Integrated soluble solid and nitrate content assessment of spinach plants using portable NIRS sensors along the supply chain.

Irina Torres[a], María-Teresa Sánchez[a,*], Dolores Pérez-Marín[b,*]

[a] *Department of Bromatology and Food Technology, University of Cordoba, Rabanales Campus, 14071 Córdoba, Spain.*

[b] *Department of Animal Production, University of Cordoba, Rabanales Campus, 14071 Córdoba, Spain.*

**Corresponding authors*. Tel.: +34 957 212576; fax: 34 957 212000

E-mail addresses: teresa.sanchez@uco.es (M.T. Sánchez) or dcperez@uco.es (D. Pérez-Marín).

Abstract

There has been increased interest in the implementation of near infrared spectroscopy (NIRS) as a non-destructive analytical technique to monitor the quality and safety of vegetables during their growing season and after harvest throughout the food supply chain. The aim of this work was to evaluate the feasibility of using a portable NIR spectrophotometer (the MicroNIR^{TM} Pro 1700 (spectral range 908–1676 nm) working in reflectance mode) based on Linear Variable Filter (LVF) technology to analyse soluble solid content (SSC) and nitrate content in spinach plants *in situ,* in the field and during the supply chain. A total of 77 spinach plants were analysed at three control points of the supply chain: 1) in the field, during the growing season and after harvest, 2) in the lab, simulating conditions at receipt at the processing industry and 3) on the leaves in the lab, after washing, thus simulating the analysis of the processed product ready to be packaged, as a previous step for the novel application of NIRS at delivery points and in the supermarkets. The results confirmed the feasibility of using the spectrophotometer throughout the supply chain to establish product quality and safety, which would allow to make real-time decisions related to the agricultural practices, optimum harvest time, industrial uses and commercial shelf-life. The comparison between the models developed for the NIRS analysis in the three control points studied indicated that the recommended procedure would be to take a single spectrum per plant as a suitable way of predicting quality and safety parameters in the field and at the reception points in the industry. Two spectra on each of the two leaves should be taken after the washing operation in the industry, with values of the standard error of cross validation of 1.0 % for SSC and 766 mg kg^{-1} for nitrate content.

Keywords: Spinach; supply chain control; NIRS; quality and safety assessment; real-time decision making

1. Introduction

There is an increasing need for the productive sector and the food industry to provide information on their products and production processes to satisfy quality standards and to guarantee the safety of the products that reach the consumers.

Near infrared spectroscopy (NIRS) sensors, which combine fast spectrum acquisition, accurate measurement, versatility, simplicity of sample presentation and low cost, provide a unique digital signal of each product analysed and enable non-destructive analysis of the product. They have shown great potential for monitoring quality and safety and for ensuring traceability in horticultural products (Nicolaï et al., 2007; Sánchez and Pérez-Marín, 2011; Cortés et al., 2019; Cattaneo and Stellari, 2019).

In addition, the characteristics of NIRS sensors make them highly suitable for establishing an integrated control system for horticultural products along the supply chain, i.e. from the field to the market. Incorporation of these sensors along the food supply chain could be favoured by the development of portable, compact and light-weight instruments, ideally suited for use not only in the field but also at an industrial level (Pasquini, 2018; Yan and Siesler, 2018). However, the implementation of a new generation of NIRS sensors for quality and safety monitoring of a particular horticultural product requires testing, in-depth study and a previous simulation of the conditions under which the sensor would be used.

In the case of spinach, a high perishable vegetable with a commercial shelf-life of about two weeks, it is essential to monitor and control soluble solid content (SSC), related with optimum harvest quality (Reid, 2002; Conte et al., 2008) and nitrate content, linked with the lighting received by the leaves and certain agricultural practices (mainly nitrogen fertilization) and related to the safety of the product (Anjana and Iqbal, 2007). Although high doses of this nutrient favour crop growth and produce more vigorous plants (Wang and Li, 2004), nitrate accumulation, which is common in leafy vegetables such as spinach plants, affects food safety, since high levels of nitrates can have detrimental effects on human health (Jaworska et al., 2005). Additionally, the nitrate content determines the industrial use of this vegetable after harvest (OJEU, 2011), as it determines whether it is used for baby food production, preserved, frozen spinach or as fresh spinach.

