Monitoring texture and other quality parameters in spinach plants using NIR spectroscopy.

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

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

[b] *Department of Animal Production, University of Cordoba, Campus of Rabanales, 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

Green colour, texture and dry matter are important attributes to appreciate freshness and quality in spinach. However, there is currently no fast, economical and non-destructive method which allows producers to measure these parameters simultaneously in the plant, in a matter of seconds. However, Near-infrared (NIR) spectroscopy might bridge this gap. NIR spectra of intact spinach leaves and modified partial least square regression models were developed for colour (a* and b*), texture (maximum fracture force, toughness, stiffness and displacement) and dry matter. A calibration equation with a high prediction performance was devised for dry matter content (r^2_{cv} = 0.74), while calibration models for all the textural parameters analysed were considered suitable for screening purposes (r^2_{cv} > 0.6). For colour-related parameters, the models allowed test samples to be rough screened. We, therefore, suggest that the analysis of green colour, texture and dry matter of spinach leaves *in situ* on the plant using NIRS technology could prove to be a valuable tool for optimizing cultural practices such as fertilization and irrigation and to assess the quality of the spinach leaves when harvested.

Keywords: Portable NIRS; *In situ* analysis; Spinach texture; MEMS instrument

1. Introduction

In horticultural products, quality is the sum of the characteristics, attributes and properties that give the fruit or vegetable its food value. The relative importance of each quality component depends on the product itself and on the use for which it is intended, fresh vs. processed (Bruhn, 2002; Kader, 2002b).

Colour is among the chief attributes used to assess the commercial quality of a horticultural product (Joseph et al., 2002). This is a physical concept that simultaneously involves observer psychology, the physiology of vision and the radiant energy emitted by the light source (Zelanski and Fisher, 1989). In the case of spinach, environmental factors, primarily temperature, humidity and light intensity, are essential for colour development (Fan et al., 2014). Optimum air temperatures for spinach growth range from 16 to 20ºC; low temperatures can damage the photosynthetic apparatus and thylakoid membranes and can inhibit protein synthesis (Decoteau, 2000), while Gruda (2005) reported that an increase in temperature during cultivation drastically alters plant development and negatively influences crop quality. Other contributory factors include soil type (Liu et al., 2016).

In fresh spinach, external colour is generally assessed visually, using standard colour-charts specific to this vegetable (Kader, 2002b). One drawback to the subjective appreciation of colour is that it is difficult to standardize; moreover, the shape, size and other superficial characteristics of the product can influence the effect produced by a colour on the observer (Francis, 1991). This method is also labour-intensive and time-consuming and cannot be used for routine analysis, although nowadays, values such as colour parameters L* (from white to black or light to dark), a* (from green to red) and b* (from blue to yellow) can be measured using digital colorimeters (Barrett et al., 2007).

Leaf texture is fast becoming another of the key parameters in spinach quality control (Gutiérrez-Rodríguez et al., 2013). Senescence in vegetables is a degradation process whereby the cell walls are broken down, leading to cell death; water and solids are also released into the intercellular space, resulting in loss of texture (Toivonen and Brummell, 2008). The texture of spinach is measured using a punch test technique, which utilizes a rounded probe that distributes the force homogeneously across a given area. From punch tests, force-displacement curves are generated and used to derive the mechanical properties of the material being evaluated: these include firmness, toughness, stiffness and displacement of the probe (Read and Sanson, 2003; Schopfer, 2006). The degree of firmness is usually associated with ripeness, freshness, retention of good quality and, therefore, with saleability, since firmness gradually declines during ripening and subsequent shelf storage (Blankenship et al., 1997; Kader 2002a). Moreover, in this crop, excessive nitrogen fertilization, which is generally used to achieve increased production**,** causes a drop in cell wall strength due to rapid growth, diminished macro- and micronutrient absorption and greater allocation of N to the cell wall (Reeve, 1970; Wright and Cannon, 2001; Onoda et al., 2004).

