEP = 0.47%; r^2 = 0.71).

The best results for firmness prediction were in all cases obtained in spectral regions of up to 1650 nm. The prediction of a parameter of a physical nature, such as firmness, is related to loss of cell wall structures such as pectin, cellulose, and hemicellulose (*19*); these compounds have various absorption speaks over the spectrum.

Here, the best results for firmness prediction were obtained with set C2, using the second-derivative treatment in the 1100–1650 nm range (SECV = 2.54 N; r^2 = 0.52). The predictive capacity of firmness prediction models was lower than that recorded for SSC, as was to be expected, since this is a physical parameter whose measurement using the reference method is already prone to considerable error, particularly since results obtained using a hand-held penetrometer may be quite subjective (*27*). This fact was reflected in the correlation found between the reference and the near-IR-predicted data, which for this parameter was 72%. Sohn and Cho (*28*) also report that with an appropriate reference analysis and long-wavelength spectra, a sufficiently precise calibration can be achieved for predicting firmness in apples.

No published studies address the prediction of firmness in plums. However, a number of authors have developed models for predicting firmness in other fruits, including apples (*28–31*), kiwi (*27*), and mandarins (*32*). The predictive capacity of the models obtained in these studies is similar to that noted here, with values for the coefficient of determination ranging from 0.5 to 0.9; these studies also stress the difficulty involved in predicting this physical parameter.

The applications developed here for the quality control of individual plums would enable not only the quantification of

sugar levels but also fruit classification by sugar content and firmness, the latter being of major interest to the fruit industry, since large distribution chains generally demand specific SSC and firmness values for a fruit in order to be considered acceptable. The minimum acceptable soluble solid content in plums is around 12 °Brix (*2*), although it depends on the target market. The 26 N threshold is the minimum firmness with which plums should be harvested to avoid bruising during standard postharvest handling (*33*); between 13 and 26 N, plums can be classed as "ready to buy" (*6*).

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