Although the feasibility of using handheld NIRS instruments for the non-destructive measurement of these quality and safety parameters in spinach plants has been demonstrated (Itoh et al., 2011; Pérez-Marín et al., 2019 and Entrenas et al., 2020), these studies were carried out as simulation studies at a laboratory level. The incorporation of NIRS sensors directly in the field or at one of the main steps of the production chain, such as the reception points in the processing industries, has not been addressed.

The objective of this study was to evaluate the feasibility of a new generation NIRS sensor to be incorporated throughout the supply chain as a tool for quality assurance and safety of spinach plants. For this purpose, three key steps in the supply chain were studied: the growing period of the spinach plants in the field and the simulation of the reception of the spinach plants in the industry and the step after the spinach leaves were removed and washed.

2. Material and methods

2.1. Sampling and reference analysis

A total of 77 spinach (*Spinacia oleracea* L. cv. '1194', 'Gorilla' and 'Solomon') plants grown outdoors on different farms in the province of Cordoba, were used in this study. The spinach plants were harvested manually during the months of February and March 2019.

SSC and nitrate content were measured following Pérez-Marín et al. (2019), using between 4 and 10 spinach leaves from each plant. All the measurements were performed in duplicate and the standard error of laboratory (SEL) was estimated from these replicates (Table 2).

2.2. NIR spectrum acquisition

NIR spectra of spinach plants were collected using a MicroNIR™ Pro 1700 LVF spectrophotometer (VIAVI Solutions, Inc., San Jose, California, USA), a portable miniature instrument adapted to *in situ* measurements. This instrument works in reflectance mode (log 1/R) in the spectral range 910 to 1676 nm, with a constant interval of 6.2 nm. It is light (64 g, not including the handle which weighs 150 g and the acquisition and data processing device), with an optical window of around 227 mm^2. The sensor integration time was 10.5 ms and each spectrum was the mean of 200 scans.

Initially, the spinach plants were analysed before harvest (Step I, set 1). Spectral analysis was performed once again on the plants in the laboratory before the leaves were removed and washed, to simulate the receipt by the industry (Step II, set 2), and after the leaves were removed and washed, to simulate the different steps of processing after conditioning (Step III, set 3).

In the case of Steps I and II in the supply chain, in which NIRS analysis was carried out on the plants both in the field and in the laboratory, 5 spectra were taken per plant (1 spectrum per leaf, in one position on the leaf blade relative to the main vein and close to the petiole on the adaxial side), on 5 leaves per plant.

In Step III, in which the spectra were taken of the washed leaves, a total of 6 spectra were taken per leaf (4 in the blade and 2 in the petiole) following the methodology proposed by Entrenas et al., (2020). Between 4 and 10 leaves were used for the reference analysis for each plant.

For the different steps tested, the instrument's performance was checked every 10 minutes. A white reference measurement was obtained using a NIR reflectance standard (Spectralon™) with a 99 % diffuse reflectance, while the dark reference was obtained using a black plate for the field analysis (Step I) and from a fixed point in the room when the measurements were taken in the laboratory (Steps II and III).

2.3. Spectral repeatability

Spectrum quality was evaluated using the root mean square (RMS) statistic, defined as the averaged root mean square of differences between the different subsamples scanned at n wavelengths (Shenk and Westerhaus, 1995a, 1996). This statistic indicates the similarity between different spectra of a single sample.

To evaluate spectral repeatability, different procedures were followed depending on the sample set studied. For measurement on the plant, both in the field (Step I) and at the reception point (Step II), the repeatability was calculated analysing 20 plants and taking 5 spectra for each of them, one per leaf on 5 different leaves. For measurement on the leaves after the washing operation (Step III), the repeatability was obtained by analysing 20 leaves and taking 6 spectra for each leaf (Entrenas et al., 2020). An admissible limit for spectrum quality and repeatability was calculated following the procedure described by Martínez et al. (1998) to calculate the standard deviation limit (STD_{limit}) from the RMS statistic and obtain an RMS cut-off value.