Another texture-related parameter measured in spinach is water content, as an indicator of succulence or turgidity (Kader, 2002b). Leafy vegetables are highly susceptible to water loss after harvest.

In spinach, colour, texture and water content have traditionally been assessed using destructive instrumental or sensory techniques (Conte et al., 2008; Gutiérrez-Rodríguez et al., 2013), thus permitting the quality evaluation of only a small number of samples from any given batch. To address this issue, numerous efforts have been made over recent years to develop non-destructive, environmentally-friendly analytical methods that will neither damage nor spoil the product, which can subsequently be sold or used for other measurements (Nicolaï et al., 2007; Saranwong and Kawano, 2007; Teixeira Dos Santos et al., 2013; Yan and Siesler, 2018). Rapid and non-destructive techniques permit the constant monitoring of spinach leaves directly on the plant and enable action to be taken immediately when any deviation from the product standard is observed at any point in the growing process.

Measuring the optical properties of food products has always been one of the most successful non-destructive techniques for quality assessment and is able to provide a number of quality readings simultaneously. In this area, NIR spectroscopy has shown great potential for the non-invasive measurement of quality parameters in horticultural products (Nicolaï, et al., 2007; Sánchez and Pérez-Marín, 2011; Magwaza et al., 2012). It combines fast, accurate measurement with considerable versatility, simplicity of sample presentation, speed of data (spectrum) collection and low cost, making it one of the approaches best suited to the needs of the horticultural sector (Walsh et al., 2000). The technology is simple, so fewer errors are introduced than in conventional analytical techniques (Osborne et al., 1993). Moreover, the use of NIR spectroscopy for quality control and assurance purposes during spinach growth and in the fresh spinach industry enables greater quantities of this vegetable to be analysed and also allows for large-scale individual analysis. At the same time, NIR spectroscopy is a powerful tool for general process monitoring in real time (De la Roza et al., 2017; Zhang et al., 2017); this is of particular interest for many agro-industrial applications such as quality control systems or for making real-time decisions during spinach cultivation.

Hence, the objective of this study was to evaluate the feasibility of NIR spectroscopy for predicting *in-situ* colour, texture and dry matter content of intact spinach at harvesting using a low-cost miniaturised, handheld, near-infrared device based on micro-electrical-mechanical system (MEMS) technology, ideal for measuring *in-situ* the quality of the plants.

2. Material and methods

2.1. Sampling

A total of 149 spinach plants (*Spinacia oleracea* L, cv. 'Solomon', 'Novico', 'Meerkat' and 'Gorilla'), 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 2017.

The harvested spinach was kept in refrigerated storage at 4°C and 85% RH until the following day, when laboratory testing was performed. Prior to each test, the spinach was allowed to reach room temperature. Both the NIR spectral acquisition and the reference analyses were carried out using a single leaf chosen from each plant registered (Gutiérrez-Rodríguez et al., 2013).

2.2. NIR spectrum acquisition

Spectra were collected on spinach leaves in reflectance mode (Log 1/R) using a handheld MEMS spectrophotometer (Phazir 2400, Polychromix, Inc., Wilmington, MA, USA). The instrument scans at non-constant intervals of approximately 8 nm across the range of NIR wavelengths 1600–2400 nm, with a scan time per sample of 3 s. Instrument performance was checked every 10 min, following the diagnostic protocols provided by the manufacturer, and white reference measurement was carried out using Spectralon as the reference. Using the MEMS-NIR instrument and in order to assess the spinach leaves analysed, four spectral measurements were made 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. In all evaluations the NIRS spectra were collected on blade tissue without conspicuous veins. The average distance between measurements was 3 cm. The four spectra were averaged to provide a mean spectrum for each plant.

