Universidad de Córdoba

# Innovación en el aseguramiento de la calidad y seguridad de productos hortícolas mediante sensores espectroscópicos de infrarrojo cercano

## Innovation in the quality assurance and safety of horticultural products using near infrared spectral sensors

Innovación en el aseguramiento de la calidad y seguridad de productos hortícolas mediante sensores espectroscópicos de infrarrojo cercano

TESIS DOCTORAL

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José Antonio Entrenas de León

UNIVERSIDAD DE CÓRDOBA

TITULO: *Innovation in the quality assurance and safety of horticultural products using near infrared spectral sensors*

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**UNIVERSIDAD DE CÓRDOBA**

***Programa de Doctorado Ingeniería Agraria, Alimentaria, Forestal y del Desarrollo Rural Sostenible por la Universidad de Córdoba y la Universidad de Sevilla***

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**Directoras:**

**Dra. Mª Teresa Sánchez Pineda de las Infantas**
**Dra. Dolores Pérez Marín**

*Noviembre 2019*

# *Innovación en el aseguramiento de la calidad y seguridad de productos hortícolas mediante sensores espectroscópicos de infrarrojo cercano*

**TESIS DOCTORAL**

para aspirar al grado de Doctor por la Universidad de Córdoba presentada por D. José Antonio Entrenas de León, Graduado en Ingeniería Agroalimentaria y del Medio Rural y Máster en Ingeniería Agronómica por la Universidad de Córdoba

*El Doctorando*

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Vº Bº Las Directoras

*Fdo.: Profª. Dra. Mª Teresa Sánchez Pineda de las Infantas*

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*Noviembre 2019*

Mª Teresa Sánchez Pineda de las Infantas, Catedrática de Universidad del Departamento de Bromatología y Tecnología de los Alimentos de la Universidad de Córdoba y Dolores Pérez Marín, Catedrática de Universidad del Departamento de Producción Animal de la Universidad de Córdoba

**I N F O R M A N**:

Que la Tesis titulada "**Innovación en el aseguramiento de la calidad y seguridad de productos hortícolas mediante sensores espectroscópicos de infrarrojo cercano**", del que es autor D. José Antonio Entrenas de León, ha sido realizada bajo nuestra dirección durante los años 2018 y 2019; y cumple los requisitos académicos exigidos por la Legislación vigente para optar al título de Doctor por la Universidad de Córdoba.

Y para que conste a los efectos oportunos firman el presente informe en Córdoba a 28 de octubre de 2019

*Fdo.: Profª. Dra. Mª Teresa Sánchez Pineda de las Infantas*

*Fdo.: Profª. Dra. Dolores Pérez Marín*

**TÍTULO DE LA TESIS:**

INNOVACIÓN EN EL ASEGURAMIENTO DE LA CALIDAD Y SEGURIDAD DE PRODUCTOS HORTÍCOLAS MEDIANTE SENSORES ESPECTROSCÓPICOS DE INFRARROJO CERCANO

**DOCTORANDO:**

JOSÉ ANTONIO ENTRENAS DE LEÓN

## INFORME RAZONADO DE LAS DIRECTORAS DE LA TESIS

La Tesis Doctoral cuyo título se menciona arriba se ha adaptado, desde sus inicios, a la metodología y al diseño programados, derivando todo ello en la obtención de resultados de indudable relevancia científica y tecnológica.

En primer lugar, hay que destacar que del trabajo de esta Tesis Doctoral se han establecido las bases científico-técnicas para el desarrollo de modelos de predicción NIRS cuantitativos, y se han obtenido un amplio abanico de aplicaciones NIRS relativas a la determinación de parámetros de calidad, principalmente aquellos relacionados con parámetros morfológicos (peso, longitud, diámetro), el color, la textura, y los contenidos en materia seca y en sólidos solubles en hortalizas, concretamente en espinaca y calabacín, para la cuantificación no destructiva de los cambios físico-químicos que tienen lugar durante el desarrollo de hortalizas en campo, en la mata, facilitando la toma de decisiones sobre el momento óptimo de cosecha, así como para establecer su categorización en la industria procesadora, y la vida útil durante su conservación poscosecha en cámaras frigoríficas. Con este fin se han utilizado estrategias de regresión lineal, específicamente, se ha empleado la regresión en mínimos cuadros parciales modificada.

En segundo lugar, se ha llevado a cabo la determinación cuantitativa de parámetros relacionados con la seguridad alimentaria; concretamente, el contenido de nitratos presentes en espinaca y calabacín ha sido establecido mediante espectroscopía NIR, con el objetivo de determinar el destino — alimentación infantil, procesado industrial (producto en conserva, refrigerado o congelado), o consumo en fresco — de las dos hortalizas anteriormente citadas. Asimismo, se ha determinado la distribución longitudinal de nitratos en calabacín, para establecer aquella zona o zonas susceptibles de su utilización en la elaboración de alimentos infantiles.

En tercer lugar, se han determinado, para las distintas aplicaciones desarrolladas y productos analizados (espinaca y calabacín) en esta Tesis Doctoral, los instrumentos NIRS más idóneos de utilización en función, tanto del momento (durante el desarrollo y maduración de hortalizas, en la cosecha, y en su conservación poscosecha) como del lugar (en la mata, en las líneas de manipulación y clasificación en la industria, y en poscosecha en cámaras frigoríficas) para la realización de dichas determinaciones, teniendo en cuenta, asimismo, los parámetros de interés elegidos y las características intrínsecas de los productos seleccionados. Se ha establecido una metodología de actuación para cada uno de los instrumentos NIRS evaluados.

Lo anteriormente expuesto justifica plenamente que la forma más idónea de presentación de esta Tesis Doctoral sea el compendio de publicaciones científicas.

Asimismo, destacar que el doctorando ha tenido la posibilidad de formarse, no sólo en aspectos científicos-técnicos ligados a la tecnología NIRS, sino también en los relacionados con la ingeniería y tecnología pre y poscosecha de frutas y hortalizas, realizando los siguientes cursos:

- Espectroscopía de Infrarrojo Cercano (NIRS). Aplicaciones en el Control de Calidad y Trazabilidad de Productos y Procesos. 12 a 25 de febrero de 2018. Universidad de Córdoba (Córdoba, España).
- 12º Curso Internacional de Tecnología Postcosecha y Procesado Mínimo Hortofrutícola. 7 a 13 de marzo de 2018. Universidad Politécnica de Cartagena (Cartagena, Murcia, España).
- Online Course 'Fundamentals and Applications of Near Infrared Spectroscopy'. 1 de noviembre de 2018 a 15 de enero del 2019. Organizado por la Universidad de Córdoba y por el International Council for Near Infrared Spectroscopy (ICNIRS).

Los trabajos publicados en forma de artículos científicos relacionados con los resultados de la Tesis Doctoral son los siguientes:

1. 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. Computers and Electronics in Agriculture 155, 446-452. DOI: 10.1016/j.compag.2018.11.004. Journal Impact Factor: 3.171. Rank: 5/56, Q1, Agriculture, Multidisciplinary-SCIE; Rank: 31/106, Q2. Computer Science, Interdisciplinary Applications-SCIE.

2. Entrenas, J.A., Pérez-Marín, D., Torres, I., Garrido-Varo, A., Sánchez, M.T. 2020. Simultaneous detection of quality and safety in spinach plants using a new generation of NIRS sensors. Postharvest Biology and Technology 160, February, 111026. DOI: 10.1016/j.postharvbio.2019.111026. Journal Impact Factor: 3.927. Rank: 5/87, Q1, Agronomy-SCIE; Rank: 18/135, Q1, Food Science and Technology-SCIE; Rank: 1/36, Q1, Horticulture-SCIE.

3. Entrenas, J.A., Pérez-Marín, D., Torres, I., Garrido-Varo, A., Sánchez, M.T. 2019. Safety and quality issues in summer squashes using handheld portable NIRS sensors for real-time decision making and on-vine monitoring. Journal of the Science of Food and Agriculture 99, 6768-6777. DOI: 10.1002/jsfa.9959. Journal Impact Factor: 2.422. Rank: 9/56, Q1, Agriculture, Multidisciplinary-SCIE; Rank: 23/71, Q2, Chemistry, Applied-SCIE; Rank: 43/135. Q2, Food Science and Technology-SCIE.

El doctorando ha participado en los siguientes congresos, simposios y conferencias:

- IV Congreso Nacional de Ingenieros Agrónomos "Retos Tecnológicos, Innovación y Apuestas de Futuro en Ingeniería Agroalimentaria y Medio Rural", organizado por la Asociación Nacional de Ingenieros Agrónomos, ANIA. Córdoba, España. 15 a 17 de octubre de 2018. Presentación de la comunicación oral: "Predicción *in situ* de la calidad de frutas mediante el uso de sensores NIRS".
- 5th Food Integrity Conference "Assuring the Integrity of the Food Chain: Delivering Real World Solutions", organizado por Eurofins. Nantes, France. 14th to 15th November 2018. Presentación de la comunicación escrita: "*In situ* application of near infrared spectroscopy in bell pepper quality determination".
- VI International Symposium on Applications of Modelling as an Innovative Technology in the Horticultural Supply Chain - Model-IT 2019, organizado por la Università degli Studi di Foggia. Molfetta, Italy. 9-12 June 2019. Presentación de la comunicación escrita: "Application of near infrared reflectance spectroscopy to assess online quality in summer squashes".

Por todo ello, se autoriza la presentación de la Tesis Doctoral.

Córdoba, 28 de octubre de 2019

*Fdo.: Profª. Dra. Mª Teresa Sánchez Pineda de las Infantas*

*Fdo.: Profª. Dra. Dolores Pérez Marín*

*A mi familia*

# Agradecimientos

Deseo expresar mi sincera gratitud y reconocimiento a todas las personas que han hecho posible la realización de esta Tesis Doctoral:

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A mi familia, por su confianza y su apoyo incondicional durante todo el tiempo que ha durado la realización de esta Tesis Doctoral.

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# Resumen

## RESUMEN

Los productores, la industria, los responsables de realizar las inspecciones de control de calidad tanto en campo como en la industria, así como los consumidores necesitan la puesta a punto y el desarrollo de tecnologías que proporcionen información exacta y útil sobre los parámetros que influyen directamente sobre la calidad y la seguridad de los productos hortícolas, siendo clave el que dichas tecnologías no estén limitadas por sus costes o tiempos de análisis.

La espectroscopía en el infrarrojo cercano (en inglés, near infrared spectroscopy, NIRS) se puede definir como una técnica no invasiva que combina rapidez, facilidad y precisión en la medida, con un bajo coste de análisis por muestra y una gran versatilidad, lo cual permite su incorporación en distintos niveles de decisión a lo largo de la cadena agroalimentaria, tanto en la precosecha, en campo, como en la industria, en poscosecha, relativos a la calidad, seguridad y vida útil de los productos frescos durante su desarrollo, posterior conservación frigorífica y transformación industrial. Asimismo, la tecnología NIRS ha sido empleada con éxito en el sector hortícola para la categorización de hortalizas.

La instrumentación NIRS ha ido evolucionando desde finales de los años sesenta hasta nuestros días. En la actualidad existe una amplia variedad de equipos NIRS con diferentes características, como diseño óptico, rango espectral de trabajo, portabilidad, coste, etc. Esto hace que, dependiendo de la aplicación, las soluciones que ofrece esta tecnología estén cada vez más adaptadas a las necesidades. No obstante, en este ámbito de enormes potencialidades quedan numerosos aspectos en los que profundizar, principalmente, los relativos a la optimización de la medida, al procesado de los datos espectrales y a su conexión con sistemas de apoyo a la decisión, que posibiliten que este tipo de aplicaciones sean una realidad.