2.4. Principal component analysis

Principal Component Analysis (PCA) was carried out to study the differences between the spinach NIRS sets obtained at the key steps. PCA was performed using the average spectrum for plants derived from each of the days and steps analysed. Matlab software (version 2015a, The Mathworks, Inc., Natick, MA, USA) was used applying mean centre as signal pre-treatment, which subtracts the mean spectrum of the group from each spectrum (Wise et al., 2006).

2.5. Definition of the calibration set for the development of NIRS models

Data pre-processing and chemometric treatments were performed using the Matlab version 2015a and WinISI II version 1.50 (Infrasoft International LLC, Port Matilda, PA, USA) (ISI, 2000) software packages.

To structure and compress the data matrix, the CENTER algorithm was applied; this algorithm determines the centre of the spectral population and calculates the Mahalanobis distance (GH) between each sample and the centre of the population, expressed in principal components (Shenk and Westerhaus, 1995a). Samples with a GH value greater than 4 were considered outliers. A combination of mathematical pre-treatments, Standard Normal Variate (SNV) and De-trending (DT) was applied for scatter correction (Barnes et al., 1989), together with the 1,5,5,1 derivative treatment, where the first digit is 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 (Shenk and Westerhaus, 1995b).

2.6. Fine-tuning of the spectrum-taking procedure in spinach plants throughout the food supply chain

For the optimization of the spectral acquisition process at different steps of the spinach supply chain using the MicroNIR™ Pro 1700, by first establishing the optimum number of spectra per plant that must be taken routinely. Different strategies were used for the analysis in the field and at receipt (Steps I and II) to develop the prediction models:

a. Selecting a single spectrum per plant, using only one leaf.
b. Using the average spectrum obtained after taking 3 spectra per plant on 3 different leaves.
c. Using, for each spinach plant, the average of the 5 spectra taken on 5 different leaves.

The spectra for strategies a and b were randomly selected from the 5 available using the Matlab software.

Calibration models for the prediction of SSC and nitrate content were constructed using modified partial least squares (MPLS) regression (Shenk and Westerhaus, 1995a). Four cross validation groups were used to avoid overfitting (Shenk and Westerhaus, 1995a). For each analytical parameter, different mathematical pre-treatments were evaluated. For scatter correction, SNV and DT methods were applied (Barnes et al., 1989). Additionally, two derivative mathematical treatments were tested: 1,5,5,1 and 2,5,5,1 (Shenk and Westerhaus, 1995b; ISI, 2000).

The best models for each parameter and each control point in the supply chain were selected by statistical criteria, using the coefficient of determination for cross validation (R^2_{cv}), the standard error of cross validation (SECV) and the RPD_{cv} (ratio of the standard deviation of the reference data for calibration to the SECV).

The SECV values obtained for the best equations for each parameter and control point studied, with a different number of spectra per plant, were compared using Fisher's F test (Massart et al., 1988; Naes et al., 2002). Since several SECV values were compared, a $SECV_{confidence\ limit}$ was calculated using the following formula: $SECV_{confidence\ limit} = SECV_{min} \cdot \sqrt{F_{critical}}$ where $SECV_{min}$ is the smallest SECV. Consequently, none of the models with a SECV between $SECV_{min}$ and $SECV_{confidence\ limit}$ were significantly different ($P < 0.05$).