2.3. Reference data

Leaf colour was measured with a Minolta CR-400 chroma meter (Konica Minolta Sensing INC., Osaka, Japan), using illuminant C as an illuminant (Glowacz et al., 2015) with an observation angle of 2º (CIE, 2004). Leaf colour changes were quantified for the leaf chosen from each sample following the same procedure previously described for the NIR spectra acquisition, in the a* (−a* = greenness and +a* = redness) and b* (−b* = blueness and +b* = yellowness) colour space (Koukounaras et al., 2009).

Later, the leaves were analysed using the punch test to assess their textural properties. This procedure involves forcing a probe of known cross-sectional area through a section of a leaf, as described by Read and Sanson (2003). The punch test was conducted at room temperature using a universal testing machine (Model 3343, Single column, Instron Corporation, Norwood, MA, USA), fitted with a 1000N load-cell.

A 6 mm diameter probe was used to penetrate the spinach leaf, using a pre-test speed of 2 mm s-1, a test speed of 1 mm s-1 when the probe came into contact with the leaf and a post-test speed of 10 mm s-1. Each leaf was placed between two clamped metal plates with coinciding holes (area of 0.95 cm^2) to keep the leaf flat. The probe moved a standard distance of 8 mm. The clearance between the probe and the hole in the plates was 0.15 mm, following the protocol of Gutiérrez-Rodríguez et al., (2013).

A force-displacement graph for each selected spinach leaf was generated from this test and the fracture properties (1) maximum force required to puncture the leaf, (2) toughness, (3) stiffness, and (4) the displacement of the probe necessary to fracture each leaf were recorded. The maximum force was measured as the force needed to puncture the leaf, toughness as the area under the force-displacement curve and stiffness as the slope of that curve. Punch test measurements were performed at the same locations on the leaf as for NIRS analysis. The four measurements were averaged to provide mean data of the texture parameters selected for each plant.

Dry matter (DM) content was determined gravimetrically by desiccation at 105ºC for 24 h (AOAC, 2000), and the final dry weight was calculated as a percentage of the initial wet weight.

Samples were analysed in duplicate and the standard error of laboratory (SEL) was estimated from these duplicates (Table 2). All measurements were performed immediately after NIRS measurements.

2.4. Data analysis: definition of calibration and validation sets

Prior to carrying out NIR calibrations, the CENTER algorithm included in the WinISI II software package ver. 1.50 (Infrasoft International LLC, Port Matilda, PA, USA) was applied to ensure a structured population selection based solely on spectral information, for the establishment of calibration and validation sets (Shenk and Westerhaus, 1991). This algorithm performs an initial principal component analysis (PCA) 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 statistical value greater than 3 were considered outliers or anomalous spectra.

The CENTER algorithm was applied in the spectral region 1600–2400 nm. The mathematical treatments SNV (Standard Normal Variate) and DT (De-trending) were applied for scatter correction (Barnes et al., 1989), together with the mathematical derivation treatment '1,5,5,1', where the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in a running average or smoothing, and the fourth is the second smoothing (Shenk and Westerhaus, 1995b; ISI, 2000).

Once spectral outliers had been removed (i.e., 4 of the original 149 samples), a set consisting of 145 samples was used to build the calibration models. These samples were selected following the method outlined by Shenk and Westerhaus (1991), using the CENTER algorithm included in the WinISI software package to calculate the Global Mahalanobis distance (GH). Samples were ordered based on the Mahalanobis distance to the centre of the population, where three of every four were selected to be part of the calibration set (N = 109 samples) and the test set was made up of the remaining 25% (N = 36 samples).

Modified partial least squares (MPLS) regression (Shenk and Westerhaus, 1995a) was used to obtain equations for predicting colour, texture and dry matter content. Six cross-validation steps were included in the process in order to avoid overfitting (Shenk and Westerhaus 1995a). For each analytical parameter, different mathematical treatments were evaluated. For scatter correction, the standard normal variate (SNV) and detrending (DT) methods were tested (Barnes et al., 1989). Additionally, four derivative mathematical treatments were tested in the development of NIRS calibrations: 1,5,5,1; 2,5,5,1; 1,10,5,1; 2,10,5,1 (Shenk and Westerhaus, 1995b).

