Simultaneous detection of quality and safety in spinach plants using a new generation of NIRS sensors

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Abstract

Near infrared (NIR) spectrophotometers require study of the spectral acquisition process, so that they can be used for quality and safety assessment of horticultural products. The aim of this work was to optimize the use of two NIR spectrophotometers for analysing spinach plants *in situ* and online: a manual, portable instrument based on Linear Variable Filter (LVF) technology (MicroNIR^{TM} 1700), suitable for analysis in the field, and during harvest and storage; and a Fourier Transform (FT)-NIR instrument (Matrix-F) suitable for the online analysis in the sorting lines. 195 spinach plants were used to predict the quality (texture, dry matter and soluble solid contents) and safety (nitrate content) parameters. Using the MicroNIR^{TM} 1700 to take 6 spectra per spinach leaf resulted in NIRS models of predictive capacity which enable to screen spinach plants *in situ* and decide on their industrial destination according to their nitrate content. For the Matrix-F instrument, a single spectrum taken online for the intact product (either moving or not) on the conveyor belt was sufficient to establish product quality and safety during industrial processing. The results also showed that the use of both instruments could form a complementary strategy for global monitoring, allowing spinach plants to be analysed throughout the food supply chain.

Keywords: Spinach plant; New generation NIRS sensors; NIRS analysis optimization; Quality and safety assessment.

1. Introduction

The commercial value of horticultural products depends on their quality, which can be defined as the sum of properties and characteristics that determine its marketability and shelf-life (Bruhn, 2002). However, fruit and vegetables constitute a unique class of food items in a sense that their size, colour, shape and physical-chemical composition vary, even when harvested at the same place and same time. Consequently, individual, non-destructive assessment is a key objective for these products (Huang et al., 2008; Lorente et al., 2012).

When assessing the eating quality and safety of spinach leaves, the main parameters to take into account are their texture, as well as dry matter content (DMC), soluble solid content (SSC) and nitrates (Jaworska et al., 2005; Conte et al., 2008; Gutiérrez-Rodríguez et al., 2013). These attributes depend not only on genotypic characteristics, but also on a number of other factors, including cultural practices, harvesting date and postharvest handling practices (Aked, 2000; Gutiérrez-Rodríguez et al., 2013).

NIR spectroscopy has become one of the most widely-used, flexible techniques for in-field measurements and online analysis on conveyor belts in the industry due to its swift response, precision, applicability to multiple products and analytes (Nicolaï et al., 2007; Saranwong and Kawano, 2007; Teixeira Dos Santos et al., 2013; Porep et al., 2015; Yan and Sisler, 2018).

Sánchez et al. (2018) and Pérez-Marín et al. (2019) demonstrated the feasibility of using NIRS technology for the *in situ* measurement of quality parameters (colour, firmness, DMC, SSC and ascorbic acid) and safety (nitrate content) in spinach using a handheld, near infrared device, the Phazir 2400, which is based on micro-electricomechanical system (MEMS) technology. However, technological development of NIRS instrumentation has resulted in phasing out of the original portable, handheld NIRS devices, such as the Phazir 2400. New portable micro-spectrophotometer devices based on LVF technology, which are characterized by their extremely small size and weight, as well as their excellent performance, due to the high-precision implementation of the key elements in their final device, are now available. The main goal is the successful use of these sensors to analyse the product directly in the field, in order to carry out the quality and safety monitoring of products in the field to facilitate real-time decision-making for crop management practices and harvest decisions. Similarly, these instruments can also be used by the industry for product evaluation during storage.

There are no reports in the literature about the use of NIRS instruments in spinach to classify it according to its destination, decided by the maximum level of nitrates legally permitted for the different processes (baby food production, preserved, deep-frozen or frozen spinach and fresh spinach) by the European Union (OJEU, 2011). Evaluation of NIRS for online analysis requires methodology of this analysis has to be established.

The objective of this study was to develop, evaluate and optimize a NIRS analysis methodology to assure quality and safety in spinach production along the food supply chain, *in situ* in the field and after harvest, and online during sorting using two new generation NIR spectrophotometers, one a handheld instrument based on LVF technology (MicroNIR™ 1700), suitable for the analysis of plants in the field and in storage, and another based on FT-NIR technology (Matrix-F), which can be incorporated in product sorting belts.

2. Materials and methods

2.1. Sampling and reference methods

A total of 195 spinach plants (*Spinacia oleracea* L. cv. 'Solomon', 'Novico', 'Meerkat' and 'Gorila'), grown outdoors on different farms in the provinces of Cordoba and Seville (Spain) were used in this study. The spinach plants were harvested during the months of January, February and March 2018.

Nitrate content and SSC were measured following Pérez-Marín et al. (2019) using between 4 and 10 spinach leaves from each plant, while texture, evaluated using the maximum puncture force (MPF) parameter - defined as the maximum force required to puncture the leaf - and DMC were measured following Sánchez et al. (2018), using a single leaf per plant. All measurements were performed in duplicate immediately after NIR spectrum collection (Pérez-Marín et al., 2019). The standard error of laboratory (SEL) was calculated from these duplicates (Table 4).

2.2. NIR spectrum acquisition

NIR spectra of spinach plants were collected using two instruments adapted to *in situ* and online applications, respectively.

A MicroNIR™ 1700 LVF spectrophotometer (VIAVI Solutions, Inc., San Jose, California, USA), designed for analysis *in situ*, was used in reflectance mode (log 1/R). This portable miniature spectrophotometer is extremely light (only 64 g, excluding the 150 g handle and the acquisition/data processing device). Its optical window measures around 227 mm^2, a 910 to 1676 nm spectral range, with a constant interval of 6.2 nm. 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™) with a 99% diffuse reflectance, while a dark reference was obtained from a fixed point in the room.

