In situ ripening stages monitoring of Lamuyo pepper using a new generation NIRS sensor

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Abstract

BACKGROUND: Near infrared spectroscopy (NIRS) was used as a non-destructive sensor to assess the quality of freshly-harvested Lamuyo peppers. 144 Lamuyo peppers which were in a range of colors (green, chocolate, orange and red) when harvested, were analyzed. In this study the evolution of the main quality parameters during the harvest period was analyzed. Additionally, NIRS predictive models using a portable manual spectrophotometer to evaluate quality parameters together with color index, were developed. Moreover, two procedures for taking NIR spectra: 1) static, taking of point spectra readings around the equator of the fruit; 2) dynamic, spectra taken by scanning the entire length of the pepper were tested.

RESULTS: Green peppers and those harvested at the beginning of the campaign presented significantly lower values (*P* < 0.05) of dry matter and soluble solid contents and titratable acidity, while those with red coloration and those harvested at the end of the campaign showed significantly higher values of these three quality parameters (*P* < 0.05). The predictive capacity of the NIRS models showed that the static mode proved to be the most suitable for measuring the quality of Lamuyo peppers.

CONCLUSIONS: The viability of NIRS for measuring dry matter content and soluble solid contents *in situ*, using a new generation NIRS sensor, was demonstrated. However, the high water content, the irregular shape of the vegetable and the fact that it is hollow inside, all point to the need for using larger samples sets so as to increase the robustness of the models obtained.

Keywords: Lamuyo pepper; New generation NIRS sensor; *In situ* determination; Quality parameters.

INTRODUCTION

Lamuyo peppers are hybrids originating from France which have gradually overtaken other types of peppers traditionally grown in Spain, with annual production figures of around 75,000-80,000 kg/ha, due to the fact that the plants are bushy, vigorous and less sensitive to cool than bell peppers. The fruits are around 12-14 cm in length and 6-8 cm in equatorial diameter and are formed by 4 distinct lobes. The flesh is thick and consistent, and the average weight of the fruit is 200-300 g.[1]

Arguably, the most influential factor in the quality and postharvest life of fruit and vegetable products is the degree of ripeness at the harvest time. Any fruit and vegetable products harvested too early or too late in relation to their optimum state of maturity are more susceptible to disease and have a shorter shelf-life than those which are harvested at the best moment.[2]

Pepper fruits are considered to be in optimal condition for consumption when they have attained the typical morphological characteristics of the cultivated type (length, equatorial diameter and thickness of the pericarp) and have a smooth, shiny skin which can be pressed without damaging the fruit. However, in peppers, the most obvious aspect of the ripening process is the color change from green to red. Peppers are non-climacteric fruits, and are therefore, unable to produce ethylene, the hormone required for the ripening process to continue after the fruit is separated from the plant. Thus, the commercial maturity of the pepper coincides with its physiological maturity, and the fruits only turn red when they are on the plant.[3]

Color is, therefore, a reliable indicator of the ripeness of the pepper. In general, consumers prefer dark green or bright red fruits, depending on whether they are harvested before or after physiological maturity, respectively.[4] Thus, López-Camelo and Gómez[5] suggested that the a*/b* ratio could be used for practical purposes as an objective ripening index in peppers in order to give a realistic view of consumer perceptions.

Therefore, for this type of vegetable, if a non-destructive quality control is set up initially in the field, on the plant, this will allow to implement staggered harvesting strategies in order to establish the optimum harvest time of the fruits and thus cater for the demand from the industry and from consumers.

NIRS technology meets all the necessary requirements to be used to determine the optimal harvest time for Lamuyo peppers in the field, directly on the plant itself, enabling to study simultaneously various specific quality indicators of the fruit and its state of maturity. At the same time, the study of the maturity curve of the pepper on the plant with NIRS allows to take real-time decisions to increase production efficiency and ensure product quality. All agri-food companies usually try to use these types of selective harvesting strategies to adapt their production to the preferences and specifications of the different markets.

The development in recent years of manual, portable, compact NIRS instruments has enabled to make great progress in the analysis of vegetables. They can be used to take agronomic decisions about the optimum harvest time as the product is growing in the field, thus avoiding the need to harvest the product first and take it to the laboratory.[6-8] These advances have led to a wide range of portable instruments appearing on the market, whose suitability, and that of the spectra-taking process, needs to be tested before analyzing different vegetables *in situ*, such as the Lamuyo pepper.

The aim of this study was therefore to study the viability for measuring, in real time and on the plant, the quality of outdoor-grown Lamuyo peppers of different colors (i.e. at different stages of ripeness), using a manual, portable, Linear Variable Filter (LVF)-based NIR instrument, which allows spectra to be taken both statically (static mode), or by scanning the surface of the product (dynamic mode), in order to study the evolution of different quality parameters and organize a staggered harvest, thus obtaining optimum acceptance of the harvested fruits by the consumer.

MATERIALS AND METHODS

Sampling

A total of 144 Lamuyo peppers (*Capsicum annum* L.) of different colors (green = 36, chocolate = 36, orange = 36 and red = 36) grown outdoors in Santaella (Córdoba, Spain) were harvested manually during the months of September and October 2017.