El objetivo principal de esta Tesis Doctoral ha sido desarrollar modelos NIRS para la predicción de parámetros de calidad y seguridad en hortalizas (espinacas y calabacines) durante el seguimiento de maduración, en la recepción en la industria, así como en las líneas de clasificación en la industria. Con este propósito se han evaluado tres espectrofotómetros comercialmente disponibles, dos muy adecuados para efectuar mediciones *in situ*, directamente sobre producto en la mata — espectrofotómetro basado en la tecnología de filtros variables lineales, en inglés conocida por sus siglas LVF y espectrofotómetro basado en sistemas microelectromecánicos, en inglés conocido por sus siglas MEMS — y el otro idóneo para su utilización en las líneas industriales de procesado — espectrofotómetro basado en espectroscopía NIR por Transformada de Fourier, en inglés, FT-NIR.

Los resultados obtenidos en los distintos trabajos de investigación que forman parte de esta Tesis Doctoral han puesto de manifiesto el potencial de la tecnología NIRS para su incorporación *in situ* y "online" en el sector hortícola, como sensor y herramienta de apoyo a la decisión que proporcionará una huella espectral única de cada producto, de utilidad para su trazabilidad y categorización y, asimismo, como un registro óptico de enorme interés para asegurar que el producto cumple los estándares de calidad y de seguridad determinados por la normativa vigente para sus distintos usos.

# Summary

## SUMMARY

Producers, consumers and the industry are demanding the development of rapid, accurate, economical and above all non-destructive technologies for determining *in situ* food-produce quality and safety, being a key factor that these technologies are not limited by their response time or cost. Additionally, a feasible quality and safety control in the handling and processing industries must be implemented.

Near infrared spectroscopy (NIRS) is recognised as a precise and reproducible technique for quantitative and qualitative analysis in the agrifood sector, being considered as a promising technology for the agrifood industry, because it meets all the requirements for modern and automatic quality and safety control. This technology represents a marked change from the conventional analytical methods, because a single spectrum allows the simultaneous characterization of different physicochemical properties, in a matter of seconds and without sample preparation, thus allowing real-time decision-making.

Over recent years, due to its swift response, precision, applicability to multiple products and analytes (it is able to provide a number of quality and safety readings simultaneously) and the advanced level of development of the latest generation of instruments, near infrared (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.

Nevertheless, the continuous technological evolution of the NIRS instrumentation has meant that some of the original portable, handheld NIRS devices appeared on the market, have now been phased out. For this reason, it is essential to evaluate the use in horticultural products of the new portable spectrophotometers to analyse the product directly in the field, in order to carry out the quality and safety monitoring of the horticultural products during their

growing and to facilitate real-time decision-making for crop management practices and thus decide on the optimal time to harvest. Additionally, these instruments can also be used by the industry for the reception and postharvest conservation of horticultural products in refrigerated chambers.

It is also important to consider that the incorporation of NIRS for online analysis in the industry needs the instrument to be previously evaluated, and a methodology of this analysis must be established and adopted first.

Therefore, the main objective of this PhD dissertation was to develop, evaluate and optimize NIRS models aimed at assuring quality and safety in horticultural products (spinach plants and summer squashes) along the food supply chain, *in situ* (directly on the plant in the field or after harvest in the industry), as well as online, in the sorting processes in the industry. For this purpose, three new generation NIR spectrophotometers, two handheld instruments — one based on Linear Variable Filter (LFV) technology and another based on micro-electro-mechanical systems (MEMS) technology, suitable for the *in situ* analysis of the horticultural products in the field and in the cold chambers — and another based on Fourier Transform (FT)-NIR technology, which can be incorporated in the processing industry, mainly in the product sorting belts, were evaluated.

The results obtained in the research papers that form part of this PhD dissertation showed that NIR spectroscopy can favour the decision-making process in the horticultural sector, allowing to set the optimum harvest time and carry out harvesting strategies in stages, depending on the industrial destination of the product. In particular, in vegetables such as spinach plants and summer squashes, where the nitrate content is a key factor when establishing the destination of the harvested product, the use of NIRS sensors *in situ*, directly on the plant, and online, in the sorting lines in the industry, would facilitate the selection of these vegetables for their possible use in making baby foods, according to the European Union legislation.

In summary, NIR spectroscopy has shown great potential for the non-invasive measurement of quality and safety parameters in vegetables, being one of the approaches best suited to the needs of the horticultural sector and enabling analysis of a larger volume of vegetables. At the same time, NIR spectroscopy has proven to be a powerful tool for process monitoring in real time; this is of interest for industrial applications requiring process control and real-time decision-making.

# Chapter 1

## Chapter 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).

Kader (2002) notes that appearance-related factors are the most-highly rated by consumers when buying fresh products. Texture and flavour are also important for eating quality in horticultural products. Firmness is linked to resistance to mechanical stress during transport. Evaluating flavour quality involves the perception of tastes and aromas of many compounds.

Over recent years, moreover, increasing attention has been paid to the properties of vegetables as a means of preventing disease and staying healthy, in short to what is termed the "nutritional quality". Postharvest losses in nutritional quality, can be substantial, and increase with physical damage, extended storage, high temperatures, low relative humidity, and chilling injury.

Additionally, it must be considered that various quality components are used to evaluate horticultural products in terms of their compliance with specifications for commercial categories, their value for genetic breeding programmes, and their response to a range of environmental factors and postharvest treatments.

Safety factors include levels of naturally-occurring toxicants in certain crops, contaminants such as chemical residues, and heavy metals, and microbial burden; all these must be monitored to ensure compliance with established maximum tolerance levels.

Over recent years, consumers have become increasingly aware of the presence of nitrates in foods, among which are vegetables, since nitrates are serious threat to human health, due to the conversion of nitrate to nitrite, which may produce methaemoglobin due to the oxidation of Fe+2 in haemoglobin (Elia et al., 1998). In response to this growing public concern about nitrates, the European Union passed Commission Regulation (EC) No 1258/2011 of 2 December 2011 setting maximum levels for nitrates in vegetables (OJEU, 2011). Thus, the maximum level for nitrates for processed cereal-based foods and baby foods for infants and young children was set at 200 mg $NO_3$/kg. These regulations highlight the need for nitrate content determination in horticultural products at harvest in order to establish their industrial use.

Due to its swift response, precision, applicability to multiple products and analytes (it is able to provide a number of quality and safety readings simultaneously) and the advanced level of development of the latest generation of instruments, 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 (Nicolaï et al., 2007; Saranwong and Kawano, 2007; Sanchez and Pérez-Marín, 2011; Teixeira Dos Santos et al., 2013; Porep et al., 2015; Yan and Siesler, 2018; Cortés et al., 2019).

Nowadays, there is a wide range of portable instruments of different types in terms of working spectral range, cost and optical design, which are based on different technologies such as MEMS or LVF (Teixeira Dos Santos et al., 2013; Pasquini, 2018; Yan and Siesler, 2018). They represent a clear evolution in the use of NIRS technology: previously, the sample had to be taken to the lab, but now, *in situ* analysis is carried out. The successful use of these sensors allows to analyse the product directly in the field, in order to carry out the quality and safety monitoring of the horticultural products during their growing and to facilitate real-time decision-making for crop management practices and thus decide on the optimal time to harvest. Similarly, these instruments can also be used by the industry for the reception and postharvest conservation of vegetables in refrigerated chambers.

Lately, there has also been an increasing demand in terms of quality and safety assurance by the horticultural products handling and processing industry, and this requires the NIRS applications to be developed in advance, simulating the industrial processes of the horticultural industries (Pasquini, 2018; Cortés et al., 2019).

Faced with such a wide diversity in the characteristics and features of these new generation NIRS sensors, there is a need for them to be evaluated in order to choose which the most suitable for a certain application or a specific product is. Additionally, it is important to consider that the incorporation of NIRS for *in situ* and online analysis needs that a methodology of these analysis must be established and adopted first, including all the issues related to taking spectra and the selection of the optimal spectral region.

In summary, and taking into account the above mentioned, it is necessary to incorporate NIRS technology aimed at assuring quality and safety in vegetables production along the food supply chain, *in situ* — directly on the plant in the field or after harvest in the industry —, as well as online, in the sorting processes in the industry, using new generation NIRS sensors, ones suitable for the analysis of the vegetables in the field and in the cold chambers, and others, which can be incorporated in the vegetables processing industry, mainly in the product sorting belts.

The Departments of Animal Production and Bromathology and Food Technology of the College of Agricultural and Forestry Engineering at Cordoba University (Spain), after a long experience in the traditional analysis of horticultural products, developed a research line in the frame of the research project: Excellence Program, Project P09-AGR-5129 'MEMS and NIRS-image sensors for the *in situ* non-destructive analysis of food and feed'; and also with the assistance of two research projects focussed on the characterization of vegetables (spinach plants and summer squashes) grown in open-air fields in Andalusia founded by Gelagri Ibérica, S.L., in which this PhD dissertation is framed, whose objective is the establishment of a decision-making support system for the horticultural sector based on the NIR spectra fingerprint for the classification and quality assurance and safety of vegetables along the food supply chain. In this content, the objectives of this PhD dissertation are as follows (Chapter 2 of this research work).

Due to the fact that the main objective of this PhD dissertation is the generation of new scientific knowledge for the horticultural sector and with the object of facilitating its dissemination, the results here obtained are shown as a compendium of research articles published in scientific journals.

In order to facilitate the reading, this PhD dissertation has been divided in different chapters:

- In Chapter 1, a general introduction to the different research papers of this PhD dissertation is written.
- In Chapter 2, the objectives of this PhD dissertation are clarified and exposed.
- In Chapter 3, the simultaneous quality assurance and safety in spinach plants in the field and in the industry using a new generation of NIRS sensors, is studied.
- In Chapter 4, instrumental comparison and *in situ* prediction of quality and safety parameters in summer squashes morphotype zucchini, are carried out.
- In Chapter 5, the conclusions of this PhD dissertation are written.

- In Chapter 6, final considerations and recommendations for future R&D&I works, are exposed.
- In Chapter 7, references used are included.

# Chapter 2

# Chapter 2. OBJECTIVES

## 2.1. General objective

The general objective of this PhD dissertation is to analyse the feasibility of NIR spectroscopy as a non-invasive, environment-friendly, versatile (applicable to multiproduct and multiparameter analysis) and accurate technology, capable of being implemented for the *in situ* analysis, in the field and after harvest, and for the online determination, in the industrial processing lines, for the quality assurance and safety of horticultural products and their classification according to reference standards.

## 2.2. Specific objectives

The specific objectives of this PhD dissertation are:

1. Development, evaluation and optimization of 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 a new generation of NIR spectrophotometers. [*This objective was reached in the research articles: 'Monitoring texture and other quality parameters in spinach plants using NIR spectroscopy'. Computers and Electronics in Agriculture 155, 446–452 (2018); 'Simultaneous detection of quality and safety in spinach plants using a new generation of NIRS sensors'. Postharvest Biology and Technology 160, 111026 (2020)*].