To measure MPF and DMC, four spectral measurements were taken on each spinach leaf in two locations (distal and proximal), on both sides (right and left) of the leaf blade relative to the main vein, on the adaxial side, with an average distance between measurements of 3 cm (Sánchez et al., 2018). The four spectra were averaged to provide a mean spectrum for each plant.

In those leaves used for measuring SSC and nitrate content, in addition to the 4 spectra per leaf previously mentioned (Pérez-Marín et al., 2019), two additional spectra were taken at the end of the blade/beginning of the petiole, one on each side of the main vein on the adaxial side, making a total number of 6 spectra per leaf. As between 4 and 10 leaves per plant were used for the chemical analyses of SSC and nitrates, a mean spectrum was obtained for these parameters from the six spectra for each leaf.

The online instrument used for the spectrum acquisition was the FT-NIR spectrophotometer Matrix-F (Bruker Optik GmbH, Ettlingen, Germany). This equipment was interfaced to a fibre optic NIR illumination and detection head containing a 10 mm diameter detector and two NIR light sources which illuminate a sample area around 153.94 cm^2. The scattered light was collected and guided via fibre optic cable (5 m in length) to the spectrometer. Furthermore, the system was equipped with a conveyor belt to move the sample, with the speed set at 15 kHz. Additionally, a distance of 10 cm between the instrument head and the conveyor belt was established, which remained constant throughout the process of taking spectra. The spectra were collected in reflectance mode in the spectral range from 4000 to 12000 cm^{-1} (834–2502.40 nm), with a resolution of 16 cm^{-1}. An internal white reference was also collected every thirty minutes.

Since a single leaf was used per plant to measure MPF and DMC, the NIR spectral acquisition was made when the conveyor belt had been stopped (static mode). Each spectrum was the mean of 16 scans and 2 spectra were taken per leaf, always on the adaxial side.

For SSC and nitrate content, online analysis was carried out with the conveyor belt in motion (dynamic mode), with 16 scans and 2 spectra taken per plant, always on the adaxial side of the leaf.

2.3. Optimization of the spectrum-taking procedure

Data pre-processing and chemometric treatments were performed using the WinISI II software package version 1.50 (Infrasoft International LLC, Port Matilda, PA, USA) (ISI, 2000).

Firstly, the optimum spectral range for both instruments (MicroNIR^{TM} 1700 and Matrix-F), after eliminating signal noise at the beginning and end of the spectrum, was selected. To achieve this, the 1,1,1,1 derivation treatment was applied (the first digit being the number of the derivative, the second the gap over which the derivative 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 (Hruschka, 2001).

In the case of the Matrix-F, once the best suitable spectral range had been selected, with optimization of the procedure of taking spectra in spinach plants in industrial sorting processes, two strategies were used to perform the chemometric analysis of the spectra obtained with this instrument:

1. Selecting at random a single spectrum per plant with Matlab v. 2017a (The Mathworks, Inc., Natick, Massachusetts, USA).
2. Using the average spectrum of the 2 spectra taken for each plant.

The first of the two established strategies used attempted to simulate the sorting processes of the spinach plants carried out in the industry and aimed at allowing to establish the viability of the full incorporation of NIRS technology in the processing lines. It is important to note that in the industry, the product travels along the classification conveyor belt only once, and that is when its quality and destination are measured and determined, depending on the levels of nitrates present.

The total number of spectra used for the development of the predictive models for the parameters analysed (MPF, DMC, SSC and nitrate content) was 195, regardless of the strategy followed (a single spectrum per plant or the average spectrum of the 2 spectra taken for each plant). NIRS calibration models for the parameters tested were developed using modified partial least squares (MPLS) regression (Shenk and Westerhaus, 1995a). Six cross validation steps were included in the process in order to avoid overfitting (Shenk and Westerhaus, 1995a).

For each analytical parameter, different mathematical pre-treatments were evaluated. For scatter correction, standard normal variate (SNV) and de-trending (DT) methods were tested (Barnes et al., 1989). Additionally, a total of two mathematical derivation treatments were tested: 1,5,5,1 and 2,5,5,1 (Shenk and Westerhaus, 1995b; ISI, 2000).

The statistics employed to select the best equations using MPLS were the coefficient of determination for cross validation (R^2_{cv}) and the standard error of cross validation (SECV) (Shenk and Westerhaus, 1996; Williams, 2001).

The SECV values for the best equations obtained for both strategies were compared using Fisher's F test (Massart et al., 1988; Naes et al., 2002). 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 cross validation of two different models and $SECV_1 < SECV_2$. F is compared to $F_{\mathrm{critical}\ (1-P,\ n1-1,\ n2-1)}$, as read from the table, with $P = 0.05$ and n_1 is the number of times the measurement is repeated with method 1, while 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.

2.4. Construction and validation of prediction models for the two instruments tested using a linear regression strategy

Once the optimal spectral ranges of both instruments and the optimum number of spectra per plant were established (Matrix-F), the CENTER algorithm was applied to ensure a structured population selection based solely on the spectral information, in order to establish the calibration and validation sets (Shenk and Westerhaus, 1991). This algorithm performs an initial principal component analysis to calculate the centre of the population and the distance of samples (spectra) from that centre in an n dimensional space, using the Mahalanobis distance (GH); samples with a GH value > 4 were considered spectral outliers. A combination of mathematical pre-treatments, SNV and DT was applied for scatter correction (Barnes et al., 1989), together with the 1,5,5,1 derivate mathematical treatment (Shenk and Westerhaus, 1995b; ISI, 2000). Once the spectral outliers had been removed and after ordering the sample sets by spectral distances (from smallest to greatest distance from the centre), three of every four were selected to be part of the calibration sets (C1 for nitrate content and SSC and C2 for DMC and MPF, the same for both instruments), while the validation sets were made up of the remaining 25 % (V1 for SSC and nitrate content and V2 for DMC and MPF) (Table 3). NIRS calibration models for the prediction of the four parameters tested were constructed with the calibration sets C1 and C2 using MPLS regression. The spectral pre-treatments were the same as those indicated in section 2.3. Lastly, the best models obtained for the calibration sets, selected by statistical criteria (Shenk and Westerhaus, 1996; Williams, 2001), were subjected to external validation using samples not involved in the calibration procedure (V1 for SSC and nitrate content and V2 for DMC and MPF), following the validation protocol outlined by Windham et al. (1989).