The fruit was harvested first from the top of the plants and then from the lower parts, since the bottom of the plant is where the peppers with a more advanced state of ripeness are found (red peppers), while the unripe peppers (green peppers) are found on the upper parts of the plant.

On arrival at the laboratory, the fruits were left at room temperature to stabilize at the laboratory temperature of 20 ºC.

Spectral data acquisition

The spectra were taken using the MicroNIR™ Pro 1700 (VIAVI Solutions, Inc., San Jose, California, USA) instrument in reflectance mode (Fig. 1). This portable miniature spectrophotometer is extremely light (64 g, not including the 150 g handle and the acquisition and data processing device). The optical window measures around 227 mm^2. This microspectrometer covers the spectral range from 910 to 1676 nm (taking data every 6.2 mm), incorporating LVF. The sensor integration time was 11 ms and each spectrum was the mean of 200 scans. The instrument's performance was checked every 10 min. A white reference measurement was obtained using a NIR reflectance standard

(Spectralon^{TM}) with a 99% diffuse reflectance, while a dark reference was obtained from a fixed point on the floor of the room.

Two modes of analysis were tested: static and dynamic. The analysis in static mode was carried out thus: the sensor was placed at any given point located at the equatorial diameter, at one side of the fruit, and the equipment was kept still while the spectrum was being recorded; next, measurements were taken at the height of the equatorial diameter, on the four sides of the fruit, rotating the fruit 90º, thus producing a total of 4 measurements per fruit. The sample was placed over a black plastic sheet when the spectra were taken. In the dynamic mode analysis, the four spectra taken per sample were obtained thus: the sensor was moved along each of the faces of the pepper analyzed, covering the area from the peduncle to the apical end of the fruit, rotating the fruit 90º between measurements.

In both cases, the 4 spectra were averaged to provide a mean spectrum per fruit.

Reference data

To measure the a*/b* color index of the fruits, which is given by the relation between the color parameters a* (red-green variation) and b* (yellow-blue variation), 4 measurements were taken around the fruit's equatorial diameter at 90º intervals, at the same points where the NIRS spectra were taken, using a Chroma METTER CR-400 colorimeter (Konica Minolta Sensing INC., Osaka Japan), with illuminant C and an observation angle of 2°.[9]

For dry matter content (DMC), 5 g of the sample was weighed on an electronic scale (0-1,000 ± 0.1 g, model P1000 N, Metter-Toledo, GmbH, Greifensee, Switzerland), and then dried in a hot-air oven at a temperature of 105°C until the weight was constant.[10] The final dry weight was calculated as a percentage of initial fresh weight.

The soluble solid content (SSC) in ºBrix was taken from the refractometer reading for the pepper juice, using a temperature-compensated digital Abbe-type refractometer (model B, Zeiss, Oberkochen, Würt, Germany).

For titratable acidity (TA), 5 g of pepper juice was used, to which 50 ml of distilled water was added. Titratable acidity was measured by titration using 0.1 N NaOH up to pH 8.2. The results were expressed as a percentage of citric acid.[10] An automatic titrator was used (Crison pH burette 24, Crison, Adella, Barcelona, Spain) to take these measurements.

All the samples were analyzed in duplicate and the standard error of laboratory (SEL) was estimated from these duplicates (Table 3). All the measurements were taken immediately after the NIRS measurements.

Data processing

Data pre-processing and chemometric treatments were performed using the WinISI II software package version 1.50 (Infrasoft International LLC, Port Matilda, PA, USA).[11]

Before the spectral data were processed, a study was conducted to select the most suitable spectral range for the instrument to carry out the quality control of Lamuyo peppers. To achieve this, the 1,1,1,1 derivation treatment was applied (the first digit being the number of the derivate, the second the gap over which the derivate is calculated, the third the number of data points in a running average or smoothing, and the fourth the second smoothing) without scatter correction, which allows to highlight the areas of the spectrum where the signal/noise ratio is degraded.[12,13]

Spectral repeatability

To calculate the spectral repeatability, the statistic Root Mean Square (RMS) was used, which refers to the difference in absorbance values between several spectra taken in the same sample, thus providing the mean value of the square root of the differences between the spectra of a sample - in this case four - which is analyzed using the same instrument, throughout the entire spectral range used.[14,15] To establish a threshold for the static and dynamic procedures, 16 Lamuyo peppers were selected, from which four spectra were taken in the equatorial region both statically and by scanning the fruit's surface, rotating the fruit 90° after each measurement. An admissible limit for spectrum quality and repeatability was set following the procedure described by Martinez *et al.*[16] to calculate the standard deviation limit (STD_{limit}) from the RMS statistic and obtain an RMS cut- off value.