Best equations were selected according to the following statistics: coefficient of determination for calibration (r^2_c), Standard Error of Calibration (SEC), coefficient of determination for cross-validation (r^2_{cv}) and Standard Error of Cross-validation (SECV). However, in order to standardize the SECV value; other statistic such as the Residual Predictive Deviation (RPD_{cv}), calculated as the ratio between the standard deviation (SD) of the calibration set to the SECV, was also calculated (Williams, 2001).

The best models obtained for the calibration set, as selected by statistical criteria, were subjected to evaluation using samples not involved in the calibration procedure and evaluated following the protocol outlined by Windham et al. (1989).

3. Results and discussion

3.1. Population characterization

Calibration and validation set characteristics, i.e. number of samples, mean, range, SD, and CV for the parameters analysed, are shown in Table 1. Structured selection based wholly 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 study parameters; moreover, the ranges of the validation set lay within those of the calibration set.

Table 1 shows how the parameters with the greatest variability were those linked to leaf texture (CV for calibration = 58.52–77.85%; CV for validation = 58.80–81.76%), while the parameters with the least variability were those related to colour (CV for calibration = 10.57–15.44%; CV for validation = 9.22–12.26%), because, as shown in

Table 1, the SD values for the colour parameters are negligible compared to their mean value, due to the great uniformity in colouration shown by the plants analysed.

3.2. Prediction of quality parameters using MPLS regression and NIR spectra

Table 2 shows the results of the best prediction models obtained for each parameter analysed (colour, texture and dry matter content) using different pre-treatments of the spectral signal. For each of the parameters studied, a total of 4 calibration models were obtained, the best of which was selected by statistical criteria: priority was given to those with lower SECV and CV values and higher r^2_{cv} and RPD_{cv} values.

As regards the predictive capacity of the models designed for colour, it is worth noting that for parameter a* (green-red variation), the model (r^2_{cv} = 0.47; RPD_{cv} = 1.36) allowed spinach leaves to be separated into high and low values, as indicated by Shenk and Westerhaus (1996) and Williams (2001). It is also important to note that the plants were mature and ready for sale, with their characteristic deep-green leaf colour and with parameter a* showing a low standard deviation.

Fearn (2014) points out, while the r^2_{cv} statistic can be a useful measure of the performance of a calibration, it does have its limitations. One major constraint is its dependence on the range of values of the calibration set, as well as on the standard deviation (SD) of the reference values.

No articles have been found in the scientific literature which deal with using NIR spectroscopy to measure this parameter in spinach, despite the fact that predicting the colour parameter in this vegetable is of great importance, since it is a highly influential parameter in consumer choice (Ferrante et al., 2004).

It should be stressed that the accuracy of the model obtained for parameter a* is limited, since the working range of the MEMS-NIR equipment does not include wavelengths in the visible region, which is important when measuring parameters related to colour, although the results do allow us to distinguish between two types of values for parameter a* measured *in situ* on the plant, which is particularly useful for spinach growers. Greenness intensity related with parameter a* in leafy vegetables is attributed to chlorophyll pigmentation, which is a measure of the photosynthetic potential and of plant productivity (Xue and Yang, 2009; Gilbert and Martin, 2015), as well as being a direct measure of the nutrient status, because much of the leaf nitrogen is contained in chlorophyll (Filella et al., 1995). Xue and Yang (2009) show that chlorophyll pigments in green plants are gaining increasing importance in the human diet, not only as food colorants, but also as healthy food ingredients, and so the *in-situ* measurement of parameter a* linked to the presence of chlorophyll would seem to be of major importance when deciding on the best time to harvest spinach.

It is also important to note that during postharvest senescence, the green chlorophyll pigments are oxidized into colourless substances, revealing yellow carotenoids (Toivonen and Brummell, 2008), so the non-destructive measurement of parameter b* would be of great use when measuring the different stages of the plant's senescence. Here, the model designed allows to distinguish between high and low values of this parameter, following Shenk and Westerhaus (1996) and Williams (2001), which shows that this model could be considered acceptable for screening purposes.