Due to the fact that the MicroNIR^{TM} 1700 instrument can be used both in the field and in the industry, a comparison was performed between the predictive models obtained for the parameters analysed, using the two instruments tested, as they could be used in a complementary manner in the industry – the Matrix-F for controlling the quality and safety of the spinach plants at sorting lines level and the MicroNIR^{TM} 1700 for checking the quality and safety of the spinach plants during storage. For this purpose, the residual predictive deviation for cross validation (RPD_{cv}) values, calculated as the ratio of the standard deviation (SD) of the reference data to the SECV values of the models obtained, were compared using Fisher's F test, as mentioned above.

3. Results and discussion

3.1. Optimal NIR spectral regions for the spectrophotometers tested

Before the prediction models were developed, both instruments were evaluated to establish the optimal spectral work region, so that representative, high-quality spectra could be obtained which would allow to construct robust models. This aspect is especially relevant for the Matrix-F, since with this equipment, the spectral signal is transmitted by fibre optics, which commonly produce a loss of signal quality on extreme wavelengths (Garrido-Varo et al., 2018; Torres et al., 2019). In the Matrix-F (Fig. 1A), the regions removed were those between 834-1251 nm and 2425-2502 nm. In the case of the MicroNIR^{TM} 1700 instrument, as shown in Fig. 1B, the full spectral range of the instrument was used.

3.2. Selection of the best spectrum capture strategy for online NIRS analysis of spinach plants with the Matrix-F instrument

Table 1 shows the statistical characteristics of the initial sample set for the four parameters analysed using the Matrix-F instrument. This set was used for the development of the initial prediction models which would allow to optimize the method of taking spectra online with this instrument.

Table 2 shows the SECV values of the best calibration models obtained using the Matrix-F instrument with different strategies for the number of spectra to be taken (1 and 2 spectra per plant analysed), for each of the parameters studied.

No significant differences were found for any of the parameters analysed between the SECV values of the predictive models developed for the different strategies tested. Therefore, in view of the results, and since, in the future, the Matrix-F instrument is likely to be incorporated in industry for the sorting lines, it is clear that the procedure of taking a single NIR spectrum per plant would be sufficient to measure online the quality and safety parameters of spinach plants tested. The results obtained agree with those reported by McCarthy and Kemeny (2008) and Torres et al. (2019), who showed that when using FT-NIR instruments, due to the improved signal/noise ratio in these instruments, a smaller number of spectra per analysed sample was sufficient for the measurement to yield relevant information.

3.3. Population characterization for quality and safety prediction of spinach plants

To obtain the same calibration and validation sets in both instruments, the samples considered as outliers (GH > 4) for the Matrix-F were removed for the portable equipment MicroNIR^{TM} 1700 and *vice versa*. In the group of samples used to measure the SSC and nitrate content parameters, 2 were considered spectral outliers, while in the group used for the DMC and MPF parameters, 4 were considered outliers.

A detailed study of the spectral outliers in the group of spectra used to measure SSC and nitrate content showed that the two samples considered as outliers presented a low nitrate content (below 315 mg kg^{-1}), as well as an atypical chromaticity, which could affect the representativeness of the spectra obtained. Likewise, for the group of spectra used to measure DMC and MPF, three of the outlier samples presented a DMC percentage of over 14.5 %. In the fourth sample, no physical-chemical differences were found which might account for the anomaly. After removing the outliers, the sets for the parameters tested were split into calibration (C1 = 146 samples and C2 = 144 samples) and validation (V1 = 47 samples and V2 = 47 samples), whose statistical characteristics are shown in Table 3.

This structured selection based wholly on spectral information proved suitable, in that the calibration and validation sets displayed similar values for range, mean and SD for all the study parameters.

Similarly, Table 3 shows that the parameter with the greatest variability is nitrate content ($CV_{calibration}$ = 64.30 %, $CV_{prediction}$ = 68.43 %). This variability is due to the different varietal behaviour in assimilating nitrates and the heterogeneity in the level of fertilization carried out on the different farms, as well as the fact that the samples were collected throughout the harvesting period, in which the level of nitrates progressively decreases. SSC, DMC and MPF all show a lower variability of between 18-30%, which could be explained by the fact that all the spinach plants tested were collected at the stage of commercial maturity.

3.4. Prediction of quality and safety parameters using MPLS regression and NIR spectra

Table 4 shows the results of the best prediction models obtained for each parameter analysed (nitrate content, SSC, DMC and MPF) for both instruments using different pre-treatments of the spectral signal.

For the nitrate content, in the case of the MicroNIR^{TM} 1700 instrument, the models allow differentiation between high, medium and low values, while the models developed with the Matrix-F only allow differentiation between high and low values (Shenk and Westerhaus, 1996, Williams, 2001).

If this parameter is measured in a non-destructive way both in the field and after harvest, such as in the sorting lines, it would allow to make a first screening of the product, by which those plants with a nitrate content below 200 mg kg^{-1} could be used in the production of baby foods (OJEU, 2011).

There are few scientific references available on the use of NIRS technology to measure nitrates in spinach, and all of these used suitable NIRS instruments exclusively to analyse the product *in situ*. Itoh et al. (2011) measured the nitrate content in spinach plants, using the FANTEC NIR Gun instrument working on transmittance mode in a spectral range of 600-1100 nm, obtaining values of $RPD_p = 2.14$ and 2.17 with the PCR and PLS regressions, respectively, which are higher than those obtained in this study. However, the size and characteristics of the sample group, the form of measurement and the optical characteristics and range of the instrument are significantly different from those used in this study. Pérez-Marín et al. (2019) also used a Phazir 2400 based on MEMS technology, in the spectral range 1600-2400 nm, to obtain values of $RPD_{cv} = 1.29$, which in that case were slightly lower than those obtained here, both for the *in situ* and the online analysis.