Quantitative models: sets, calibration and validation procedures

First, the structure and spectral variability of the sample population was studied to select the samples that would form the sample set. To achieve this, the CENTER algorithm was used, which was applied to the 144 spectra collected both statically and dynamically. This algorithm performs an initial principal component analysis (PCA) and determines the center of the population and the distance between each sample and the center using the Mahalanobis distance (GH). Samples with a GH value over 4 were considered outliers or anomalous spectra.[17] As spectral pre-treatments, Standard Normal Variate (SNV) and Detrending (DT) were used for scatter correction,[18] together with the first derivative treatment '1,5,5,1'.[14]

Once spectral outliers were removed for each mode of analysis, 111 samples were selected to form part of the calibration set and the remainder (29 samples) constituted the validation set (Table 2).

Modified partial least squares (MPLS) regression (Shenk and Westerhaus, 1995a) was used to obtain NIRS calibration models for the prediction of the color index, (a*/b*) and quality parameters (DMC, SSC and TA) in Lamuyo peppers using the MicroNIR^{TM} Pro 1700. All regression equations were obtained using SNV + DT for scatter correction[18] and different derivative mathematical treatments were tested: 1,5,5,1; 1,10,5,1; 2,5,51 and 2,10,5,1.[14]

The statistics used to select the best equations with MPLS were the coefficient of determination for calibration (r^2_c), the standard error of calibration (SEC), the coefficient of determination for cross-validation (r^2_{cv}) and the standard error of cross-validation (SECV). Furthermore, the Residual Predictive Deviation (RPD_{cv}) for cross-validation was calculated as the ratio of the standard deviation (SD) of the reference data to the SECV. This statistic enables SECV to be standardized, facilitating the comparison of results obtained with sets of different means.[19]

Once the best predictive model was selected by statistical criteria for each parameter analyzed using the two modes of analysis, tests were run for significant differences between models for each parameter, with a view to identifying the most suitable mode of analysis for routine use in Lamuyo peppers during the growing period on the plant. The SECV values for the best equations obtained for each parameter were compared using Fisher's F test.[20,21] The values for F were calculated as:

$$F = \frac{(SECV_2)^2}{(SECV_1)^2}$$

where $SECV_1$ and $SECV_2$ are the standard error of prediction of two different models and $SECV_1 < SECV_2$. F is compared to $F_{critical}$ (1- P, n_1-1, n_2-1) as read from the table, with $P = 0.05$ and n_1 the number of times the measurement is repeated with method 1; n_2 is the number of times the measurement is repeated with method 2. If F is higher than $F_{critical}$, the two SECV values are significantly different.

Finally, once the best equations for each of the two established analysis modes were selected according to statistical criteria, and the best spectral sampling strategy was chosen (static or dynamic mode), the models were subjected to an external validation process, according to the protocol outlined by Windham *et al.*[22] based on the following statistics: standard error of prediction (SEP), standard error of prediction corrected for bias ($SEP_{(c)}$), bias and coefficient of determination for external validation (r^2_p). Generally, for calibration groups comprising 100 or more samples, and validation groups containing nine or more samples, the following control limits are assumed: Limit Control $SEP_{(c)}$ = 1.30 x SEC, Limit Control bias = ± 0.60 x SEC and minimum value of 0.6 for r^2_p. Furthermore, the Residual Predictive Deviation (RPD_p) for prediction was calculated as the ratio of the standard deviation (SD) of the validation data to the SEP.

Statistical analysis

In order to study the influence of the coloration at harvest on the DMC, SSC and TA of Lamuyo pepper, a one-factor analysis of variance (ANOVA) was carried out, using Statgraphics Centurion XV (StatPoint Inc., Warrenton, North Virginia, USA).

Next, the differences between the means were compared with the Fisher's Least Significant Difference (LSD) test, and differences at $P < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Influence of the coloration at harvest on the quality of Lamuyo peppers

The result of the ANOVA test pointed to the existence of significant differences ($P < 0.05$) between the different colorations of Lamuyo peppers analyzed for the three parameters tested. The results of Fisher's LSD test for DMC, SSC and TA are shown in Table 1.

As regards DMC, green Lamuyo peppers had a significantly lower value of the parameter ($P < 0.05$) than that found in chocolate-colored and red peppers, with no significant differences being found in orange-colored peppers. Similarly, red peppers had a significantly higher DMC ($P < 0.05$) than green and orange peppers, although no significant differences were found with chocolate-colored peppers.

As for the SSC parameter, the green peppers presented significantly lower values for this parameter ($P < 0.05$) than those found in the other colors, while red Lamuyo peppers had the highest content in this parameter, which was significantly higher ($P < 0.05$) than the other colors. Chocolate- and orange-colored peppers had the same content in soluble solids, in between the value of the other two colors. These results coincide with those of Sánchez *et al.*[23] who analyzed the dry matter and soluble solid contents just after harvesting bell peppers of different colors.

Regarding to the TA parameter, the green peppers were the least acidic, followed by chocolate, orange and lastly red peppers, which had the highest values of this parameter, with significant differences ($P < 0.05$) in TA found between the different colors, which represent different stages of the fruit's ripeness. These results are similar to those obtained by Ghasemnezhad *et al.*[24] who showed an increase in the titratable acidity of bell peppers throughout the ripening process, since while the fruit ripens, the metabolic reactions increase, increasing the concentration of organic acids involved in the Krebs cycle. For the green bell peppers, these organic acids are present in small quantities, as the ripening process has not yet started.