2. Development and evaluation of NIRS calibration models for the prediction of quality and safety parameters in spinach plants and in summer squashes using handheld portable NIRS sensors for real-time decision-making and on-vine monitoring. [*This objective was reached in the following research articles*: *'Monitoring texture and other quality parameters in spinach plants using NIR spectroscopy'. Computers and Electronics in Agriculture 155, 446–452 (2018); 'Simultaneous detection of quality and safety in spinach plants using a new generation of NIRS sensors'. Postharvest Biology and Technology 160, 111026 (2020); 'Safety and quality issues in summer squashes using handheld portable NIRS sensors for real-time decision making and on-vine monitoring'. Journal of the Science of Food and Agriculture 99, 6768–6777 (2019)*].

3. Study of the longitudinal nitrate accumulation in summer squash to stablish which zone of the vegetable determines its destination in the processing industry. [This objective was reached in the research article: *'Safety and quality issues in summer squashes using handheld portable NIRS sensors for real-time decision making and on-vine monitoring'. Journal of the Science of Food and Agriculture 99, 6768–6777 (2019)*].

# Chapter 3

# Chapter 3. NIR SPECTROSCOPY FOR *IN SITU* AND ONLINE DETERMINATION OF QUALITY AND SAFETY PARAMETERS IN SPINACH PLANTS

## 3.1. Monitoring texture and other quality parameters in spinach plants using NIR spectroscopy. Computers and Electronics in Agriculture 155, 446–452 (2018)

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

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**Computers and Electronics in Agriculture 155, 446–452 (2018)**

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**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, 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 crosssectional 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, range, mean, 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 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.

The regression coefficients for the best predictive models obtained for the quality parameters analysed are shown in Fig. 1. The regression coefficients for the colour parameters (a* and b*) show that the areas of the spectrum with greater weight in the models are located around 1950 and 2100–2200 nm, which are associated to N–H and O–H stretching modes besides C=O vibration bands. Furthermore, for the texture parameters (maximum puncture force, toughness, stiffness and displacement) analysed, the area at 1650–1780 nm, which corresponds to the first overtone of C–H stretching, also has a great importance. For dry matter, important wavelengths were at around 1790 and 2180 nm which are related to O−H combination and N−H bend second overtone, respectively (Shenk et al., 2008).

### *3.3. External validation*

Validation statistics for the prediction of the quality parameters analysed in intact spinach are shown in Fig. 2.

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. 2, 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**.

Number of samples, 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

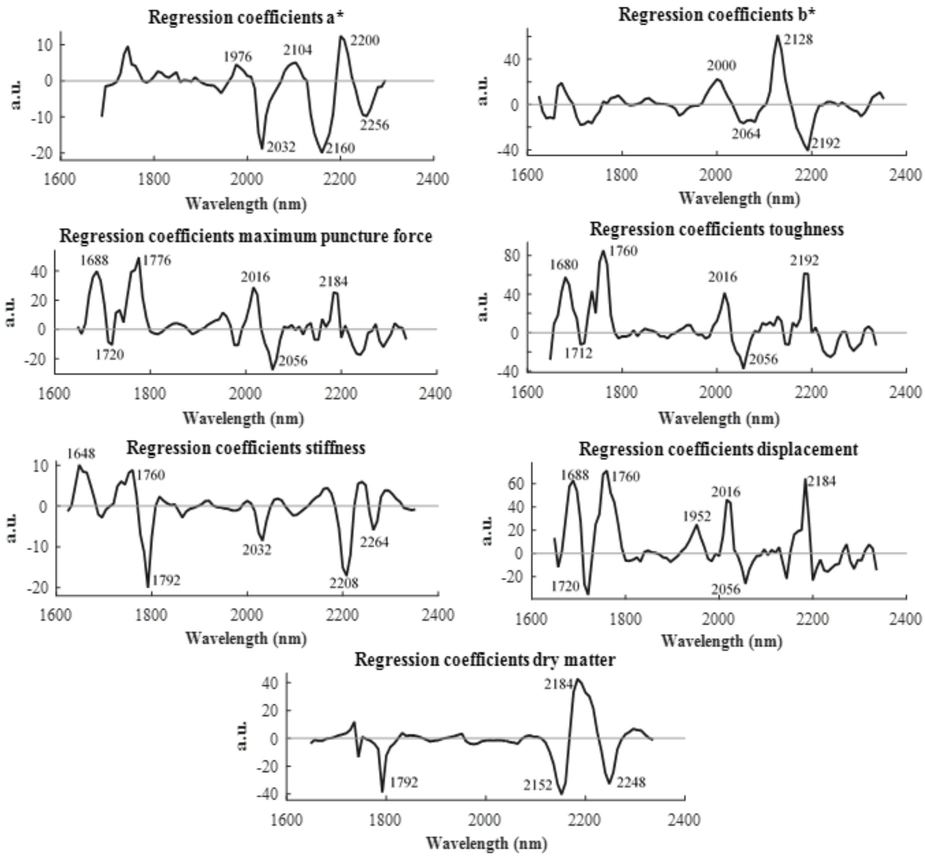
| Parameter | Set | N | Range | Mean | SD | CV (%) |
|---|---|---|---|---|---|---|
| a* | Calibration | 109 | –18.33–(–8.66) | -13.05 | 1.38 | 10.57 |
| | Validation | 36 | –17.32–(–10.78) | -13.34 | 1.23 | 9.22 |
| b* | Calibration | 109 | 11.55–26.40 | 17.94 | 2.77 | 15.44 |
| | Validation | 36 | 13.77–23.02 | 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

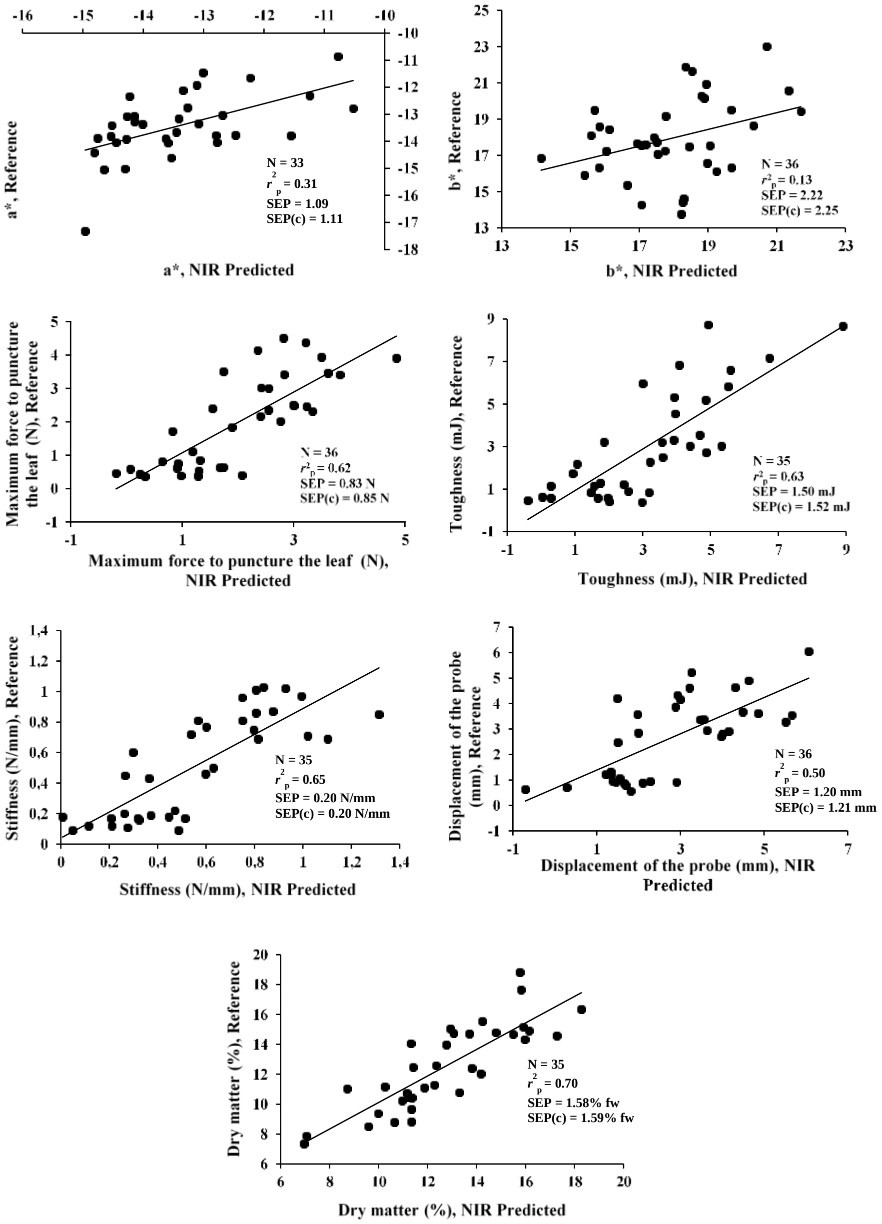
| Parameter | Mathematic treatment | N | Range | Mean | SD | $r^2_{vc}$ | SECV | $RPD_{cv}$ | SEL |
|---|---|---|---|---|---|---|---|---|---|
| a* | 2,10,5,1 | 108 | -18.33-(-8.66) | -13.06 | 1.38 | 0.47 | 1.01 | 1.36 | 0.29 |
| b* | 1,5,5,1 | 105 | 11.55-25.71 | 17.89 | 2.56 | 0.38 | 2.02 | 1.26 | 0.62 |
| 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.** Regression coefficients for colour (a*, b*), texture (maximum puncture force, toughness, stiffness, displacement) and dry matter of intact spinach leaves




* a.u.= arbitrary units

**Fig. 2.** Reference values *versus* NIR-predicted data for the validation set.



### 3.2. Simultaneous detection of quality and safety in spinach plants using a new generation of NIRS sensors. Postharvest Biology and Technology 160, 111026 (2020)

# 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™ 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™ 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-electrico-mechanical 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 $F_{critical\ (1-P,\ n1-1,\ n2-1)}$, 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™ 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™ 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™ 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™ 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™ 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™ 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™ 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™ 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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**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 | $F_{critical}$ |
|---|---|---|---|---|
| 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™ 1700 (B).