For SSC, which is a crucial parameter when choosing the optimum time for harvesting, for measuring the shelf-life of spinach and for classifying the product in the industry, the predictive capacity of the models obtained with the two instruments tested can be considered to be good when interpreting the coefficient of determination, as proposed by Shenk and Westerhaus (1996) and Williams (2001), while Nicolaï et al. (2007) stated that a RPD_{cv} value of between 2 and 2.5 indicates that coarse quantitative predictions are possible (Matrix-F) and a RPD_{cv} value between 2.5 and 3 corresponds to good prediction accuracy (MicroNIR^{TM} 1700).

Perez-Marín et al. (2019) using the instrument Phazir 2400 for the *in situ* analysis of the spinach plants, obtained models of predictive capacity (RPD_{cv} =2.54) similar to that obtained here (RPP_{cv} = 2.62) with the MicroNIR^{TM} 1700 instrument, which is also suitable for the *in situ* analysis of the product.

For DMC, the best model developed with the MicroNIR^{TM} 1700 showed a predictive capacity that can be considered as good, while the best model developed with the Matrix-F was able to distinguish between high, medium and low values (Shenk and Westerhaus, 1996; Williams, 2001). Nicolaï et al. (2007) indicated that the RPD$_{cv}$ between 1.5 and 2 means that the model can discriminate between low and high values of the response variable.

Conte et al. (2008) showed the importance of the analysis of this parameter in spinach plants for growers and also for postharvest, since DMC values of around 10-12 % ensure a good resistance to handling and allow a high visual quality to be maintained during storage.

Sánchez et al. (2018), obtained similar results using the handheld MEMS spectrophotometer Phazir 2400 for DMC (RPD$_{cv}$ = 1.96) to those found in this work (RPD$_{cv}$ = 1.83) when analysing spinach plants *in situ*.

For MPF, the predictive capacity of the models developed with the micro-spectrophotometer allowed differentiation between high, medium and low values, while the FT-NIR instrument only allowed to distinguish between high and low values (Shenk and Westerhaus, 1996; Williams, 2001).

Sánchez et al. (2018), who used the Phazir 2400, obtained slightly higher results for MPF (RPD$_{cv}$ = 1.72) than those obtained in this study (RPD$_{cv}$ = 1.44) with the MicroNIR^{TM} 1700. This difference can be attributed to the fact that those authors used calibration groups with a wider variability (CV = 65.15 %) than those used here.

Finally, it is important to note that for the nitrate content and SSC parameters, the analysis with the Matrix-F instrument was performed in dynamic mode (with the conveyor belt in movement), which means that these results are of particular interest to the industry, since they reinforce the potential use of this equipment as a tool to measure safety and quality parameters in moving production lines.

3.5. Comparison between the best models developed with the Matrix-F and MicroNIR^{TM} 1700 instruments.

Table 4 includes the F values obtained from the comparison between the RPD_{cv} of each spectrophotometer. For the nitrate content, no significant differences between the RPD_{cv} values were detected, although the highest RPD_{cv} value was found with the MicroNIR^{TM} 1700. It is important to take into consideration that the MicroNIR^{TM} 1700 took a greater number of spectra for this parameter (6 spectra * number of leaves per plant) than the Matrix-F (1 spectrum per plant), and that two of these 6 were specifically taken in the petiolar area of the leaf, which has the greatest nitrate accumulation (Qiu et al., 2014).

It is also important to note that with the portable equipment, the measurement is taken with the head in direct contact with the blade and that the analysis was carried out in static mode, without the sample moving; while in contrast, with the Matrix-F instrument, the spectra were taken with the plants in motion and a separation of 10 cm between the head and the sample. These aspects should be taken into account when creating robust models in the case of highly complex parameters such as nitrate content.

The results obtained are of particular interest to the industry, as NIRS technology could be carried out online in the sorting lines and *in situ* in cold chambers as a routine method of analysis, in order to measure not only parameters associated with quality, but also those associated with safety, such as nitrate content. However, for future work, a much larger number of samples must be provided to develop more robust calibrations with both instruments.

For SSC, the highest values were obtained with the MicroNIR^{TM} 1700, while for DMC and MPF, differences between the RPD$_{cv}$ values were not significant.

3.6. External validation

Validation statistics for the prediction of the safety and quality parameters using the Matrix-F and the MicroNIR^{TM} 1700 are shown in Fig. 2.

Some samples (1 sample for nitrate content and 3 samples for MPF with Matrix-F; 3 samples for MPF with MicroNIR^{TM} 1700), which were initially part of the V1 and V2 validation sets, were eliminated before the validation procedure since they were barely represented in the calibration sets with which the predictive models were designed.

As regards the prediction of nitrate content, 3 samples were predicted by the models, with negative values assigned for this parameter. However, the predictive NIRS values for these samples were shown as zero (Fig. 2). According to the validation protocol established by Windham et al. (1989) and once the results shown in Fig. 2 were analysed, the models constructed for predicting SSC in intact spinach with both instruments, and DMC with MicroNIR^{TM} 1700, met the validation requirements in terms of the coefficient of determination for prediction, R^2_p ($R^2_p > 0.6$), and both the standard error of prediction corrected for bias (SEP$_{(c)}$) and the bias were within confidence limits: the equations thus ensure accurate prediction, and can be applied routinely.

For the rest of the parameters analysed, for both instruments, the models developed did not attain the recommended minimum value of 0.60 for R^2_p. However, it should be stressed that for nitrate content with the MicroNIR^{TM} 1700 and for DMC with the Matrix-F, they were close ($R^2_p = 0.51$ and $R^2_p = 0.55$ respectively). Moreover, the SEP(c) and bias lay within the confidence limits. The equations can therefore be taken as an initial approximation to the measurement both *in situ* and online of quality and safety parameters in intact spinach.