It is important to note that for Lamuyo peppers, no study has been found in the literature which evaluates the quality of the fruits in different stages of ripeness, as reflected by their colors. Janse[25] however, studied the influence of the degree of ripeness represented by the different colors at harvest time (green, red, yellow and orange colors) in bell peppers. In that study, the author showed that dry matter and total acid contents were around 25% and 60% lower, respectively, in green peppers than in the other colors, and that these peppers were the least sweet, the least aromatic and had the least pleasant taste. It was the red peppers that presented the highest percentages of dry matter content (8.4%) and acids (3.7 mmol/100 g), the best aroma and a higher content of glucose and fructose, which gave them a better flavor. Orange and yellow fruits showed no significant differences between them.

Optimum spectral region and spectral repeatability

Prior to the development of the models, it was necessary to optimize the NIRS analysis by means of the spectrum quality and repeatability measurement.

For this purpose, the existence of noise in the spectrum (spectral range 910-1676 nm) was evaluated. To achieve this, the derivate treatment 1,1,1,1 was applied in both analysis modes in order to determine the area of the spectral range affected by noise, as this it degrades the signal/noise relationship. After this process, the spectral range between 1459-1676 nm was eliminated due to the high level of noise detected and all the models were designed using the spectral range 910–1458 nm.

Spectral repeatability is crucial to the construction of models that are both accurate and robust. The mean STD for the samples analyzed was 52,244 µlog (1/R) (static mode) and 52,337 µlog (1/R) (dynamic mode), representing a STD_{limit} of 65,702 µlog (1/R) (static mode) and 68,131 µlog (1/R) (dynamic mode). As can be seen, the values obtained for mean STD and STD_{limit} for both modes of analysis were practically identical. However, any slight differences detected in the dynamic mode could be accounted for by the movement of the instrument during the NIR analysis, which could cause slight deviations in the measurements.

When the RMS value of the each of the 4 spectra of each sample for the two strategies devised did not exceed the value of the STD_{limit}, these spectra were then averaged and subsequently used to perform the calibrations. In this way, a high sample repeatability was achieved, which is essential for obtaining robust equations.

Spectral features

Second-derivative spectra (D_2log (1/R)) for Lamuyo peppers in different stages of ripeness represented by the different colors at harvest time (green, chocolate, orange and red), captured by the instrument MicroNIR^{TM} Pro 1700, together with the most relevant absorption bands, are shown in Fig. 2.

In the NIR region between 910 and 1458 nm, absorption peaks at 978 nm, 1065 nm, 1120 nm, 1164 nm, 1294 nm, 1338 nm, 1369 nm and 1400 nm, mainly related with C−H combination and O−H first overtone,[26,27] appear to be especially relevant for the classification of Lamuyo peppers by ripeness stage.

Prediction of color and quality parameters using MPLS regression

After using the CENTER algorithm to study the structure and spectral variability, 2 and 3 anomalous samples were detected in the static and dynamic modes, respectively, one of which was anomalous for both strategies. Therefore 4 anomalous samples were obtained for the two modes of spectral analysis, which were then removed.

Table 2 shows the characteristics of the calibration and validation sets used to develop the predictive models for the parameters analyzed.

Structured selection based on spectral information, using the CENTER algorithm, proved suitable, in that the calibration and validation sets displayed similar values for range, mean and SD for all the study parameters; moreover, the established ranges of the validation lay within those of the calibration set.

The calibration statistics for the best models for predicting color index and quality parameters in Lamuyo peppers analyzed in static and dynamic modes are shown in Table 3.

Regarding the color index (a*/b*), the predictive capacity of the model developed from the spectral reading taken statically allowed to distinguish between high, medium and low values for this parameter, while with the predictive capacity obtained through surface scanning the product, enabled to distinguish between high and low values.[15,19]

No previous studies have been found in the scientific literature on measuring the color index (a*/b*) in peppers using NIRS technology, although values for a*/b* increase significantly during ripening due to higher carotenoid levels, thus also providing a useful indicator of the fruit's ripeness.[28] However, Clément *et al.*[29] and Torres *et al.*[30] used NIRS technology to predict the color index in tomatoes using Varian Cary 500 UV-VIS-NIR (spectral range 400–1000 nm) and Perten DA-7000 (spectral range 400 a 1700 nm) spectrophotometers, obtaining models whose predictive capacities (RPD_{cv} = 2.81 and RPD_{cv} = 2.23, respectively) were higher than those obtained here, which shows how difficult it is to take pepper color measurements during the ripening process on the plant given the irregular distribution of this parameter throughout ripening, as well as the convenience of using instruments which focus on the visible region of the fruit.