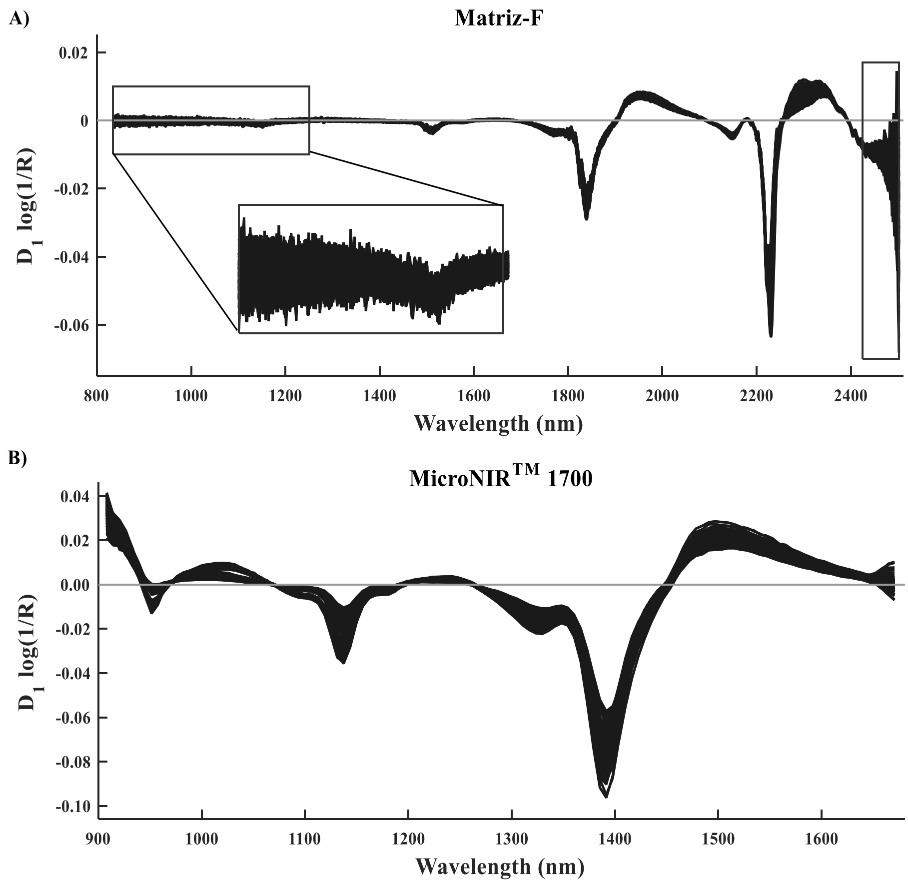
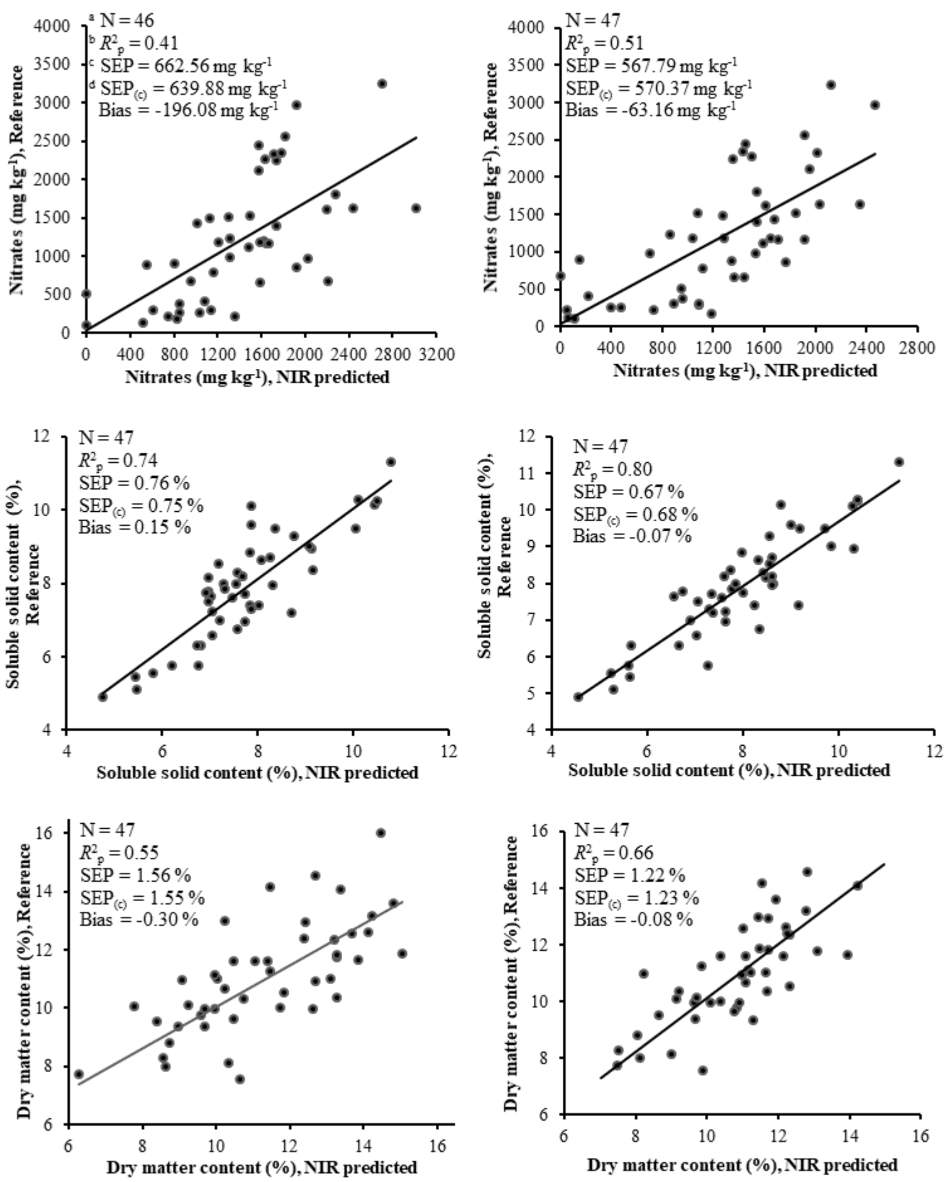
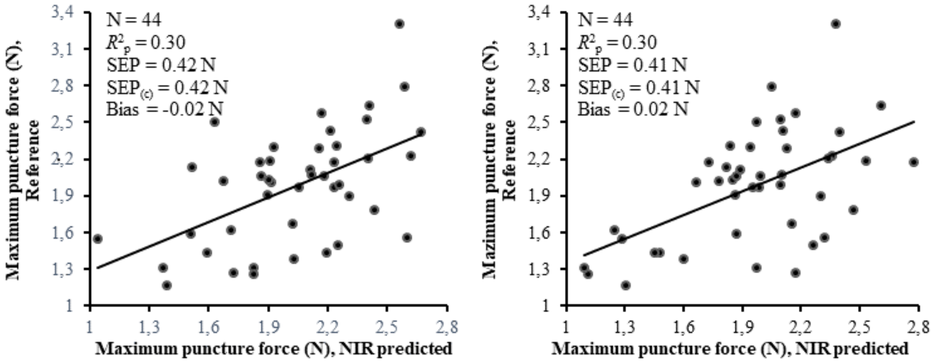


**Fig. 2.** Reference and NIR predicted values for quality and safety parameters with Matrix-F (A) and MicroNIR™ 1700 (B) instruments.

**A) Matrix-F** **B) MicroNIR™ 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.

# Chapter 4

# Chapter 4. NIRS ANALYSIS OPTIMIZATION, INSTRUMENTAL COMPARISON AND ON-VINE PREDICTION OF SAFETY AND QUALITY PARAMETERS IN SUMMER SQUASH

## 4.1. Safety and quality issues in summer squashes using handheld portable NIRS sensors for real-time decision making and on-vine monitoring. Journal of the Science of Food and Agriculture 99, 6768–6777 (2019)

# Safety and quality issues in summer squashes using handheld portable NIRS sensors for real-time decision making and on-vine monitoring

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**Abstract**

**BACKGROUND: Portable handheld near infrared spectroscopy (NIRS) instruments currently present enormous advantages in terms of size, weight and robustness. They also provide fast, precise information that can be obtained *in situ*, and represent a viable option for controlling vegetable safety and quality during the growth period. The aim of this research was to evaluate three handheld portable NIRS instruments for *in situ* and real time analysis of intact summer squashes. 221 summer squashes were analyzed by traditional methods and used to develop calibration models for morphological, safety and quality parameters. Additionally, the longitudinal distribution of nitrate content in summer squashes weighing over 400 g was also studied, and the evolution of this parameter during the harvest period was also tracked to determine which summer squashes and which zones (peduncle, equatorial or stylar) of the vegetable could be earmarked for baby food production.**

**RESULTS: The robustness of the calibration models obtained confirmed the expectations raised by NIRS technology for morphological, safety and quality control of individual summer squashes, and the models developed with the MicroNIR-1700 instrument were those which proved more accuracy and precision, being the peduncle zone the part that presents a higher content in nitrates.**

**CONCLUSIONS: It is in the peduncle zone, therefore, where measurements of this parameter must be carried out to decide on the destination of the harvested product. Additionally, summer squashes picked at the end of the harvest are those which must be used for baby food production.**

**Keywords:** Summer squash; Portable NIR sensor; *In situ* determination; Safety and quality parameters; Nitrate content; Baby foods.

## INTRODUCTION

Near infrared spectroscopy, which can be defined as a non-invasive environmentally-friendly technique which combines versatility, speed, ease of use and accurate measurements with the low cost of each analysis, offers the possibility of measuring safety and quality attributes in fruits and vegetables.[1] This facilitates its incorporation at different decision-making steps in the agri-food chain, both in the pre-harvest and harvest periods in the field, and in the post-harvest period, in the processing industry.[1-4]

Currently, portable, handheld and compact-design NIRS instruments are in full development and expansion.[5,6] These portable devices run on batteries and offer huge advantages in terms of size, weight and robustness of the analysis in uncontrolled environmental conditions, in addition to being cheaper to acquire in comparison with the classic laboratory instruments.

Nowadays, there is a wide range of portable instruments of different types in terms of working spectral range, cost and optical design, which are based on different technologies such as micro-electro-mechanical system (MEMS) or linear variable filters (LVF). They represent a clear evolution in the use of NIRS technology: previously, the sample had to be taken to the lab, but now, an *in situ* analysis is carried out.[7] Faced with such a wide diversity in the characteristics and features of these portable NIRS sensors, there is a need for them to be evaluated in order to choose which is the most suitable for a certain application or a specific product.

The use of portable NIRS sensors can favor the decision-making process in the horticultural sector, allowing to set the optimum harvest time and carry out harvesting strategies in stages, depending on the industrial destination of the product.[8-10] In particular, in vegetables such as summer squashes, where the nitrate content is a key factor when establishing the destination of the harvested product, the use of a handheld NIRS sensor, *in situ*, directly on the plant, would facilitate the selective harvesting of this vegetable for its possible use in making baby foods (the maximum level for nitrates in processed cereal-based foods and baby foods for infants and young children is set at 200 mg $NO_3$ $kg^{-1}$), according to the European Union legislation.[11]

Likewise, despite the fact that numerous studies carried out on nitrate accumulation in plants have found that the concentration of this substance depends on a number of different factors – plant biology, daylight intensity, soil type, temperature, humidity, sowing density, plant maturity, vegetation period, harvesting period and nitrogen source[12] –the order of nitrate content accumulation in the different organs has only been established (petiole > leaf > stem > roots > inflorescence > tuber > fruit > seed), while the nature of nitrate accumulation inside the fruit and which edible part contains the nitrate has not been studied.[13] This differs for this parameter and for summer squashes from other quality parameters such as dry matter and soluble solid content (SSC) and from other fruits such as melon, where the variation inside the fruit has been widely researched.[14,15] This is a vital factor in determining the key zones for analysis and also for saving certain parts of this vegetable for more critical destinations, such as baby foods.

Sánchez *et al.*[9] determined safety and quality parameters in summer squashes on the plant, using an NIRS instrument, Phazir 2400, based on MEMS technology. However, the recent arrival of modern commercial sensors has led to the phasing out of many of the earlier models, such as the Phazir 2400 mentioned above. For this reason, the efficiency of these new portable devices in horticultural applications needs to be assessed.

The main objective of this research work is therefore to evaluate and compare handheld, portable NIRS instruments when used to assess the safety and quality of summer squashes on the plant. It also aims to study the longitudinal nitrate accumulation in this fruit to stablish which zone of the vegetable (peduncle, equatorial or stylar) contains a greater accumulation of nitrates and is, therefore, the key zone to be analyzed, and the one which determines the destination of the vegetable in the processing industry.