Furthermore, the NIRS analysis process was also optimized on the leaves, to simulate the analysis after the industrial washing step. To achieve this, a possible reduction in the number of spectra taken per leaf was considered, following different strategies:

a. Using the average spectrum of taking 2 spectra per leaf, one from the blade and one from the petiole. In this case, one spectrum was selected from the 4 taken in the blade and one of the 2 from the petiole, since it is in the latter area where the highest accumulation of nitrates occurs and in the industry blades and petioles are processed together. Both spectra were taken on one side of the central nerve.
b. Using the average of the 3 spectra taken per leaf. In addition to the 2 spectra taken in the previous strategy, a further spectrum was taken from the leaf blade, on the other side of the central nerve.

c. Using the average spectrum of the 6 spectra (4 from the blade and 2 from the petiole) measured per leaf.

Next, the number of leaves needed to predict the parameters to be analysed was optimized. To achieve this, starting with one leaf, the number of leaves used to develop predictive NIRS equations were increased, until models were not significantly affected ($P > 0.05$) by the number of leaves analysed.

For the optimization of the NIRS analysis in already-washed spinach, considering both the number of spectra per leaf and the number of leaves, different models for the prediction of the SSC and nitrate content, without the elimination of chemical outliers, were developed and evaluated following the same methodology previously described for Steps I and II. The SECV values obtained for the best equations for each parameter and each strategy were also compared using Fisher's F test.

Finally, once the optimum analysis procedures for the three steps in the supply chain were decided on, the optimization of the NIRS models to predict SSC and nitrate content in spinach plants was carried out. The best equations were selected according to the statistical criteria mentioned above.

3. Results and discussion

3.1. Spectral repeatability

Prior to developing the models, it is crucial to optimise the NIRS analysis by means of the spectrum quality and repeatability measurement. For this purpose, the

STD_{limit} for each analysis step or control point was calculated, as described in Section 2.3.

The mean STD and STD_{limit} for the three different NIRS analysis steps are shown in Table 1. The STD_{limit} values obtained for Steps I and II were higher than those obtained for Step III. This could be due to the fact that the spectral measurements were taken in the plants without pre-washing the leaves, so these may have contained traces of dirt and dust. In addition, the spectra were taken on 5 different leaves (1 spectrum per leaf) and from 20 plants, while for Step III, the 6 spectra were taken on the same leaf and on 20 leaves, which could have resulted in a wider variation in the material analysed in Steps I and II.

The difference in spectral repeatability values obtained between Steps I and II, in which the same number of spectra were taken on the plants and before the leaves were washed, may be due to the fact that in Step I, the plants were analysed in the field, under variable and uncontrolled environmental conditions. Furthermore, obtaining spectra from the plant in the field is a far more complex task, which may lead to the analysis having lower repeatability, mainly due to the residual moisture that could remain on the leaf surfaces, even after they are dried before taking the spectra.

In a previous study, Pérez-Marín et al. (2019) calculated the value of $STD_{límit}$ in spinach leaves analysed in the laboratory. These authors took 4 sub-samples per leaf on the adaxial side of the blade and obtained a STD_{limit} value (128,437 µlog (1/R)), greater than those obtained in this work for the three analysis steps. These differences could be due to the instruments used, since Pérez-Marín et al. (2019) used a NIRS instrument based on MEMS technology (Phazir 2400), with a smaller window size (~ 55 mm^2) than that of the MicroNIR^{TM} Pro 1700 (~ 227 mm^2) used in the present work.

3.2. Population characterization

Before the predictive models were developed, PCA on raw spectra (Fig. 1) was used to carry out a study into the population structure.

Fig. 2A displays the scores of the first and third principal components (PCs), which represents 85.24 % and 1.66 % of the explained variance, respectively. A clear distinction must be drawn between the group of samples analysed in the field (Set 1) and those analysed in the laboratory (Sets 2 and 3).

For the samples analysed in the field, a grouping can be seen when the PC1 scores show a positive trend and the PC3 scores show a negative trend. The representation of the loadings for PC1 and PC3 (Fig. 2B) shows that in these areas, corresponding to the spectral range around 900–1300 nm and 1400–1500 nm (PC1 > 0 and PC3 < 0), the main absorption peaks for the distinction between the different sets are related to water content, since for these PCs, the loading plot exhibits one main band around 1450 nm (Shenk et al., 2008).