To measure the quality parameters as indicated by Shenk and Westerhaus[15] and Williams,[19] the predictive capacity of the model obtained by static analysis for DMC, according to the values of the coefficient of determination for cross-validation, allows to distinguish between low, medium and high values for this parameter, while in the dynamic analysis of the product, the predictive capacity of the model can be considered as good. Nicolaï *et al.*[31] stated that a RPD_{cv} value of between 1.5 and 2 means that the model can discriminate low from high values of the response variable. It is important to note that this parameter is crucial as a measurement of ripening and is considered of vital importance for the pepper industry.

Ignat *et al.*[32] in bell peppers, using a diode array instrument (spectral range 477–950 nm) reported a predictive capacity (RPD_{cv} = 3.8) higher than those obtained here although these authors used a wider calibration set since they chose fruits picked during the growing season, from the 34th day after anthesis until full ripening (88th day after anthesis), and when fully grown. It should also be remembered that Lamuyo peppers show very irregular shapes and it is therefore more difficult to take NIR spectra of them than in bell peppers.

Sánchez *et al.*[23] also studied bell peppers, and analyzed them by taking spectra at the fruits' equatorial diameter using a portable manual instrument based on MEMS technology (MicroPhazir, spectral range 1600-2400 nm), obtaining predictive capacity models (RPD_{cv} = 1.64) similar to those obtained here for static analysis, which were slightly lower than those obtained for analysis by scanning the surface of the product.

For SSC, both analysis strategies enable to distinguish between high, medium and low values for this parameter[15,19] while according to Nicolai *et al.*[31] both models can discriminate between low and high values of SSC. Reid[33] pointed out the importance of measuring this parameter to determine the physiological maturity of fruit and vegetables.

Penchaiya *et al.*[34] used a diode array spectrophotometer (spectral range 780-1690 nm) to obtain models of predictive capacity (RPD_{cv} = 2.08) slightly higher than those of this research work. These authors used a wide range of sample attributes in the calibration set, obtained by random harvesting at various stages of ripeness. Ignat *et al.*[32], using the same instrument and the same spectral range as above, obtained models of predictive capacity (RPD_{cv} = 3.9) higher than ours; it is important to stress the greater variability of the fruits used, which also affected the dry matter parameter, as mentioned above.

Toledo-Martín *et al.*[35] using an instrument based on MEMS technology Phazir-1018 with a 1000-1800 nm spectral range, obtained models for SSC in 14 types of pepper with a predictive capacity (RPD_{cv} = 1.7) very similar to that obtained in this work, while Sánchez *et al.*[23] for bell peppers, obtained slightly lower values for SSC predictive capacity models (RPD_{cv} = 1.65) than those obtained in this work, using the same MEMS instrument for DMC.

Finally, it should be noted that the predictive capacity of the models obtained for TA by means of static and dynamic analysis allows to distinguish between low and high values for this parameter.[15,19] Toledo-Martín *et al.*[35] obtained models for TA in 14 types of pepper with a predictive capacity (RPD_{cv} = 1.4) similar to ours. Flores *et al.*[36] showed that the measurement of acidity-related parameters in intact fruits is notoriously difficult; nonetheless, the models developed for this parameter suggested that NIRS technology may be used for screening purposes.

Once the calibration equations for the analyzed parameters were developed for each of the modes of analysis tested, the SECV statistic values obtained for each parameter in the study were compared. As can be seen in Table 4, the SECV values corresponding to the color index parameter obtained with the MicroNIR^{TM} Pro 1700 in static mode are significantly lower ($P < 0.05$) than when the dynamic mode is used. For the rest of the parameters analyzed, no significant differences were found between the SECV values for the predictive models with the two modes of analysis tested.

Although it could be argued that initially the dynamic mode analysis may appear to result in a better fit, as it covered the whole area of the fruits analyzed and collected more information about it, the fact that the existence of lobes gives the peppers an irregular surface and that in the future, the analysis is likely to be carried out in the field, when the product is on the plant, means that it is better to take readings on the fruit statically during its development, hence the choice of this option for taking spectra in the field. In addition, it would make it easier for the producers to take spectra quicker and more comfortable. Thus, the static mode appears to be the most suitable for the analysis of the color index and quality parameters in Lamuyo pepper *in situ*, directly on the plant.

After comparison of both modes of analysis, and once the static mode was chosen, the models obtained with this analysis mode were externally validated, using a set comprising 29 samples (Fig. 3). It is important to point out that in the case of the color index (a*/b*), 2 samples which were initially part of the validation set, were eliminated before the validation procedure was carried out because they were hardly represented in the calibration set with which the predictive model were finally designed.

The models obtained to predict the parameters DMC and SSC met all the validation requirements established in the protocol established by Windham *et al.*[22], as well as the validation requirements in terms of the coefficient of determination for prediction, r^2_p ($r^2_p > 0.6$). Both the standard error of prediction corrected for bias ($SEP_{(c)}$) and the bias were within confidence limits, and so the models thus ensure an accurate prediction and can be applied routinely. However, for predicting the color index a*/b* and titratable acidity, the r^2_p and $SEP_{(c)}$ values do not comply with the validation protocol,[22] while in the case of bias, only the titratable acidity model complies. The results therefore suggest that these NIRS models produced could constitute an initial attempt to measure the maturity of Lamuyo peppers in the plant.