## MATERIALS AND METHODS

### Sampling and reference methods

A total of 221 summer squashes (126 below 400 g in weight and 95 over 400 g) *(Cucurbita pepo* subsp. *pepo* morphotype zucchini cv. Mirza), grown in an open-air plantation in the district of La Montiela, Santaella (Córdoba, Spain), were harvested between May and July 2017. On arrival at the laboratory, the fruits were promptly placed in refrigerated storage at 5 ºC and 85% relative humidity. Prior to measurement, each sample was left at room temperature to stabilize at the laboratory temperature of 20 ºC.

The summer squashes were individually weighed on an electronic balance (0–1,000 ± 0.01 g; model P1000 N, Metter-Toledo, GmbH, Greifensee, Switzerland). Their length was measured using a measuring tape and the equatorial diameter was then measured using a digital precision caliper (0–300 ± 0.01 mm; Comecta, Barcelona, Spain).

Nitrate content, dry matter and SSC were measured following Sánchez *et al.*[9]. To analyze these parameters in summer squashes weighing over 400 g, the fruit was divided into three zones: the peduncle zone (upper third of the squash starting at the peduncle), the equatorial zone (middle third in the equator of the fruit) and the stylar zone (lower third of the fruit, starting at the pistil scar). All the analytical measurements were performed immediately after NIR spectrum collection and in duplicate.

### Spectral data collection

The NIR spectra of the intact summer squashes were collected in reflectance mode (log 1/R) using three handheld NIRS instruments:

- Phazir 2400, a handheld MEMS-based NIR digital transform spectrometer (Polychromix, Inc., Wilmington, MA, USA). This compact, robust spectrometer weighing 1.7 kg is specially designed for *in situ* NIRS analysis. The equipment scans at a non-constant interval of approximately 8 nm, across the NIR wavelength range of 1600 to 2400 nm, with a window area of around 55 $mm^2$. The sensor integration time was 600 ms and each spectrum was the mean of 5 scans. This instrument is equipped with special quartz protection to prevent dirt from accumulating. The instrument's performance was checked every 10 min, following the diagnostic protocols provided by the manufacturer, and white reference measurement was carried out using Spectralon™ (a NIR reflectance standard with a 99% diffuse reflectance) as reference. Although the model has now been phased out, it was used as a reference sensor to compare with the other instruments.

- MicroPhazir, a handheld MEMS-based NIR digital transform spectrometer (Polychromix Inc., Wilmington, MA, USA). This model is an updated version of the Phazir 2400 and its instrumental design and optical features are therefore, very similar: it is a pistol-shaped device which is portable, compact and robust. The window area is at around 41 $mm^2$ and it works in the spectral range of 1600 to 2400 nm with a non-constant interval of 8 nm. However, it is much lighter (1.2 kg) than its predecessor, which makes it more comfortable when analyzing the product. Unlike the former, it has an internal reference which enables easy calibration in the field. The sensor integration time was 600 ms and each spectrum was the mean of 5 scans. The device is equipped with quartz protection to prevent dirt accumulation.
- A MicroNIR-1700 LVF spectrometer (VIAVI Solutions, Inc., San Jose, California, USA). This portable miniature spectrometer is extremely light (64 g, without including the handle of 150 g and the acquisition and data processing device). Its optical window is larger than that of the previous equipments (the measurement area is around 227 $mm^2$). This microspectrometer covers a 910 to 1676 nm spectral range, with a constant interval of 6.2 nm. The instrument's performance was checked every 10 min. A white reference measurement was obtained using Spectralon™, while a dark reference was obtained from a fixed point in the room. The sensor integration time was 11 ms and each spectrum was the mean of 200 scans.

The main features of these instruments are summarized in Table 1.

To collect NIR spectra using these three spectrometers, the fruits, regardless of weight, were divided into the three zones (peduncle, equatorial and stylar) mentioned above.

Four spectral measurements were taken in each of the three zones analyzed, the first at a random location in the center of the analyzed zone, which were then rotated 90º after each measurement, thus obtaining 12 spectra per summer squash.

The 12 spectra were averaged to provide a mean spectrum per fruit in the case of summer squashes weighing below 400 g (126 spectra), for all the parameters analyzed.

For summer squashes weighing over 400 g, the same procedure as described above for taking the spectra was carried out. To develop predictive models of the morphological (weight, length and equatorial diameter) parameters, an average was taken of the 12 spectra obtained initially, resulting in a single spectrum per fruit, which produced a total of 95 spectra. However, taking into account the fact that the analysis of nitrate content, dry matter and SSC were carried out by zones, the 4 spectra corresponding to each of the studied zones were averaged, thus obtaining an average spectrum per zone – i.e. a total of 285 spectra (95 fruits · 3 zones/fruit · 1 spectrum/zone).

**Data processing**

Data pre-processing and chemometric treatments were performed using the WinISI II software package version 1.50 (Infrasoft International LLC, Port Matilda, PA, USA).[16]

Before the spectral data were processed, a study was conducted to select the most suitable spectral range for the instruments tested to carry out the morphological, safety and quality control of summer squashes. 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 highlights the areas of the spectrum where the signal/noise ratio is degraded.[17]

*Spectral repeatability*

Spectrum quality was evaluated using the root mean square (RMS) statistic. The RMS statistic is defined as the averaged root mean square of differences between the different subsamples scanned at *n* wavelengths.[18,19] This statistic indicates the similarity between different spectra of a single sample. To establish a threshold for this statistic, two strategies were tested. In the first, the repeatability was measured considering only the spectral information collected in the center of the peduncle zone of the summer squashes, after rotating the product 90º between each measurement, using 10 samples for each type of summer squash analyzed. In the second strategy, 12 spectra (3 zones * 4 spectra/zone) were taken following the same procedure and number of samples above mentioned. An admissible limit for spectrum quality and repeatability was set following the procedure described by Martínez *et al.*[20] to calculate the standard deviation limit ($STD_{limit}$) from the RMS statistic and obtain an RMS cut-off value.

*Definition of calibration and validation sets*

Prior to carrying out NIRS calibrations, the CENTER algorithm was applied to ensure a structured population selection based solely on spectral information, for the establishment of calibration and validation sets.[21] This algorithm performs an initial principal component analysis to calculate the center of the population and the distance of samples (spectra) from that center in an n dimensional space, using the Mahalanobis distance (GH); samples with a GH value > 4 were considered outliers or anomalous spectra. A combination of mathematical pretreatments, standard normal variate (SNV) and de-trending (DT) was applied for scatter correction,[22] together with the 1,5,5,1 derivate mathematical treatment.[16,18]

To predict the morphological parameters, the CENTER algorithm was applied to the 221 spectra obtained after averaging the 12 spectra taken of each fruit while for the prediction of the safety and quality parameters, and since the analysis was performed by zones in summer squashes weighing over 400 g, the CENTER algorithm was applied to the 411 available spectra.

Having ordered the sample set by spectral distances and once the spectral outliers were removed, NIRS calibration models for the prediction of morphological, safety and quality parameters in intact summer squashes were initially constructed using the calibration sets comprising all the available samples (C1 = 217 samples for morphological parameters and C2 = 407 samples for safety and quality parameters). After analyzing the accuracy and precision of the models obtained and evaluating the three instruments, new calibration models were developed for these parameters using the most suitable instrument. For this purpose, the samples forming the validation set were selected by taking one sample out of every four from the initial sets (C1 and C2). After this procedure, the calibration (C3 = morphological parameters and C4 = nitrate content, dry matter and SSC) and validation (V3 = morphological parameters and V4 = nitrate content, dry matter and SSC) sets thus comprised the samples shown in Table 2.

*Data pre-processing and calibration model construction using a linear regression strategy*

NIRS calibration models for the prediction of morphological, safety and quality parameters in intact summer squashes were initially constructed with the calibration sets C1 and C2 respectively, using modified partial least squares regression,[23] with subsequent cross-validation. The calibration set was divided into 4 groups; each group was then validated using a calibration developed for the other samples; finally, validation errors were combined to obtain a standard error of cross-validation (SECV).

For each analytical parameter, different mathematical pretreatments were evaluated. For scatter correction, SNV and DT methods were tested.[22] Additionally, a total of two mathematical derivation treatments were tested: 1,5,5,1; 2,5,5,1.[16,18]

The statistics used to select the best equations were the coefficient of determination for cross-validation ($r^2_{cv}$), and the SECV. Furthermore, the residual predictive deviation ($RPD_{cv}$) for cross-validation was calculated as the ratio of the standard deviation (SD) of the reference data to the SECV. This statistic enables SECV to be standardized, facilitating the comparison of results obtained with sets of different means.[24]

Once the best predictive model for each parameter was selected without the elimination of physical-chemical outliers, tests were run for significant differences between models, with a view to identifying the most suitable spectrometer for routine use in on-vine summer squashes during the growing period. The SECV values for the best equations obtained for each parameter with the three instruments were compared using Fisher's F test.[25,26] The values for F were calculated as:

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

where $SECV_1$ and $SECV_2$ are the standard error of cross validation of two different models and $SECV_1 < SECV_2$. F is compared to $F_{critical}$ (1-*P*, $n_2$-1, $n_1$-1) read from the table with $P = 0.05$ and n-1 degrees of freedom. If F is higher than $F_{critical}$, the two SECV values are significantly different. When several SECV values are compared, as in this research, a $SECV_{confidence\ limit}$ is calculated using the following formula: $SECV_{confidence\ limit} = SECV_{min} \sqrt{F_{critical}}$ where $SECV_{min}$ is the smallest SECV. As a consequence, none of the models which have a SECV between $SECV_{min}$ and $SECV_{confidence\ limit}$ are significantly different.

Finally, once the best NIRS instrument was chosen, new models were developed (optimizing the performance models parameters) with that spectrometer using the C3 and C4 calibration sets. The best-fitting equations obtained for these new calibration sets, as selected by the same statistical criteria mentioned above, were subsequently subjected to external validation using the prediction sets V3 and V4, respectively, following the protocol outlined by Windham *et al*.[27]

**Statistical analysis**

In order to study the influence of both the harvest date and the zone analyzed, as well as the harvest date x zone interaction, in the nitrate content (wet analysis) of summer squashes weighing over 400 g, a two-factor analysis of variance (ANOVA) was carried out using Statgraphics Centurion XV (StatPoint Inc., Warrenton, North Virginia, USA).

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

## RESULTS AND DISCUSSION

**Nitrate content evolution during the harvest period**

Nitrate content in summer squashes weighing over 400 g were significantly influenced ($P < 0.05$) by harvest date and analyzed zone but not by the harvest date x zone interaction (Table 3). For each of the analyzed zones, the nitrate content decreased significantly ($P < 0.05$) as the harvest period progressed, reaching minimum values on the last harvesting day (07/13/2017). As regards the zone analyzed, the nitrate content was significantly higher ($P < 0.05$) in the peduncle zone, which indicates that it is here where this substance accumulates the most. It is also worth noting that there is a significantly lower nitrate content in the equatorial zone ($P < 0.05$). Therefore, when determining the destination for the summer squashes after harvesting, both farmers and the processing industry should carry out the NIRS and wet analysis to measure the nitrate content present in the peduncle zone of the vegetable. It is also recommended to use end-of-harvest summer squashes to elaborate baby foods (nitrate content < 200 mg $kg^{-1}$), since the nitrate content in the three analyzed zones of these vegetables is below the limits authorized by the European Union.[11]

**Optimal spectral region and spectral repeatability**

Before developing the models, the NIRS analysis of summer squashes had to be optimized in order to obtain a representative and quality spectrum per fruit or per zone, which in turn enable to obtain robust models when defining their safety and quality characteristics and to assess their possible industrial use in the baby food industry.