These colour measurements (a* and b*) can therefore be made using a rapid, non-destructive hand-held sensor over the whole spinach plant, thus giving the farmers an instant response and allowing the spinach harvest to be started at the optimum time.

Texture is an important point in the eating quality of spinach. The textural properties can include several parameters, such as maximum force required to puncture the leaf, toughness, stiffness and the displacement of the probe necessary to fracture each leaf. All of these are closely correlated between each other, meaning that any of these physical measurements could be effectively used for texture evaluation.

To measure parameters related to texture, the models developed for maximum force to puncture the leaf (r^2_{cv} = 0.67; RPD_{cv} = 1.72), toughness (r^2_{cv} = 0.62; RPD_{cv} = 1.62), stiffness (r^2_{cv} = 0.69, RPD_{cv} = 1.79) and the displacement of the probe necessary to fracture each leaf (r^2_{cv} = 0.62, RPD_{cv} = 1.61) allow to discriminate between low, medium and high values for these parameters, following Shenk and Westerhaus (1996) and Williams (2001).

The results obtained can be considered as satisfactory, given that various authors (Pérez-Marín et al., 2007; Flores-Rojas et al., 2009) have already shown the difficulty and complexity of predicting physical parameters related to texture in other vegetables.

As it has already been pointed out, the texture of a product is not a single, well-defined attribute, but encompasses the structural and mechanical properties of a food item and the sensory perception of that food in the hand or mouth (Abbott and Harker, 2016). Generally, assessment of texture is based on the measurement of firmness, which is in turn linked to the resistance of fresh produce to mechanical stress during transport and distribution (Thompson, 2002).

However, the use of NIR spectroscopy allows us to measure not just one textural parameter but several at the same time, which means that spinach texture can be better defined, and measurements taken directly on the plant.

No references have been found in the scientific literature on measuring texture in spinach leaves using NIR spectroscopy.

For the dry matter parameter, the calibration model showed a good predictive capacity (r^2_{cv} = 0.74; RPD_{cv} = 1.96) when interpreting the coefficient of determination and RPD_{cv} values, as proposed by Shenk and Westerhaus (1996) and Williams (2001). The non-destructive measurement of this parameter *in situ* is, in fact, of great importance both for growers and for the later handling of the post-harvest crop, since DM values of around 10-12% fw ensure a good resistance to handling and allow maintenance of visual quality at a high standard during storage (Conte et al., 2008). In addition, Bergquist et al. (2006) have underlined the positive correlation between the high content of DM and vitamin C at harvest time and the visual quality retention of spinach leaves during storage. This again reveals the importance of measuring the DM content in a non-destructive way in order to decide on the best time to harvest and ensure that the spinach has a high vitamin content.

No publications have been found in the scientific literature which deal with using NIR spectroscopy to measure this quality parameter in spinach. There are other studies on the prediction of dry matter in leaves of other vegetables (Steidle et al., 2017), although these leaves (sunflower) have very different characteristics to spinach leaves.

3.3. External validation

Validation statistics for the prediction of the quality parameters analysed in intact spinach are shown in Fig. 1.

The models constructed for predicting all the textural parameters analysed, with the exception of the displacement, and also for the prediction of dry matter in intact spinach, met the validation requirements in terms of r^2_p (r^2_p > 0.6) and both the SEP(c) and the bias were within confidence limits: the equations thus ensure accurate prediction, and can be applied routinely. For the parameter ‘displacement of the probe necessary to fracture the leaf’, it should be stressed that the SEP(c) and bias lay within the confidence limits, although r^2_p = 0.5 did not attain the recommended minimum value.