The differences between the samples analysed in the field and those of the industrial steps II and III are, therefore, mainly produced by the bands related to water content. The high respiration and water-loss rates of the spinach after the harvest result in a rapid loss of quality and tissue decay during postharvest handling, especially under non-refrigerated conditions (Salveit, 2016; Basil and Siddiqui, 2018).

Table 2 shows the range, mean, standard deviation (SD) and coefficient of variation (CV) of the population available for SSC and nitrate content. This set was used for the development of the prediction models for the three steps along the supply chain in spinach production.

SSC shows the lowest variability (Table 2), probably because the spinach plants were close to, or at, the stage of commercial maturity. The set for nitrate content shows high variability, due to the different cultivar behaviour in assimilating nitrates and the fact that the samples were collected throughout the harvesting period, where the level of nitrates decreases progressively from the first to the second cutting. Also, the plants analysed were collected from different farms, where different doses of fertilizer had been applied.

3.3. *Fine-tuning of the spectrum capture strategy for NIRS analysis of spinach plants in the field and in the industry*

Given that no previous work on the NIRS analysis of spinach plants directly in the field or at the reception step in the industry were found, the spectrum collection process was optimized for both steps of the production chain (Steps I and II), with the aim of facilitating the implementation of NIRS technology in both steps in the quickest, most trouble-free way possible, while enabling robust prediction models to be obtained.

Table 3 shows the SECV values for the best calibration models obtained for the different strategies followed based on the number of spectra to be acquired (1, 3 and 5 spectra per plant) for each parameter. To compare the SECV values obtained for the three strategies of obtaining spectra studied at the different steps of analysis, in the field and after the product reaches the industry, the NIRS models were developed without the elimination of chemical outliers.

According to the results shown in Table 3, there were no significant differences between the SECV values obtained for either parameter.

According to these results, and to make the NIRS measurement procedure as simple as possible, it was considered sufficient to take just one spectrum per plant, which would be the most suitable way to determine the quality and safety parameters in spinach plants in the field and at receipt in the industry.

3.4. *Fine-tuning of the spectrum capture strategy for the industrial NIRS analysis of spinach leaves after defoliating and washing*

Pérez-Marín et al. (2019) determined SSC and nitrate content by analysing 4-10 leaves and taking 4 spectra from the blade of each leaf, two on each side of the central nerve, while Entrenas et al. (2020), analysed the same number of leaves per plant, taking, in addition to the 4 spectra on the blade, two additional spectra in the petiolar zone, making a total of 6 spectra per leaf. However, the NIRS analysis protocols established by these authors involve taking a high number of spectra from each plant, which slows down spectra measurements. If this technology is to be used as a routine analysis method in the industry, the spectral methodology to be followed must be optimized, and it is therefore essential to look at the feasibility of reducing the number of spectra per leaf and deciding on the optimal number of leaves to analyse per plant.

To achieve this, firstly, the number of spectra to be measured on each leaf was optimized. Table 4 displays the SECV values for the best calibration models developed for each parameter using a different number of spectra per leaf (2, 3 and 6 spectra) in all the leaves used in the reference method (between 4 and 10 depending on their size).

No significant differences were found for the two parameters analysed between the SECV values of the predictive models developed using different number of spectra. Therefore, to facilitate the use of the NIR spectroscopy in the processed product, in cold chambers and also in the markets, the simplest way to measure the quality and safety parameters after the process of washing would be to take two spectra per leaf.

After selecting the optimum number of spectra per analysed leaf and to establish the minimum number of leaves to determine the quality and safety in the spinach plants, new predictive models were developed without removing chemical outliers, using 2 spectra per leaf and increasing progressively the number of leaves to be analysed.

Table 5 shows the SECV values for the calibration models developed using a different number of leaves (1, 2, 3) per plant. For the prediction of nitrate content, no significant differences were found among the SECV values obtained, and so, for this parameter, it would be sufficient to use a single leaf, with two spectra measurements.