The existence of noise in the spectrum was then evaluated (spectral range 1600-2400 nm for the two MEMS spectrometers and 910-1676 nm for the LFV instrument).[17] After this process, the spectral range between 2240–2400 nm was eliminated in the Phazir 2400 and in the MicroPhazir due to the high level of noise detected (Fig. 1). Thus, all the models subsequently developed with these instruments were designed using the spectral range 1600–2240 nm. In the case of the MicroNIR-1700 instrument, as shown in Fig. 1, it does not produce high noise levels when working between 910-1676 nm, and for this reason, the full spectral range of the instrument was used.

Table 4 shows the $STD_{limit}$ values for the two strategies tested, using the three NIRS spectrometers. It is clearly shown that the values given by the $STD_{limit}$ were lower when only 4 spectra per fruit were taken in one particular zone; in this case, the peduncle zone was chosen because of the greater accumulation of nitrates. As a result, to determine the destination of the analyzed fruit, for the two types of summer squash and NIRS instruments, it would be enough to perform the NIRS and laboratory analysis only in this zone.

As can be seen, the lowest $STD_{limit}$ values were obtained with the MicroNIR-1700 instrument for the two strategies and types of summer squashes, while the results were reasonably similar in the two MEMS devices compared.

Once the RMS value did not exceed the value of the $STD_{limit}$, the spectra were then averaged.

The calculation of the RMS statistic is of extreme importance because it aims to ensure the spectral repeatability, which is essential for obtaining high quality spectral data, and therefore constitutes an essential step in obtaining robust equations.

No values for this statistic have been found in the scientific literature for summer squashes analyzed either whole or in zones on the vine, although the

RMS statistic is extremely useful for obtaining representative spectral libraries of this vegetable, when they are analyzed on the plant.

**Spectral properties**

The mean log (1/R) spectra, together with the most relevant absorption bands for intact summer squashes scanned with Phazir 2400, MicroPhazir and MicroNIR-1700 are shown in Fig. 2.

In the 1600-2240 nm wavelength region for the two MEMS spectrometers tested, the major absorption peak at around 1920 nm is mainly related to water absorption, while there is another peak at 1780 nm, related to the first overtone of C-H stretching bonds.[28]

The mean spectrum obtained with MicroNIR-1700 shows a peak at 1450 nm related to the first overtone of the O-H group, as well as to the N-H stretch first overtone. [28] Moreover, the peak corresponding to the second overtone of O-H group can be seen at 970 nm.[29] Another peak can also be observed at approximately 1170 nm, which is linked to the second overtone of the C-H groups.[28]

**Choice of the best handheld, portable NIRS instrument for *in situ* morphological, safety and quality determinations in summer squashes**

Table 5 shows the statistics for the best calibration models obtained to predict the parameters studied using the three instruments tested. In order to compare the three spectrometers, the calibration models for the different parameters in the study were carried out without eliminating the physical-chemical outliers during their development, which means that the values for mean, range and SD for each parameter are the same (Table 2).

Once the calibration models for the analyzed parameters were developed for each of the instruments tested, the SECV statistic values obtained for each parameter were compared. As can be seen in Table 6, the SECV values corresponding to the weight and nitrate content parameters obtained with the MicroNIR-1700 are significantly lower ($P < 0.05$) than for the other two instruments used. As regards length, the SECV values obtained with the Phazir

2400 and MicroNIR-1700 are significantly lower ($P$ < 0.05) than those obtained with the MicroPhazir. For the rest of the parameters, no significant differences ($P$ > 0.05) were found between the SECV values for the predictive models with the three instruments.

In view of the results obtained, the MicroNIR-1700 instrument therefore, appears to be the most suitable for the analysis of the morphological, safety and quality parameters in summer squashes *in situ,* directly on the plant.

**New calibration models for predicting morphological, safety and quality parameters in summer squash and external validation**

After comparison of the spectrometers using the same number of samples, new calibration models with the sets C3 and C4 were constructed, but this time eliminating physical-chemical outlier samples if necessary; only the MicroNIR-1700 spectrometer was used for this purpose. The calibration statistics for the best models are shown in Table 7.

In the case of the morphological parameters, the models developed for the parameters of weight and equatorial diameter showed a predictive capacity ($r^2_{cv}$ = 0.84, $RPD_{cv}$ = 2.49) which could be considered good for both parameters, following the interpretation of the coefficient of determination values proposed by Shenk and Westerhaus[19] and Williams[24] while Nicolaï *et al*.[1] state that a $RPD_{cv}$ value of between 2 and 2.5 indicates that coarse quantitative predictions are possible. As for length, the predictive capacity of the developed model ($r^2_{cv}$ = 0.72; $RPD_{cv}$ = 1.87) can be considered good,[19,24] while in Nicolaï *et al*.[1], the $RPD_{cv}$ between 1.5 and 2 means that the model can discriminate between low and high values of the response variable.

The results obtained in this research are similar to those obtained by Sánchez *et al*.[30] for the prediction of the morphological parameters of weight ($RPD_{cv}$ = 2.88), length ($RPD_{cv}$ = 2.42) and equatorial diameter ($RPD_{cv}$ = 2.26), using the Phazir 2400 in reflectance mode in the spectral range of 1600-2400 nm.

The satisfactory predictive capacity obtained for these morphological parameters using the handheld instrument MicroNIR-1700 is associated to the correlation between the size of the product and its water content. The absorption level of the light is highly dependent on the variation of these parameters, so it is possible to correlate the NIR signal with morphological parameters.[31]

As regards the determination of nitrate content, the model's predictive capacity ($r^2_{cv}$ = 0.68; $RPD_{cv}$ = 1.78) allows to discriminate between high, medium and low values, following the guidelines of Shenk and Westerhaus[19] and Williams,[24] and between high and low values according to the $RPD_{cv}$ values suggested by Nicolaï *et al*.[1]

As far as measuring this parameter with NIRS technology, Sánchez *et al*.[9] obtained predictive capacity models ($RPD_{cv}$ = 1.91) similar to the one obtained here ($RPD_{cv}$ = 1.78) with the MicroNIR-1700 instrument.

Predicting the content in dry matter and soluble solids in summer squashes is extremely important in order to decide on the optimum moment for harvesting. The predictive capacity of the models for these parameters allows to differentiate between high, medium and low values for dry matter and between high and low values for SSC.[19,24]

As Fearn[32] 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—and on the SD of the reference values—of the calibration set. This would account for the lower $r^2_{cv}$ values recorded here for both parameters, due to the reduced SD values shown.

Dardenne[33] and Fearn[32] have also shown that the $RPD_{cv}$ statistic is equal to $1/\sqrt{1-r^2_{cv}}$ and depends to the same degree as $r^2_{cv}$ on the range and SD of the data in the calibration set. This view is borne out by the results obtained here (Table 7), which indicate a close match between the highest and lowest $r^2_{cv}$ values and $RPD_{cv}$ values for the parameters tested.

Sánchez *et al*.[9] measuring dry matter and SSC in summer squashes obtained $RPD_{cv}$ values of 1.75 and 1.56, respectively, using the Phazir 2400. The predictive capacity of both models is slightly higher than that obtained here, because the authors were able to use calibration sets with more variability.

Validations of the best calibration models obtained with calibration sets C3 and C4 were performed using the sets V3 and V4, respectively (Fig. 3). It is important to point out that some samples (N = 4 samples for weight; N = 2 samples for length; N = 3 equatorial diameter; N = 6 samples for nitrate content, N = 4 samples for dry matter, N = 1 sample for SSC), which were initially part of the V3 and V4 validation sets, were eliminated before the validation procedure was carried out with the calibration models developed with the MicroNIR-1700 instrument, due to they were hardly represented in the calibration sets with which the predictive models were finally designed.

Likewise, as regards the prediction of nitrate content, 4 samples which had a lower nitrate content (values below 103 mg $kg^{-1}$) were predicted by the models assigning them negative values for this parameter. However, the predictive NIRS values for these samples were shown as zero in Fig. 3.

After studying the results of the validation models, it can be affirmed that the standard error of prediction (SEP) values obtained are comparable to those from SECV, for the parameters tested. It is confirmed that the SECV is a good estimator of the SEP.[34]

According to the validation protocol established by Windham *et al*.[27] and once the results shown in Fig. 3 were analyzed, the models constructed for predicting all the morphological parameters analyzed, and also for the prediction of dry matter in intact summer squashes, 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 models thus ensure accurate prediction, and can be applied routinely. For the parameters nitrate content and SSC, it should be stressed that the $SEP_{(c)}$ and bias lay within the confidence limits, and although, $r^2_p$ values did not attain the recommended minimum value ($r^2_p$ =0.55 and 0.57 for nitrate content and SSC, respectively), they were close. Therefore, the results obtained suggest that the NIRS models developed can be regarded as a useful preliminary trial for obtaining accurate on-vine morphological, safety and quality predictions for intact summer squashes.

Furthermore, the external validation results obtained in this research for the morphological parameters of weight ($RPD_p$ = 3.09), length ($RPD_p$ = 2.37) and equatorial diameter ($RPD_p$ = 3.10) are superior to those reported by Sánchez *et al*.[30] ($RPD_p$ = 2.49, $RPD_p$ = 1.59 and $RPD_p$ = 1.67 for the three parameters mentioned above, respectively). For the nitrate content, the external validation value of RPD statistic ($RPD_p$ = 1.60) is slightly lower than that obtained by Sánchez *et al*.[9] ($RPD_p$ = 1.93) while for dry matter and SSC, the $RPD_p$ values here obtained (1.52 and 1.84, respectively) were higher than those reported by the authors cited ($RPD_p$ = 1.32 for dry matter; $RPD_p$ = 1.22 for SSC).

## CONCLUSIONS

The results obtained suggest that the greatest accumulation of nitrates in summer squashes takes place in the peduncle zone, and it is this area which must be analyzed to determine the destination of the harvested product. In addition, the summer squashes harvested at the end of the harvesting time should be the ones which are destined for baby food production, since they have nitrate values of below 200 mg $kg^{-1}$.

The findings also confirm the expectations raised that NIRS technology can enable intact summer squashes to be selectively harvested according to their morphological, safety and quality characteristics and to establish their industrial destination non-destructively. Additionally, the three NIRS instruments tested provided a similar level of accuracy for the measurement of equatorial diameter, dry matter and SSC. However, for weight, length and nitrate content, significantly more accurate models were obtained with the LVF instrument. The MicroNIR-1700 instrument is therefore, the most suitable for measuring, *in situ*, the morphology, safety and quality of summer squash.