In general, the standard error of prediction (SEP) is considered a valuable statistical parameter to evaluate the predictive capacity of an equation, and it is widely accepted that an SEP value of less than 2*SEL shows that the model has an excellent predictive capacity (Westerhaus, 1989; Williams, 2001). The SEL values for the parameters analysed in this work are shown in Table 4. For both instruments and for DMC and MPF parameters, the SEP values were between 1 and 2, show excellent predictive capacity of the NIRS models.

The SEP values for the nitrate content and SSC using both devices are much higher than the measured SEL values, which shows a low predictive capacity of the models (Westerhaus, 1989; Williams, 2001). However, when interpreting the low SEL values for SSC and nitrate content in comparison with the SEP values obtained for the prediction, it should be taken into account that the reference value has been obtained by liquefying all the analysed leaves. For this reason, a sampling error was not included in the SEL value. Nevertheless, it is important to stress that all the limits and values recommended in the literature and mentioned above refer to other NIRS analysis conditions, e.g. using at-line instruments and using pre-dried and ground samples. In this study, models were developed with portable or online instruments, using intact and complex samples with a high level of moisture and a high perishable character. In this case, the comparison with the limits indicated may be too restrictive.

4. Conclusions

The results obtained showed the feasibility of NIRS technology for measuring DMC and SSC in spinach plants along the food supply chain using two new generation instruments. Additionally, both instruments were able to give accurate information about high and low levels of nitrate content, allowing to establish the industrial destination of this vegetable, and also about texture – degree of firmness – which is usually associated with freshness, the retention of good quality in the spinach plant and its final saleability.

The Matrix-F instrument is ideally suited for online measurements. The results showed that a single spectrum of the spinach leaves taken when the product is on the sorting belts in static or dynamic mode would be sufficient to establish product quality and safety, which would facilitate the incorporation of this NIR instrument in the processing industries of horticultural products.

For the MicroNIR^{TM} 1700, taking 6 spectra per leaf, including 2 spectra taken on the petiole of the leaf, is suitable for measuring nitrates, both in the field and after harvest. For industry, the blades and the petioles are processed together, and the largest accumulation of nitrates occurs in the petioles, which serve to determine the industrial use of the spinach leaves (baby food, preserved, deep-frozen or frozen spinach, or fresh spinach).

Finally, it must be mentioned the importance of optimization of the new generation NIR instruments before their use for *in situ* and online analysis. The two instruments tested here can be used in a complementary way: the MicroNIR^{TM} 1700 for the analysis of spinach plants while they are growing in the field, during and after harvest, and the Matrix-F for quality and safety control of the product on the conveyor belts, allowing the monitoring of product along the food supply chain.

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References

Aked, J., 2000. Fruits and vegetables. In: Kilcast, D., Subramaniam, P. (Eds.), The Stability and Shelf-life of Food. Woodhead Publishing Ltd. and CRC Press LLC, Boca Raton, Florida, pp. 249–278.

Barnes, R.J., Dhanoa, M.S., Lister, S.J., 1989. Standard normal variate transformation and de-trending of near infrared diffuse reflectance spectra. Appl. Spectrosc. 43, 772–777. https://doi.org/10.1366/0003702894202201.

Bruhn, C.M., 2002. Consumer issues in quality and safety: Definition and evaluation for fresh horticultural crops. In: Kader, A.A. (Ed.), Postharvest Technology of Horticultural Crops, 3rd ed. Division of Agriculture and Natural Resources, University of California, Oakland, CA, pp. 31–37.

Conte, A., Conversa, G., Scrocco, C., Brescia, I., Laverse, J., Eliba, A., Nobile, M.A.D., 2008. Influence of growing periods on the quality of baby spinach leaves at harvest and during storage as minimally processed produce. Postharvest Biol. Technol. 50, 190–196. https://doi.org/10.1016/j.postharvbio.2008.04.003.

Garrido-Varo, A., Sánchez-Bonilla, A., Maroto-Molina, F., Riccioli, C., Pérez-Marín, D., 2018. Long-length fiber optic near-infrared (NIR) spectroscopy probes for online quality control of processed land animal proteins. Appl. Spectrosc. 72, 1170–1182. https://doi.org/10.1177/0003702817752111.

Gutiérrez-Rodríguez, E., Lieth, H.J., Jernstedt, J.A., Labavitch, J.M., Suslow, T.V., Cantwell, M.I., 2013. Texture, composition and anatomy of spinach leaves in relation to nitrogen fertilization. J. Sci. Food Agr. 93, 227–237. https://doi.org/10.1002/jsfa.5780.

Huang, H., Yu, H., Xu, H., Ying, Y., 2008. Near infrared spectroscopy for on/in-line monitoring of quality in foods and beverages: A review. J. Food Eng. 87, 303–313. https://doi.org/10.1016/j.jfoodeng.2007.12.022.

Hruschka, W.R., 2001. Data analysis: Wavelength selection methods. In: Williams, P.C., Norris, K.H. (Eds.), Near-Infrared Technology in the Agricultural and Food Industries. American Association of Cereal Chemists, Inc., St. Paul, MN, pp. 39–58.

ISI, 2000. The Complete Software Solution Using a Single Screen for Routine Analysis, Robust Calibrations and Networking. Manual, FOSS NIRSystems/Tecator. Infrasoft International, Silver Spring, MD.

Itoh, H., Tomita, H., Uno, Y., Naomasa, S., 2011. Development of method for non-destructive measurement of nitrate concentration in vegetable leaves by near-infrared spectroscopy. IFAC Proceedings Volumes 44, 1773–1778. https://doi.org/10.3182/20110828-6-IT-1002.00738.

Jaworska, G., 2005. Content of nitrates, nitrites, and oxalates in New Zealand spinach. Food Chem. 89, 235–242. https://doi.org/10.1016/j.foodchem.2004.02.030.