In addition, the external validation results obtained for SSC ($RPD_p = 2.1$) and TA ($RPD_p = 1.3$) are higher than those obtained by Toledo-Martín *et al.*[35] for SSC ($RPD_p = 1.8$) and TA ($RPD_p = 0.9$), respectively.

To evaluate the predictive ability of models in relation to the error of the reference method, the SEL values were calculated (Table 3) and compared with the SEPs. For the parameters a*/b* and DMC, the SEP values obtained were between 1-1.5 SEL, which means that the models developed have an excellent level of accuracy.[19,37] For SSC and TA, the SEP values obtained were 5 times higher than the SEL, so the accuracy of the models obtained can be considered low.[19,37] Nevertheless, it is important to stress that all the limits and values recommended in the scientific literature and mentioned above refer to other NIRS analysis conditions, i.e. using at-line instruments and using pre-dried and ground samples. In this study, models were developed *in situ* with a handheld portable instrument, using intact fruits with a high level of moisture. In this case, the comparison with the limits indicated may be too restrictive. It is also important to consider that whereas the reference values were obtained from the Lamuyo pepper juice, the spectra were taken from a specific point of the fruits. For this reason, it could be said that a sampling error occurred which was not included in the SEL values.

The regression coefficients for the best predictive models for color index (a*/b*), DMC, SSC and TA are illustrated in Fig. 4. These regression coefficients show significant importance for the region around at 980 nm, corresponding to water absorption[26] and at around 1170–1360 nm, which correspond to the second overtone of the C-H stretching bonds.[27] These results are similar to the ones obtained by Ignat *et al.*[38] for different cultivars of bell pepper during their ripening process.

CONCLUSIONS

The results suggest that Lamuyo peppers harvested when green were those which presented a lower content in DMC and SSC as well as lower values of TA, while those picked when red had significantly higher values of the 3 parameters analyzed.

The findings also confirm the expectations raised that NIRS technology can enable Lamuyo peppers to be harvested selectively according to their content in dry matter and in soluble solids using the MicroNIR^{TM} Pro 1700. Similarly, of the two strategies for taking spectra studied, the most suitable was the NIRS analysis in the statistic mode – taking of point spectra readings in the center of the surface of the peppers analyzed, without the instrument moving during the measurement –, which allows farmers to take spectra on peppers easily while they are growing on the plant.

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Table 1. Quality of Lamuyo peppers according to color at harvest

Coloration	Quality parameters		
	Dry matter content (%)	Soluble solid content (ºBrix)	Titratable acidity (% citric acid)
Green	6.81 (0.70) $^{(a)}$	5.94 (0.56) $^{(a)}$	0.14 (0.03) $^{(a)}$
Chocolate	7.28 (0.81) $^{(b,c)}$	6.65 (0.93) $^{(b)}$	0.20 (0.03) $^{(b)}$
Orange	6.93 (0.99) $^{(a,b)}$	6.65 (1.10) $^{(b)}$	0.25 (0.03) $^{(c)}$
Red	7.48 (1.05) $^{(c)}$	7.24 (0.94) $^{(c)}$	0.27 (0.04) $^{(d)}$

Standard deviation in brackets.

$^{(a)–(d)}$Means with different superscript letters in the same column differ significantly ($P < 0.05$).

Table 2. Statistical analysis of the calibration and validation sample sets, including number of samples (N), data ranges, means and standard deviations (SD) and coefficients of variation (CV)

Parameter	Set	N	Range	Mean	SD	CV (%)
Color index (a*/b*)	Calibration	111	-0.69-2.33	0.61	1.06	173.77
	Validation	29	-0.67-1.92	0.90	0.89	98.89
Dry matter content (%)	Calibration	111	4.81-11.23	7.25	0.90	12.41
	Validation	29	5.41-9.08	6.85	0.89	12.99
Soluble solid content (ºBrix)	Calibration	111	4.70- 9.30	6.74	0.97	14.39
	Validation	29	4.80-8.90	6.37	1.04	16.33
Titratable acidity (% citric acid)	Calibration	111	0.09-0.36	0.21	0.06	28.57
	Validation	29	0.10-0.34	0.22	0.06	27.27

Table 3. Calibration statistics for NIR-based models for predicting color and quality parameters in Lamuyo peppers

Parameter	Analysis mode	Math treatment	N	Range	Mean	SD	SECV	r^2_{cv}	RPD$_{cv}$	SEL
Color index (a*/b*)	Static	2,5,5,1	108	-0.69–2.33	0.62	1.07	0.72	0.55	1.49	0.78
	Dynamic	2,5,5,1	111	-0.69–2.33	0.61	1.06	0.88	0.32	1.20	
Dry matter content (%)	Static	1,10,5,1	107	4.81-8.92	7.24	0.78	0.48	0.63	1.63	0.37
	Dynamic	2,5,5,1	108	4.81–8.92	7.22	0.83	0.43	0.72	1.93	
Soluble solid content (ºBrix)	Static	1,10,5,1	111	4.70–9.30	6.74	0.98	0.56	0.68	1.75	0.10
	Dynamic	1,5,5,1	111	4.70–9.30	6.74	0.98	0.55	0.69	1.78	
Titratable acidity (% citric acid)	Static	2,5,5,1	109	0.09–0.36	0.21	0.06	0.04	0.45	1.50	0.01
	Dynamic	2,5,5,1	108	0.09–0.33	0.21	0.06	0.05	0.37	1.20	