For SSC, using a single leaf, higher SECV value than those obtained using 2 and 3 leaves was obtained. However, no significant differences were found when 2 or 3 leaves were used. Thus, for the SSC parameter, the number of leaves to be used to develop the models would be 2, taking 2 spectra per leaf.

Therefore, for the simultaneous measurement of these quality and safety parameters, after the spinach leaves have been washed on the production line, only two leaves would have to be analysed per plant. This is a considerable improvement with respect to studies previously carried out, in which the number of spectra per plant taken was much higher (4-10 leaves/plant and 4 and 6 spectra/leaf) than those carried out in this study (4 spectra/plant). Hence, the analyses could be carried out more quickly, without any loss of accuracy, thus allowing a much greater quantity of the product or number of batches to be analysed.

3.5. *Prediction of quality and safety parameters in spinach throughout the supply chain using MPLS regression*

Once the spectral acquisition process was optimized to determine the quality and safety parameters of the spinach at different steps of the production chain, the development of predictive models for the 3 steps of the production chain studied or simulated was optimized.

Table 6 shows the statistics of the best prediction models obtained using the spectral data for prediction of the quality and safety parameters. For the SSC prediction at the three key control points, the models developed distinguished between high, medium, and low values (Shenk and Westerhaus, 1996; Williams, 2001). Nicolaï et al. (2007) indicated that RPD_{cv} values between 1.5 and 2 could discriminate between low and high values of the predicted variable.

The results show the feasibility of using new generation portable NIRS equipment to predict SSC directly in the field, permitting the use of this technology as a surveillance tool to establish the optimal harvest time. Similarly, it also confirms the viability of using NIRS technology in the different steps of the production chain, once the product has been reached the industry, thus increasing the sampling pressure of the batches of processed plants and more effective monitoring of the product shelf life.

For nitrate content prediction, regardless of the step in the production chain studied, the predictive models also could differentiate between high, medium and low values, as indicated by Shenk and Westerhaus (1996) and Williams (2001).

Research on the use of portable NIRS instruments for the simultaneous measurement of SSC and nitrate content of spinach plants was carried out in the laboratory using washed leaves. Perez-Marín et al. (2019) measured these parameters using the instrument Phazir 2400 in a spectral range of 1600–2400 nm, obtaining values of RPD_{cv} = 2.54 and RPD_{cv} = 1.29 for SSC and nitrate content, respectively. Then, Entrenas et al. (2020), using the same instrument as in this study, also obtained promising results (RPP_{cv} = 2.62 for SSC and RPP_{cv} = 1.41 for nitrate content) for the quality and safety characterization of this vegetable.

In both studies, the results obtained for the prediction of the SSC were slightly better than those obtained in our study, which could be due to the fact that the calibration sets used by these authors to develop the predictive models contained a larger number of samples and greater variability.

For nitrate content, Pérez-Marín et al. (2019) reported a model with a lower predictive ability than that obtained here, although there were differences of the equipment used by these authors in terms of optical characteristics, spectral range and the analysis window. Nevertheless, Entrenas et al. (2020) obtained models with a predictive capacity (RPD_{cv} = 1.41) very similar to that obtained here in Steps II and III. For the first control point (Step I) in the field, the results were slightly more favourable. This increase in the robustness of the model may be due to the fact that these leaves were manipulated less prior to their NIRS analysis, which is consistent with the study reported by Basil and Siddiqui (2018) who demonstrated that spinach plants decay rapidly once harvested.

Therefore, although the sets of spinach plants used in this work has only a small number of samples, they were sufficient to demonstrate the viability of using NIRS technology. The results are of great interest to producers and the industry, since they confirm the usefulness and future potential of the MicroNIR™ Pro 1700 in the analysis of spinach along the supply chain, without carrying out any prior sample preparation. This will allow all batches of spinach to be controlled throughout processing from pre-harvest, in order to be categorized according to their quality and nitrate content. However, once the suitability has been proven, in future, the number of samples must be increased to develop more robust calibrations, with samples from different seasons and cultivars. This is especially important in biological products, which have countless variations in the sources, and also in the case of minor parameters with extremely wide variability, such as nitrate content (Perez-Marín et al., 2019).