Lorente, D., Aleixos, N., Gómez-Sanchis, J., Cubero, S., García-Navarrete, O.L., Blasco, J., 2012. Recent advances and applications of hyperspectral imaging for fruit and vegetable quality assessment. Food Bioprocess Tech. 5, 1121–1142. http://dx.doi.org/10.1007/s11947-011-0725-1.

Massart, D.L., Vandeginste, B.G.M., Deming, S.M., Michotte, Y., Kaufman, L., 1988. Chemometrics: A Textbook. (Data Handling in Science and Technology 2). Elsevier Science, Amsterdam, The Netherlands.

McCarthy, W.J., Kemeny, G.J., 2008. Fourier Transform spectrophotometers in the Near-Infrared. In: Burns, D.A., Ciurczak, E.W. (Eds.), Handbook of Near-Infrared Analysis. CRC Press, Boca Raton, Florida, pp. 79–93.

Naes, T., Isaksson, T., Fearn, T., Davies, A., 2002. A User-Friendly Guide to Multivariate Calibration and Classification. NIR Publications, Chichester, UK.

Nicolaï, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K.I., Lammertyn, J., 2007. Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: a review. Postharvest Biol. Technol. 46, 99–118. https://doi.org/10.1016/j.postharvbio.2007.06.024.

Official Journal of the European Union (OJEU), 2011. Commission Regulation (EC) No 1258/2011 of 2 December 2011 Amending Regulation (EC) No 1881/2006 as Regards Maximum Levels for Nitrates in Foodstuffs. OJ L 320/15-17, 3.12.2011.

Pérez-Marín, D., Torres, I., Entrenas, J.A., Vega, M., Sánchez, M.T., 2019. Pre-harvest screening on-vine of spinach quality and safety using NIRS technology. Spectrochim. Acta A Mol. Biomol. Spectrosc. 207, 242–250. https://doi.org/10.1016/j.saa.2018.09.035.

Porep, J.U., Kammerer, D.R., Carle, R., 2015. Online application of near infrared (NIR) spectroscopy in food production. Trends Food Sci. Tech. 46, 211–230. https://doi.org/10.1016/j.tifs.2015.10.002.

Qiu, W., Wang, Z., Huang, C., Chen, B., Yang, R., 2014. Nitrate accumulation in leafy vegetables and its relationship with water. J. Soil Sci. Plant Nutr. 14, 761–168.

Sánchez, M.T., Entrenas, J.A., Torres, I., Vega, M., Pérez-Marín, D., 2018. Monitoring texture and other quality parameters in spinach plants using NIR spectroscopy. Comput. Electron. Agric. 155, 446–452. https://doi.org/10.1016/j.compag.2018.11.004

Saranwong, S., Kawano, S., 2007. Applications to agricultural and marine products: fruits and vegetables. In: Ozaki, Y., McClure, W.F., Christy, A.A. (Eds.), Near-Infrared Spectroscopy in Food Science and Technology. John Wiley & Sons, Inc., NJ, pp. 219–242.

Shenk, J.S., Westerhaus, M.O., 1991. Population structuring of near infrared spectra and modified partial least squares regression. Crop Sci. 31, 1548–1555. https://doi.org/10.2135/cropsci1991.0011183X003100060034x.

Shenk, J.S., Westerhaus, M.O., 1995a. Analysis of Agriculture and Food Products by Near Infrared Reflectance Spectroscopy. Monograph. NIRSystems Inc., Silver Spring, MD.

Shenk, J.S., Westerhaus, M.O., 1995b. Routine Operation, Calibration, Development and Network System Management Manual. NIRSystems Inc., Silver Spring, MD.

Shenk, J.S., Westerhaus, M.O., 1996. Calibration the ISI way. In: Davies, A.M.C., Williams, P.C. (Eds.), Near Infrared Spectroscopy: The Future Waves. NIR Publications, Chichester, pp. 198–202.

Teixeira dos Santos, C.A., Lopo, M., Páscoa, R.N.M.J., Lopes, J.A., 2013. A review on the applications of portable near-infrared spectrometers in the agro-food industry. Appl. Spectrosc. 67, 1215–1233. https://doi.org/10.1366/13-07228.

Torres, I., Sánchez, M.T., Entrenas, J.A., Garrido-Varo, A., Pérez-Marín, D., 2019. Monitoring quality and safety assessment of summer squashes along the food supply chain using near infrared sensors. Postharvest Biol. Technol. 154, 21–30. https://doi.org/10.1016/j.postharvbio.2019.04.015.

Westerhaus, M.O., 1989. Interpretation of regression statistics. In: Marten, G.C., Shenk, J.S., Barton II, F.E. (Eds.), Near Infrared Spectroscopy (NIRS): Analysis of Forage Quality. Agriculture Handbook nº643. USDA-ARS, Government Printing Office, Washington, DC, pp. 39.

Williams, P.C., 2001. Implementation of near-infrared technology. In: Williams, P.C., Norris, K.H. (Eds.), Near-Infrared Technology in the Agricultural and Food Industries. AACC, Inc., St. Paul, MN, pp. 145–171.

Windham, W.R., Mertens, D.R., Barton II, F.E., 1989. Protocol for NIRS calibration: sample selection and equation development and validation. In: Martens, G.C., Shenk, J.S., Barton II, F.E. (Eds.), Near Infrared Spectroscopy (NIRS): Analysis of Forage Quality. Agriculture Handbook nº643. USDA-ARS, Government Printing Office, Washington, DC, pp. 96-103.

Yan, H., Siesler, H.W., 2018. Hand-held near-infrared spectrometers: State-of-the-art instrumentation and practical applications. NIR news, 29, 8–12. https://doi.org/10.1177/0960336018796391.

Table 1

Number of samples (N), range, mean, standard deviation (SD), and coefficient of variation (CV) for the initial set for nitrate, soluble solid and dry matter contents, and maximum puncture force for the Matrix-F instrument.