N, number of samples; SD, standard deviation; SECV, standard error of cross-validation; r^2_{cv}, coefficient of determination for cross-validation; RPD$_{cv}$, ratio of the SD of the original data to SECV; SEL, standard error of laboratory

Table 4. Comparison between SECV values obtained for the best models for predicting color index and quality parameters of Lamuyo pepper using the static and dynamic modes of analysis tested; Fisher test (*P* < 0.05)

Parameter	Static mode		Dynamic mode		F	$F_{critical}$
	N	SECV	N	SECV		
Color index (a*/b*)	108	0.72	111	0.88	1.49*	1.37
Dry matter content (%)	107	0.48	108	0.43	1.25	1.38
Soluble solid content (ºBrix)	111	0.56	111	0.55	1.04	1.37
Titratable acidity (% citric acid)	109	0.04	108	0.04	1.00	1.37

*: Significant differences (*P* < 0.05).

N, number of samples; SECV, standard error of cross-validation.

Figure 1. Spectra acquisition procedure in Lamuyo pepper using the MicroNIR$^{\text{TM}}$ Pro 1700

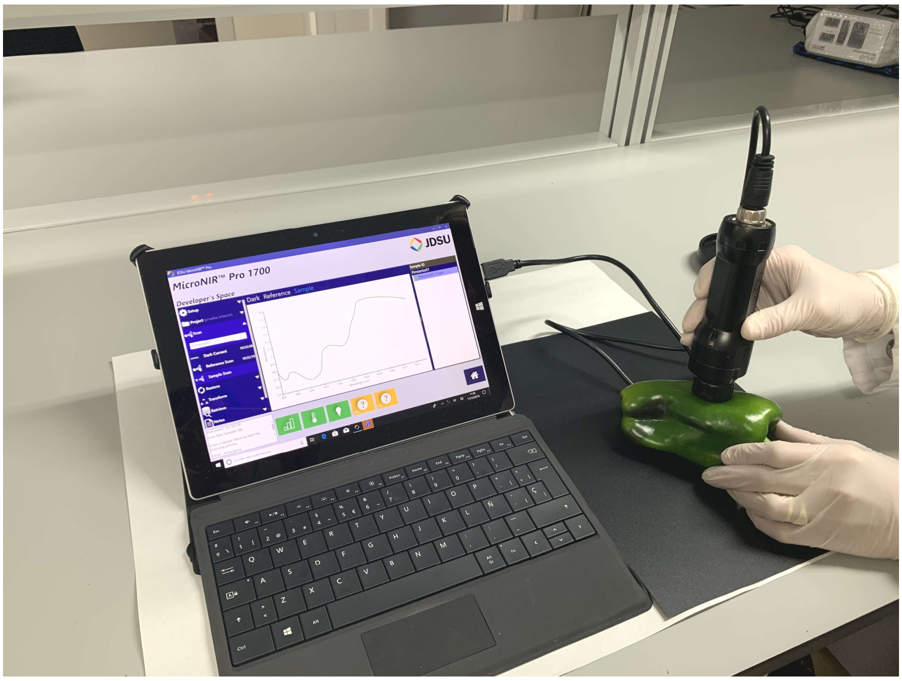

Figure 2. D_2log (1/R) spectra for Lamuyo peppers in different ripeness stages represented by the different colors (green, chocolate, orange and red) at harvest time