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**Table 1.** Technical features of the spectrometers Phazir 2400, MicroPhazir and MicroNIR-1700.

| Property | Instrument | | |
|---|---|---|---|
| | Phazir 2400 | MicroPhazir | MicroNIR-1700 |
| Detector type | Single-element InGaAs detector | Single-element InGaAs detector | 128-pixel InGaAs photodiode array |
| Dispersion element | MEMS | MEMS | LVF |
| Wavelength range (nm) | 1600-2400 | 1600-2400 | 910-1676 |
| Resolution (nm) | ≈ 8 | ≈ 8 | 6.2 |
| Sampling integration time (ms) | 600 | 600 | 11 |
| Weight (kg) | 1.7 | 1.2 | $64 \cdot 10^{-3}$ |
| Analysis mode | Reflectance | Reflectance | Reflectance |

**Table 2.** Number of samples (N), range, mean, SD, and coefficient of variation (CV) for the different calibration (C1, C2, C3 and C4) and validation (V3 and V4) sets.

| | Parameters | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Weight (g) | | | Length (cm) | | | Equatorial diameter (mm) | | | Nitrates (mg $kg^{-1}$) | | | Dry matter (% fw) | | | SSC (ºBrix) | | |
| | C1 | C3 | V3 | C1 | C3 | V3 | C1 | C3 | V3 | C2 | C4 | V4 | C2 | C4 | V4 | C2 | C4 | V4 |
| N | 217 | 163 | 54 | 217 | 163 | 54 | 217 | 163 | 54 | 407 | 306 | 101 | 407 | 306 | 101 | 407 | 306 | 101 |
| Range | 78.43-1746.49 | 78.43-1746.49 | 125.12-1135.89 | 12.83-43.50 | 12.83-43.50 | 16.60-43.00 | 28.02-89.58 | 28.02-89.58 | 30.48-83.95 | 18.50-1979.96 | 18.50-1979.96 | 55.92-1209.18 | 3.16-7.56 | 3.16-7.56 | 3.61-7.25 | 2.80-6.50 | 2.80-6.50 | 2.80-5.70 |
| Mean | 463.92 | 457.39 | 483.51 | 24.12 | 24.05 | 24.33 | 52.57 | 52.17 | 53.8 | 362.61 | 356.55 | 380.96 | 4.69 | 4.68 | 4.71 | 4.13 | 4.13 | 4.14 |
| SD | 302.38 | 300.09 | 311.21 | 5.63 | 5.67 | 5.57 | 14.29 | 14.16 | 14.75 | 292.67 | 299.06 | 272.99 | 0.72 | 0.68 | 0.83 | 0.47 | 0.46 | 0.50 |
| CV | 65.18 | 65.61 | 64.36 | 23.34 | 23.58 | 22.89 | 27.18 | 27.14 | 27.42 | 80.71 | 83.88 | 71.66 | 15.38 | 14.53 | 17.62 | 11.38 | 11.14 | 12.08 |

**Table 3.** Evolution of nitrate content in the three zones analyzed during the harvest period in summer squashes weighing over 400 g.

| Harvest date | Nitrate content (mg $kg^{-1}$) | | |
|---|---|---|---|
| | Peduncle zone | Equatorial zone | Stylar Zone |
| 17 May 2017 | 1056.55 (502.58)(a) | 758.96 (359.49)(b) | 827.43 (330.33)(a,b) |
| 22 May 2017 | 520.87 (96.49)(e) | 405.10 (34.64)(f) | 464.98 (64.30)(e,f) |
| 31 May 2017 | 750.77 (249.37)(c) | 624.78 (180.81)(d) | 694.48 (208.89)(c,d) |
| 05 June 2017 | 516.51 (225.14)(e) | 452.77 (164.12)(f) | 500.39 (193.19)(e,f) |
| 12 June 2017 | 343.34 (157.49)(g) | 283.95 (106.10)(h) | 320.45 (109.02)(g,h) |
| 20 June 2017 | 193.85 (109.82)(i,k) | 175.56 (92.51)(j,l) | 201.07 (114.18)(i,j,k,l) |
| 26 June 2017 | 240.00 (133.85)(i) | 213.71 (125.67)(j) | 225.39 (127.70)(i,j) |
| 06 July 2017 | 181.73 (43.09)(i,k) | 153.39 (49.86)(j,l) | 172.74 (61.87)(i,j,k,l) |
| 13 July 2017 | 91.25 (68.94)(k) | 66.00 (50.91)(l) | 84.25 (66.11)(k,l) |

Standard deviation in brackets.

The same letter indicates homogeneous group established by ANOVA ($P < 0.05$).

**Table 4.** $STD_{limit}$ (μlog(1/R) of the RMS statistic for summer squashes analyzed on-vine.

| Spectrometer | Summer squash | | | |
|---|---|---|---|---|
| | Weight > 400 g | | Weight < 400 g | |
| | Strategy I 4 spectra | Strategy II 12 spectra | Strategy I 4 spectra | Strategy II 12 spectra |
| Phazir 2400 | 53,822 | 65,290 | 52,659 | 62,893 |
| MicroPhazir | 44,304 | 61,560 | 49,177 | 63,818 |
| MicroNIR-1700 | 29,205 | 29,711 | 47,533 | 51,784 |

**Table 5.** Calibration statistics for NIR-based models for predicting morphological, safety and quality parameters in intact summer squash.

| Parameter | Instrument | Math treatment | SECV | $r^2_{cv}$ | $RPD_{cv}$ |
|---|---|---|---|---|---|
| Weight (g) | Phazir 2400 | 1,5,5,1 | 155.91 | 0.73 | 1.94 |
| | MicroPhazir | 2,5,5,1 | 161.07 | 0.72 | 1.88 |
| | MicroNIR-1700 | 2,5,5,1 | 142.48 | 0.78 | 2.12 |
| Length (cm) | Phazir 2400 | 1,5,5,1 | 3.31 | 0.65 | 1.70 |
| | MicroPhazir | 1,5,5,1 | 3.40 | 0.64 | 1.66 |
| | MicroNIR-1700 | 1,5,5,1 | 3.11 | 0.69 | 1.81 |
| Equatorial diameter (mm) | Phazir 2400 | 1,5,5,1 | 6.34 | 0.80 | 2.25 |
| | MicroPhazir | 1,5,5,1 | 6.70 | 0.78 | 2.13 |
| | MicroNIR-1700 | 2,5,5,1 | 6.22 | 0.81 | 2.30 |
| Nitrate content (mg $kg^{-1}$) | Phazir 2400 | 2,5,5,1 | 240.03 | 0.33 | 1.22 |
| | MicroPhazir | 1,5,5,1 | 226.02 | 0.40 | 1.29 |
| | MicroNIR-1700 | 1,5,5,1 | 198.07 | 0.54 | 1.48 |
| Dry matter (% fw) | Phazir 2400 | 2,5,5,1 | 0.53 | 0.46 | 1.36 |
| | MicroPhazir | 2,5,5,1 | 0.53 | 0.47 | 1.36 |
| | MicroNIR-1700 | 1,5,5,1 | 0.51 | 0.50 | 1.41 |
| SSC (ºBrix) | Phazir 2400 | 1,5,5,1 | 0.36 | 0.43 | 1.31 |
| | MicroPhazir | 1,5,5,1 | 0.35 | 0.45 | 1.34 |
| | MicroNIR-1700 | 1,5,5,1 | 0.33 | 0.50 | 1.42 |

**Table 6.** Comparison between SECV values obtained for the best models for predicting the morphological, safety and quality parameters of summer squashes using the MEMS and LVF spectrometers tested; Fisher test ($P$ < 0.05).

| Parameter | SECV Phazir 2400 | SECV MicroPhazir | SECV MicroNIR-1700 | $SECV_{min}$ | $SECV_{min} \cdot \sqrt{F_{critical}}$ |
|---|---|---|---|---|---|
| Weight (g)* | 155.91 | 161.07 | 142.48 | 142.48 | 154.63 |
| Length (cm)* | 3.31 | 3.40 | 3.11 | 3.11 | 3.37 |
| Equatorial diameter (mm) | 6.34 | 6.70 | 6.22 | 6.22 | 6.96 |
| Nitrate content (mg $kg^{-1}$)* | 240.03 | 226.02 | 198.07 | 198.07 | 214.94 |
| Dry matter (% fw) | 0.53 | 0.53 | 0.51 | 0.51 | 0.55 |
| SSC (ºBrix) | 0.36 | 0.35 | 0.33 | 0.33 | 0.36 |

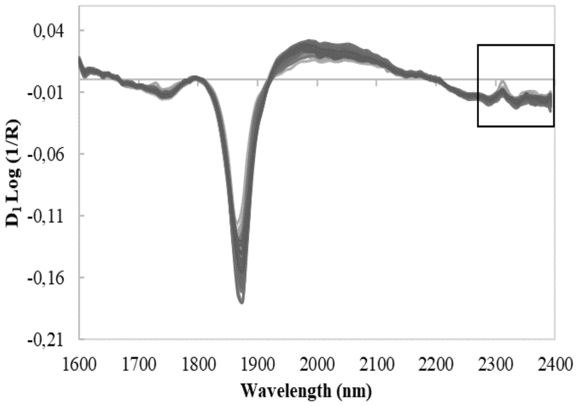
*: Significant differences ($P$ < 0.05) between the SECV values obtained.

**Table 7.** Calibration statistics for best NIR-based models for predicting morphological, safety and quality parameters in intact summer squashes using the MicroNIR-1700 instrument.

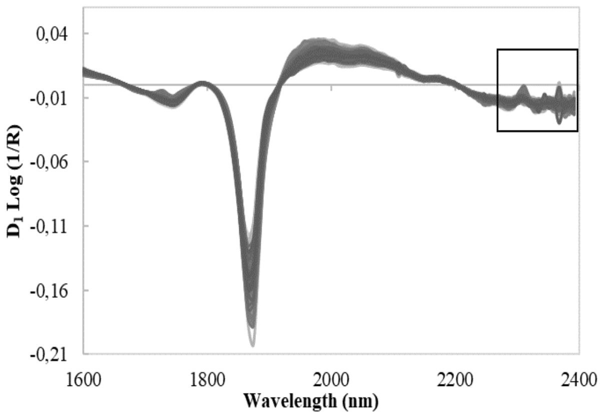
| Parameter | Math treatment | N | Range | Mean | SD | SECV | $r^2_{cv}$ | $RPD_{cv}$ |
|---|---|---|---|---|---|---|---|---|
| Weight (g) | 1,5,5,1 | 154 | 78.43-1388.16 | 420.70 | 250.33 | 100.65 | 0.84 | 2.49 |
| Length (cm) | 1,5,5,1 | 155 | 12.83-40.00 | 23.62 | 5.22 | 2.79 | 0.72 | 1.87 |
| Equatorial diameter (mm) | 2,5,5,1 | 156 | 28.02-89.58 | 52.53 | 13.87 | 5.57 | 0.84 | 2.49 |
| Nitrate content (mg $kg^{-1}$) | 2,5,5,1 | 294 | 18.50-1219.73 | 325.72 | 251.12 | 141.32 | 0.68 | 1.78 |
| Dry matter (% fw) | 2,5,5,1 | 297 | 3.16-7.51 | 4.64 | 0.62 | 0.42 | 0.54 | 1.48 |
| SSC (ºBrix) | 2,5,5,1 | 300 | 2.80-5.20 | 4.12 | 0.43 | 0.31 | 0.47 | 1.39 |

**Figure 1.** $D_1$ log (1/R) spectra for summer squash. Instruments: a) Phazir 2400, b) MicroPhazir and c) MicroNIR-1700.