	Nitrate content (mg kg^{-1})	Soluble solid content (%)	Dry matter content (%)	Maximum puncture force (N)
N	195	195	195	195
Range	67.00-3844.83	4.10-11.45	4.10-19.12	1.03-4.57
Mean	1340.50	7.81	11.42	2.11
SD	887.46	1.61	2.47	0.61
CV (%)	66.20	20.61	21.63	28.91

Table 2

Comparison between SECV values for the best calibration models for nitrate, soluble solid and dry matter contents, and maximum puncture force obtained using the Matrix-F and collecting a different number of spectra per sample; Fisher test ($P < 0.05$).

Parameter	[a] **SECV** **1 spectrum**	**SECV** **2 spectra**	**F**	$\mathbf{F_{critical}}$
Nitrate content (mg kg^{-1})	723.08	741.2	1.05	1.27
Soluble solid content (%)	0.83	0.89	1.15	1.27
Dry matter content (%)	1.64	1.59	1.06	1.27
Maximum puncture force (N)	0.48	0.47	1.04	1.27

[a] Standard error of cross validation

Table 3

Number of samples (N), range, mean, standard deviation (SD), and coefficient of variation (CV) for the different calibration (C1 and C2) and validation (V1 and V2) sets for nitrate, soluble solid and dry matter contents, and maximum puncture force in spinach plants

	Nitrate content (mg kg^{-1})		Soluble solid content (%)		Dry matter content (%)		Maximum puncture force (N)	
	C1	V1	C1	V1	C2	V2	C2	V2
N	146	47	146	47	144	47	144	47
Range	67.00-3844.83	98.00-3243.15	4.10-11.45	4.90-11.30	4.10-19.12	4.30-16.03	1.03-4.57	1.17-3.30
Mean	1405.16	1185.93	7.74	7.89	11.54	10.90	2.16	1.97
SD	903.58	811.54	1.64	1.46	2.53	2.11	0.64	0.49
CV (%)	64.30	68.43	21.19	18.50	21.92	19.36	29.63	24.87

Table 4

Calibration statistics for NIR-based models for predicting nitrate, soluble solid and dry matter contents, and maximum puncture force in spinach plants.

Parameter	Instrument	Math treatment	[a] N	Range	[b] Mean	[c] SD	[d] SECV	[e] R^2_{cv}	[f] RPD$_{cv}$	F	F$_{critical}$	[g] SEL
Nitrate content (mg kg^{-1})	Matrix-F	1,5,5,1	143	67.00-3844.83	1429.18	897.42	676.14	0.44	1.33	1.12	1.32	23.90
	MicroNIR™ 1700	2,5,5,1	143	67.00-3844.83	1430.11	896.22	633.73	0.50	1.41			
Soluble solid content (%)	Matrix-F	1,5,5,1	138	4.10-11.15	7.66	1.61	0.72	0.80	2.24	1.37*	1.32	0.10
	MicroNIR™ 1700	1,5,5,1	142	4.10-11.45	7.73	1.65	0.63	0.85	2.62			
Dry matter content (%)	Matrix-F	1,5,5,1	140	5.92-17.72	11.52	2.36	1.40	0.65	1.69	1.17	1.32	1.67
	MicroNIR™ 1700	1,5,5,1	138	5.92-17.27	11.47	2.32	1.27	0.70	1.83			
Maximum puncture force (N)	Matrix-F	1,5,5,1	140	1.03-3.43	2.12	0.59	0.44	0.44	1.34	1.15	1.32	0.36
	MicroNIR™ 1700	1,5,5,1	140	1.03-3.77	2.12	0.59	0.41	0.52	1.44			

[a] Number of samples.

[b] Mean of the calibration set.

[c] Standard deviation of the calibration set.

[d] Standard error of cross validation.

[e] Coefficient of determination of cross validation.

[f] Residual predictive deviation for cross validation.

[g] Standard error of laboratory.

Fig. 1. D_1 log (1/R) spectra for spinach samples. Instruments: Matrix-F (A) and MicroNIR^{TM} 1700 (B).