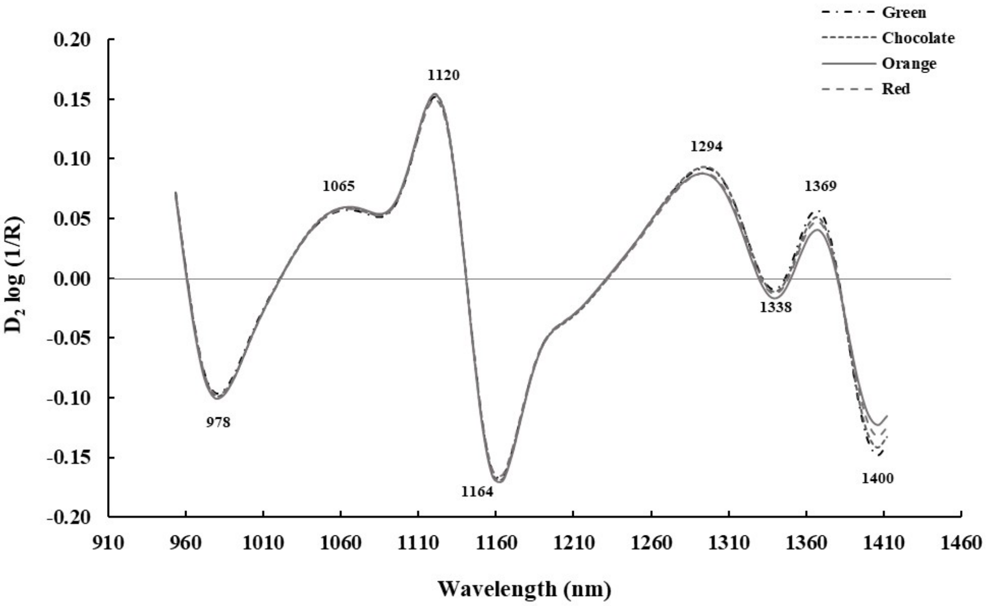

Figure 3. Reference *versus* NIR-predicted values for color and quality parameters in Lamuyo peppers. N, number of samples for the validation set; SEP, standard error of prediction; $SEP_{(c)}$, standard error of prediction corrected for bias; r^2_p, coefficient of determination for prediction; RPD_p, ratio of the SD to SEP.

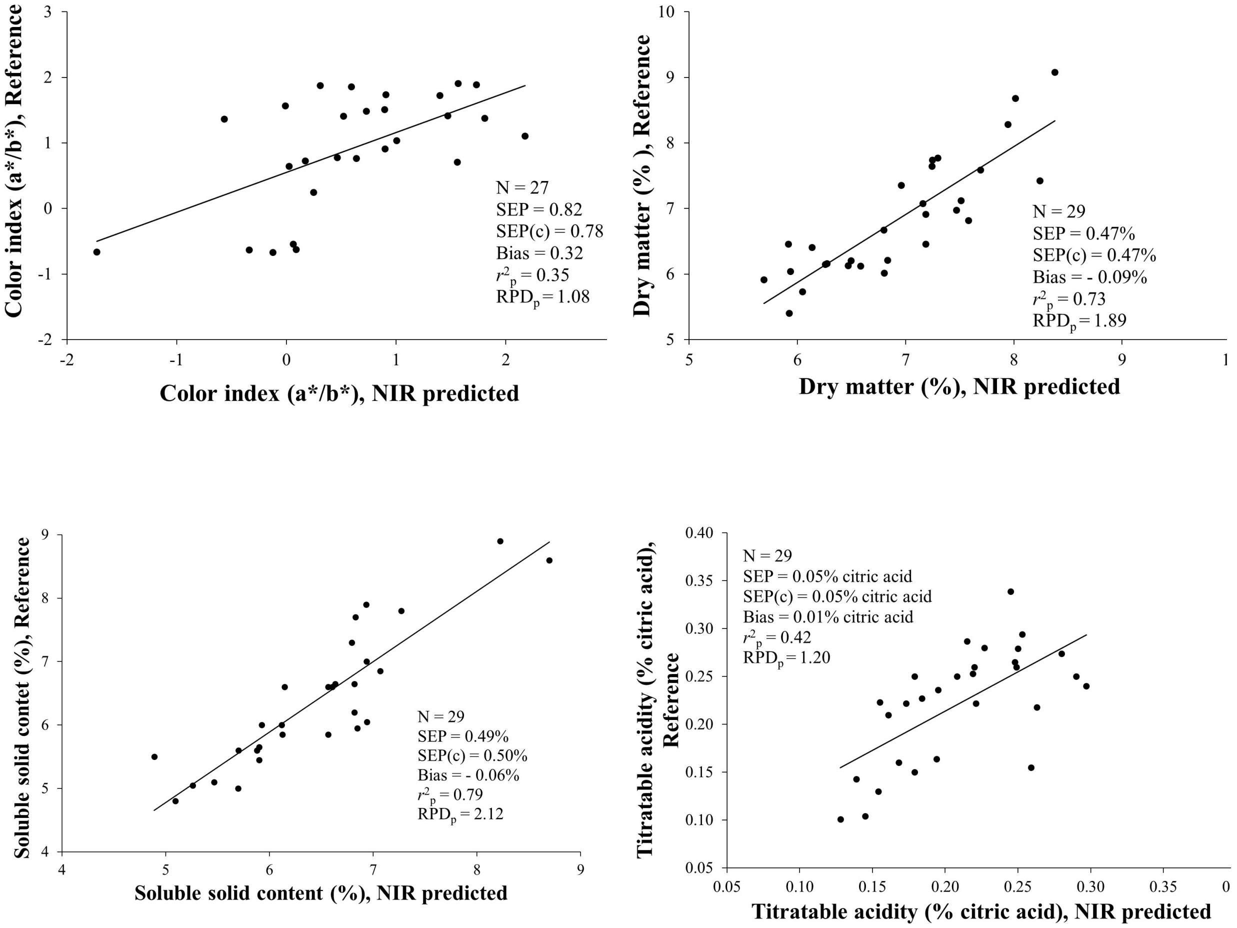

Figure 4. Regression coefficients for Lamuyo pepper color index (a*/b*), dry matter and soluble solid contents, and titratable acidity during on-vine ripening. * a.u.= arbitrary units