4. Conclusions

The results obtained show the viability of using the handheld spectrophotometer MicroNIR™ Pro 1700 for the rapid screening of quality and safety parameters in spinach plants through supply chain. A single spectrum per plant is suitable for measuring the SSC and nitrate content in the field and at the reception in the industry, which would pave the way for the routine use of NIRS technology by the growers and in the industry. In the case of the analysis of spinach leaves after the leaf removal and washing operations, it seems to be sufficient to analyse two leaves per plant, with two spectra taken in each one, one on the leaf blade and another on the petiole, thus simplifying the NIRS analysis methodology in the processed product, and facilitating its possible future use not only in the industry but also in markets.

These results are of interest, because non-destructive measurement of these parameters in a matter of seconds facilitates not only decision-making about the optimal time for harvest, mainly based on the SSC, but also the monitoring of the plant requirements of nitrogen fertilization, thus making it possible to set the quantity and optimal time for this nutrient to be applied to the crop. Further studies are needed in order to improve the robustness of the calibration models.

CRediT authorship contribution statement

Irina Torres: Formal analysis, Investigation, Software, Data curation, Writing - original draft, Writing - review & editing, Visualization. **María-Teresa Sánchez:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Dolores Pérez-Marín:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1

Mean STD and STD_{limit} values obtained from the spectral repeatability study for the different NIRS analysis throughout the spinach supply chain.

Repeatability statistics	Field	Laboratory	After washing
Mean [a] STD	81,982	57,537	41,739
STD_{limit}	88,132	63,205	45,738

[a] Standard deviation

Table 2

Range, mean, standard deviation (SD) and coefficient of variation (CV) for the soluble solid and nitrate contents calibration sets, and standard error of laboratory (SEL).

	Soluble solid content (%)	Nitrate content (mg kg^{-1})
Range	5.8–14.4	41–3526
Mean	9.2	1344
SD	1.7	1122
CV (%)	18.5	83
SEL	0.04	24

Table 3

Comparison between standard error of cross validation values for the best calibration models obtained for soluble solid and nitrate contents, taking different number of spectra per plant during the analysis in the field and at the reception point in the industry. Fisher's F test (P < 0.05).

Supply chain	Parameter	[a] SECV 1 spectrum	SECV 3 spectra	SECV 5 spectra	$SECV_{min}$	$SECV_{min} \cdot \sqrt{F_{critical}}$
Field	Soluble solid content (%)	1.2	1.3	1.2	1.2	1.5
	Nitrate content (mg kg^{-1})	862	713	741	713	863
Laboratory	Soluble solid content (%)	1.1	1.2	1.1	1.1	1.3
	Nitrate content (mg kg^{-1})	882	913	833	833	1007

[a] Standard error of cross validation.

Table 4

Comparison between standard error of cross validation values for the best calibration models obtained for soluble solid and nitrate contents, taking different number of spectra per leaf and using between 4 and 10 leaves analysed after leaf removal and washing in the laboratory. Fisher's F test ($P < 0.05$).

Parameter	[a] SECV 2 spectra	SECV 3 spectra	SECV 6 spectra	$SECV_{min}$	$SECV_{min} \cdot \sqrt{F_{critical}}$
Soluble solid content (%)	1.1	1.1	1.2	1.1	1.3
Nitrate content (mg kg^{-1})	727	739	763	727	879

[a] Standard error of cross validation.

Table 5

Comparison between standard error of cross validation values for the best calibration models for soluble solid and nitrate contents obtained by analysing different number of leaves per plant and taking 2 spectra per leaf in the NIRS analysis after leaf removal and washing. Fisher's F test ($P < 0.05$).

Parameter	[a] SECV 1 leaf	SECV 2 leaves	SECV 3 leaves	$SECV_{min}$	$SECV_{min} \cdot \sqrt{F_{critical}}$
Soluble solid content (%)	1.4*	1.2	1.1	1.1	1.3
Nitrate content (mg kg^{-1})	792	785	714	714	864

[a] Standard error of cross validation.