However, the models predicted colour parameters in validation-set samples with low values for r^2_p, in neither case meeting the recommendations of Windham et al., (1989). These models are thus not suitable for routine applications. The comparatively low r^2_p value displayed for a* and b* may be due to the narrower range and lower SD recorded for these parameters (Table 1). This is also clearly illustrated in Fig. 1, where it is evident that the a* and b* exhibited by most samples lie in the ranges of -12–(-14) for parameter a* and 16-20 for parameter b*, with very little coverage of the range for other values. These results highlight the importance not only of ensuring a sufficient number of samples in the calibration set, but also of guaranteeing the adequate distribution and structure of the sample set.

The SEL values for the parameters tested are shown in Table 2. For the parameters: a*, b*, maximum puncture force, toughness and stiffness, SEP fell between 3 and 4 SEL, indicating acceptable performance of the NIRS models developed. For the displacement, SEP fell between 2 and 3 SEL, showing good performance of the NIRS model and for dry matter, SEP was between 1 and 2 SEL, showing excellent predictive capacity of the NIRS model (Westerhaus, 1989, Williams, 2001).

4. Conclusions

It should be stressed that the NIR equations constructed should be regarded as a first step in the fine-tuning of NIR spectroscopy for the *in situ* monitoring of quality parameters in intact spinach. Given the general importance in the eating quality of spinach and consumers' general acceptance of dry matter content and textural properties, the use of the MEMS-NIR portable NIR device tested here, which is rapid, lightweight and user-friendly, should be considered for use in the routine, non-destructive analysis of spinach on the plant.

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Table 1.

Range, mean, standard deviation (SD) and coefficient of variation (CV) for the parameters of colour, texture and dry matter content studied in the calibration and validation sets

Parameter	Set	N	Range	Mean	SD	CV (%)
a*	Calibration	109	-18.33– (-8.66)	-13.05	1.38	10.57
	Validation	36	-17.32– (-10.78)	-13.34	1.23	9.22
b*	Calibration	109	11.55–26.40	17.94	2.77	15.44
	Validation	36	13.77–23.02	17.94	2.20	12.26
Maximum puncture force (N)	Calibration	109	0.20–4.98	1.98	1.29	65.15
	Validation	36	0.37–4.51	1.99	1.37	68.84
Toughness (mJ)	Calibration	109	0.16–10.79	2.98	2.32	77.85
	Validation	36	0.38–8.73	3.18	2.60	81.76
Stiffness (N/mm)	Calibration	109	0.09–1.30	0.55	0.34	61.81
	Validation	36	0.09–1.03	0.52	0.33	63.46
Displacement (mm)	Calibration	109	0.32–6.58	2.58	1.51	58.52
	Validation	36	0.57–6.05	2.67	1.57	58.80
Dry matter content (% fw)	Calibration	109	6.14–19.67	12.50	3.10	24.80
	Validation	36	7.35–18.83	12.60	2.91	23.09

Table 2.

Statistics of best calibration models to predict colour, texture and dry matter content and standard error of laboratory

Parameter	Mathematic treatment	N	Range	Mean	SD	r^2_{vc}	SECV	RPD_{cv}	SEL
a*	2,10,5,1	108	-18.33-(-8.66)	-13.06	1.38	0.47	1.01	1.36	0.29
b*	1,5,5,1	105	11.55-25.71	17.89	2.56	0.38	2.02	1.26	0.62
Maximum puncture force (N)	2,5,5,1	105	0.20-4.62	1.93	1.26	0.67	0.73	1.72	0.23
Toughness (mJ)	2,5,5,1	107	0.16-10.79	2.92	2.26	0.62	1.39	1.62	0.44
Stiffness (N/mm)	2,5,5,1	105	0.09-1.30	0.55	0.34	0.69	0.19	1.79	0.06
Displacement (mm)	2,5,5,1	103	0.32-6.58	2.54	1.50	0.62	0.93	1.61	0.51
Dry matter content (% fw)	2,5,5,1	105	6.14-19.67	12.43	2.98	0.74	1.52	1.96	0.90

Fig. 1. Reference values *versus* NIR-predicted data for the validation set.