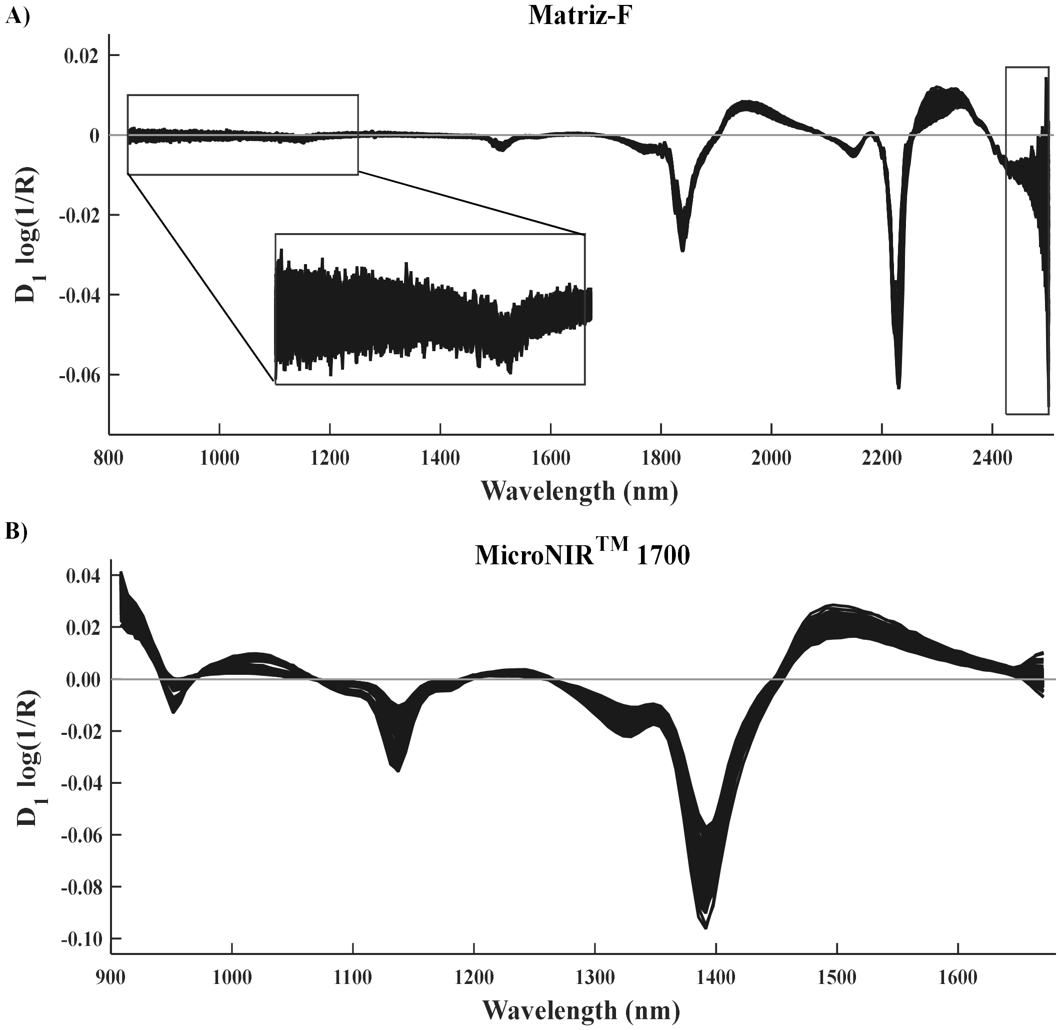
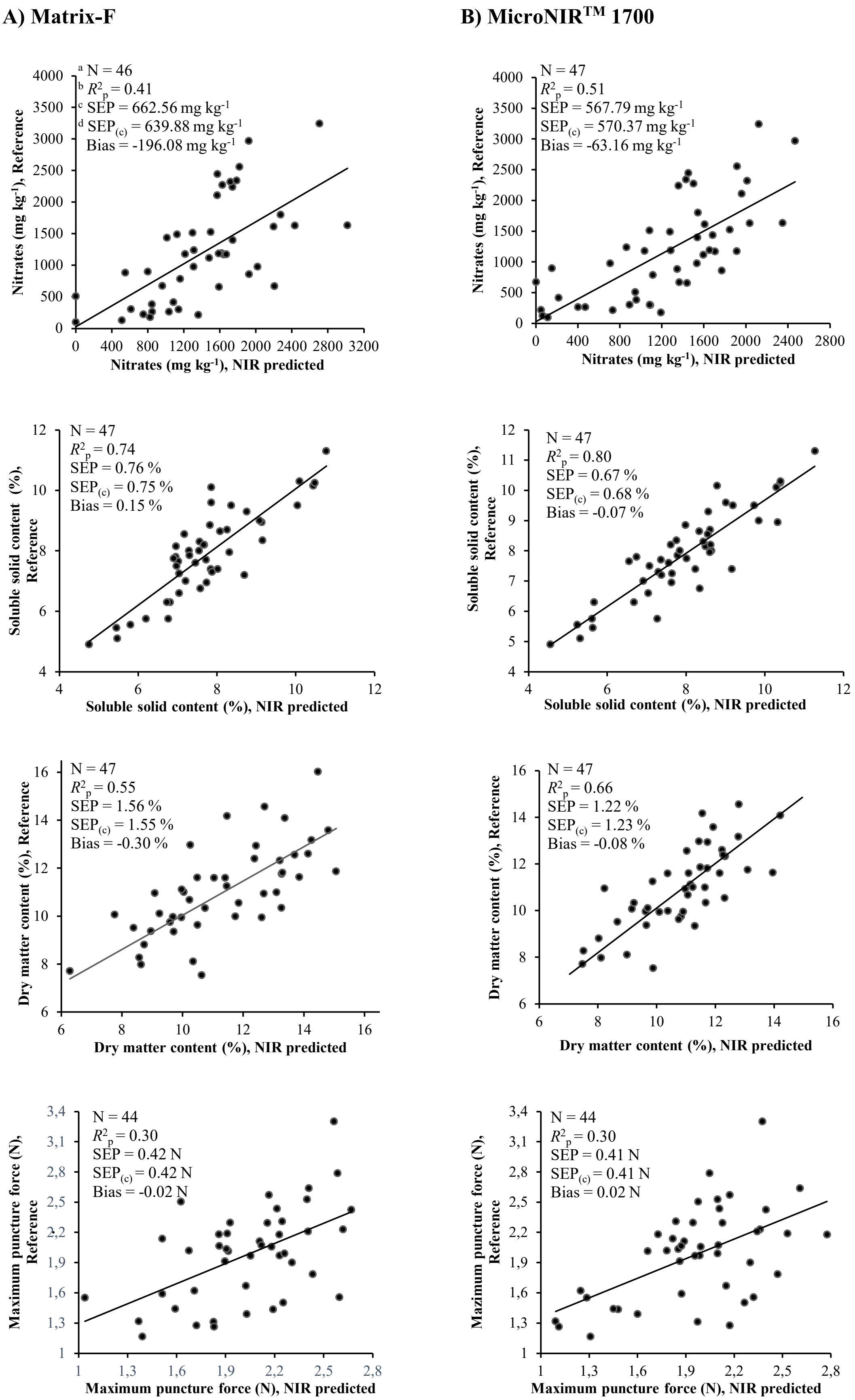


Fig. 2. Reference and NIR predicted values for quality and safety parameters with Matrix-F (A) and MicroNIR^{TM} 1700 (B) instruments.



A) Matrix-F

B) MicroNIR^{TM} 1700

[a] Number of samples for the validation set

[b] Coefficient of determination of prediction.

[c] Standard error of prediction.

[d] Standard error of prediction corrected for bias.