Table 6

Calibration statistics for predicting soluble solid and nitrate contents using the instrument MicroNIR^{TM} Pro 1700 in the field and in the laboratory.

Parameter	Control point	Mathematical treatment	[a] N	[b] Mean	[c] SD	[d] SECV	[e] R^2_{cv}	[f] RPD_{cv}
Soluble solid content (%)	Field	1,5,5,1	72	9.2	1.6	1.1	0.55	1.55
	Laboratory	2,5,5,1	71	9.1	1.6	1.0	0.60	1.66
	After washing	2,5,5,1	70	9.1	1.6	1.0	0.62	1.76
Nitrate content (mg kg^{-1})	Field	1,5,5,1	75	1341	1132	725	0.59	1.55
	Laboratory	1,5,5,1	74	1318	1116	772	0.52	1.45
	After washing	2,5,5,1	76	1359	1122	766	0.54	1.46

[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.

Fig. 1. Average spectra for spinach plants at different steps of the supply chain.

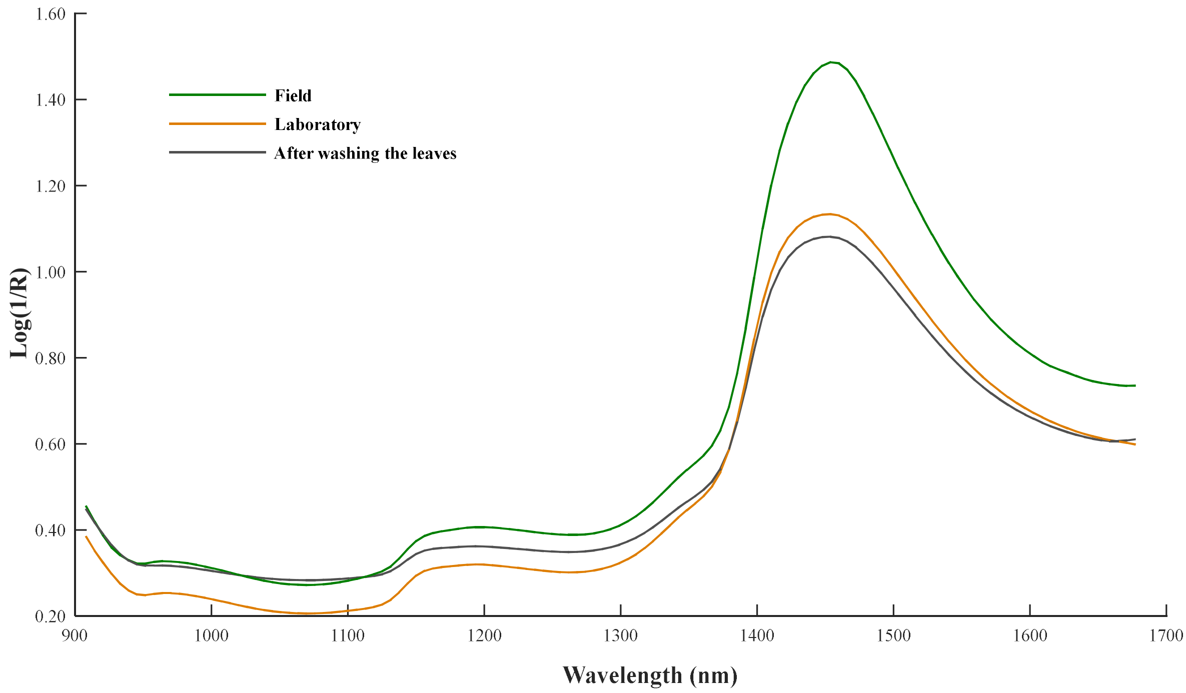

Fig. 2. Plots of scores (A) and loadings (B) for the first (PC1) and third (PC3) principal components for spinach plants analysed in the different steps of the supply chain.