**a) Phazir 2400**




**b) MicroPhazir**



**c) MicroNIR-1700**

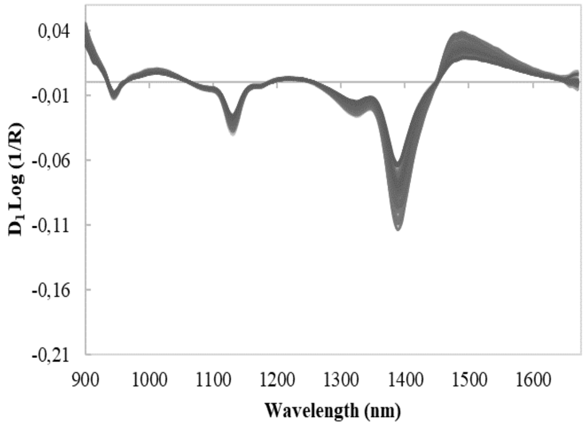
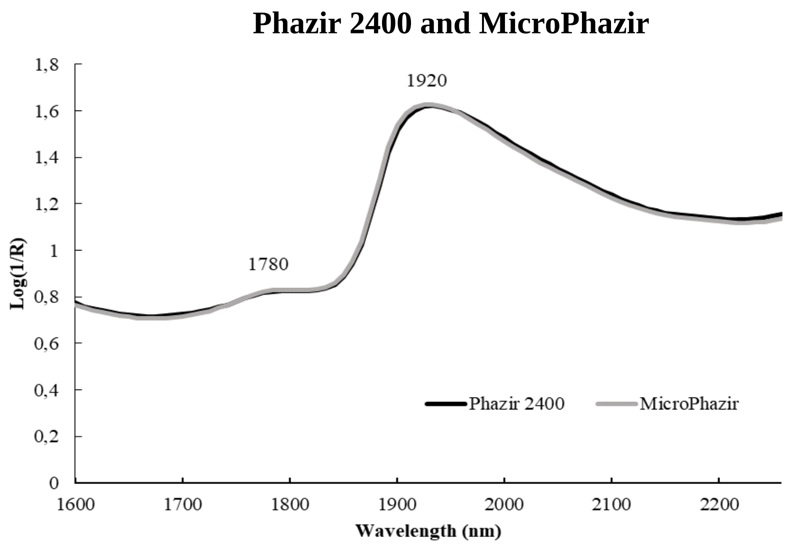


**Figure 2.** Mean spectra for summer squash. Instruments: Phazir 2400, MicroPhazir and MicroNIR-1700.



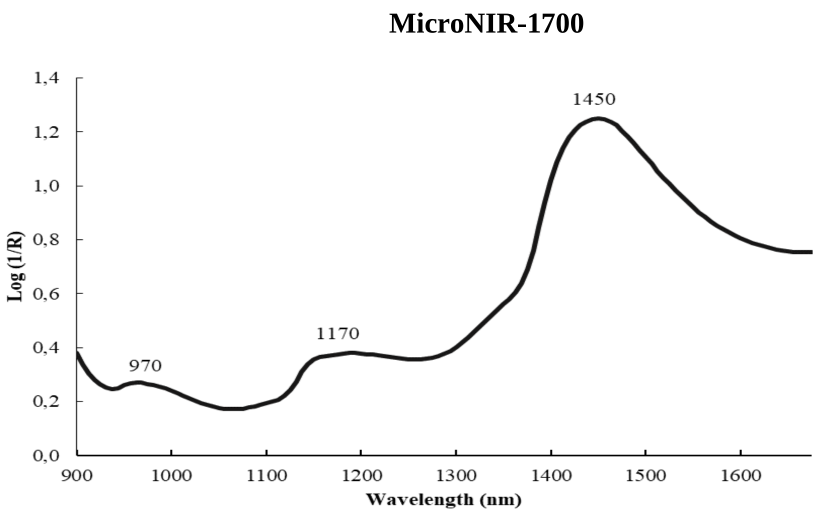
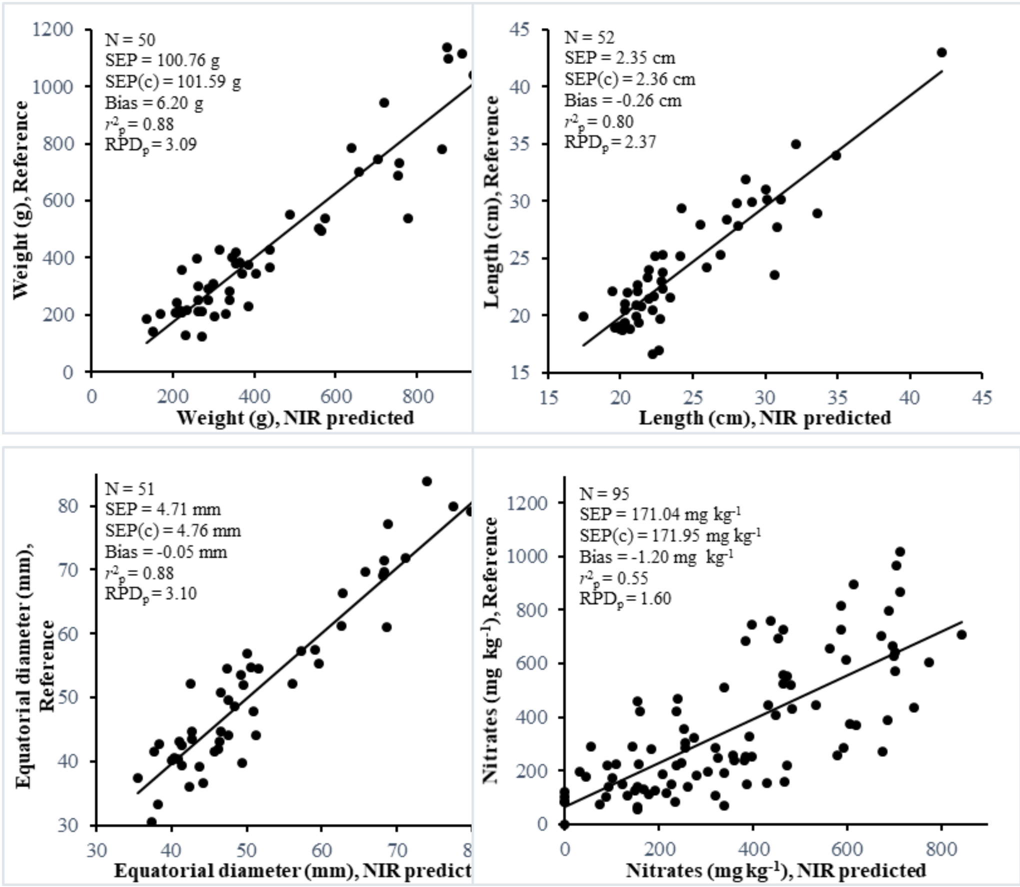
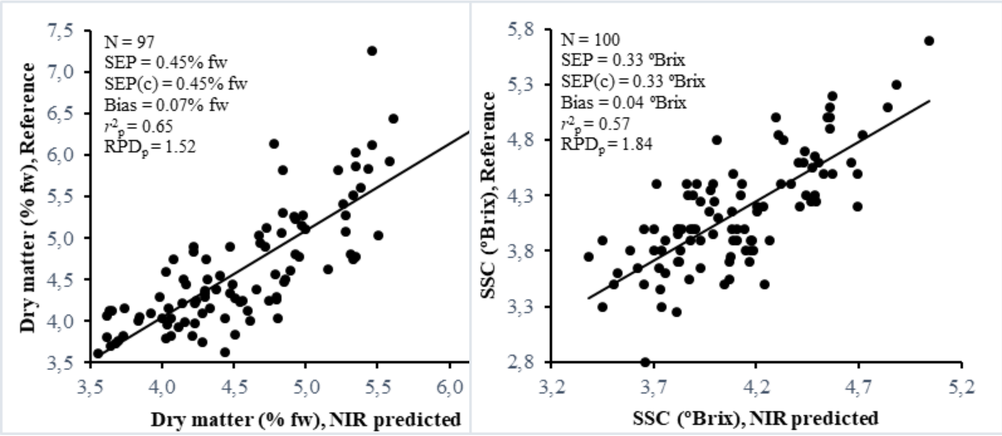



**Figure 3.** Reference *versus* NIR-predicted data for the validation sets. N, number of samples for the validation set; SEP, standard error of prediction; SEP(c), standard error of prediction corrected for bias; $r^2_p$, coefficient of determination for prediction; $RPD_p$, residual predictive deviation for prediction; fw, fresh weight




N = 97
SEP = 0.45% fw
SEP(c) = 0.45% fw
Bias = 0.07% fw
$r^2_p$ = 0.65
$RPD_p$ = 1.52
Dry matter (% fw), Reference
Dry matter (% fw), NIR predicted
N = 100
SEP = 0.33 ºBrix
SEP(c) = 0.33 ºBrix
Bias = 0.04 ºBrix
$r^2_p$ = 0.57
$RPD_p$ = 1.84
SSC (°Brix), Reference
SSC (°Brix), NIR predicted

# Chapter 5

## Chapter 5. CONCLUSIONS

The main conclusions reached, based on the research carried out, the proposed strategies and the results obtained during the development of this PhD dissertation, are:

1. In spinach plants and using the MicroNIR™ 1700, taking 6 spectra per leaf, including 2 spectra taken on the petiole of the leaf, is perfectly suitable for measuring nitrates, both for its application 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). [*This conclusion was met in the research article: 'Simultaneous detection of quality and safety in spinach plants using a new generation of NIRS sensors'. Postharvest Biology and Technology 160, 111026 (2020)*].

2. As regards the Matrix-F instrument, which 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 enough to establish the product's quality and safety, which would facilitate the incorporation of this NIR instrument in the processing industries of horticultural products. [*This conclusion was reached in the research article: 'Simultaneous detection of quality and safety in spinach plants using a new generation of NIRS sensors'. Postharvest Biology and Technology 160, 111026 (2020)*].

3. The NIRS equations constructed should be considered as a first step in the fine-tuning of NIR spectroscopy for the *in situ* and online monitoring of green colour and texture related parameters in spinach plants, which are usually associated with freshness, retention of good quality and, therefore, with saleability. [*This conclusion was met in the research articles: 'Monitoring texture and other quality parameters in spinach plants using NIR spectroscopy'. Computers and Electronics in Agriculture 155, 446–452 (2018)*; *'Simultaneous detection of quality and safety in spinach plants using a new generation of NIRS sensors'. Postharvest Biology and Technology 160, 111026 (2020)*].

4. The results obtained showed the feasibility of NIRS technology for measuring quality (dry matter and soluble solid contents) in spinach plants along the whole food supply chain. Additionally, accurate information about high and low levels of nitrate content was given, allowing to establish the industrial destination of this vegetable. [*This conclusion was met in the research articles: 'Monitoring texture and other quality parameters in spinach plants using NIR spectroscopy'. Computers and Electronics in Agriculture 155, 446–452 (2018); 'Simultaneous detection of quality and safety in spinach plants using a new generation of NIRS sensors'. Postharvest Biology and Technology 160, 111026 (2020)*].

5. The three NIRS instruments tested which are rapid, lightweight and user-friendly, provided a similar level of accuracy for the measurement of equatorial diameter, dry matter and soluble solid contents in summer squash on-vine. However, for weight, length and nitrate content, significantly more accurate models were obtained with the newer LVF instrument. The MicroNIR$^{TM}$ 1700 instrument is therefore, the most suitable for measuring *in situ*, the morphology, safety and quality of summer squashes. [*This conclusion was reached in the research article: 'Safety and quality issues in summer squashes using handheld portable NIRS sensors for real-time decision making and on-vine monitoring'. Journal of the Science of Food and Agriculture 99, 6768–6777 (2019)*].

6. The greatest accumulation of nitrates in summer squashes takes place in the peduncle zone, and therefore, this is the area that must be analysed to determine the destination of the harvested product. In addition, the summer squashes harvested at the end of the harvesting period should be the ones destined for baby food production, since they have nitrate values of below 200 mg/kg. [*This conclusion was reached in the research article: ''Safety and quality issues in summer squashes using handheld portable NIRS sensors for real-time decision making and on-vine monitoring'. Journal of the Science of Food and Agriculture 99, 6768–6777 (2019)*].

7. The results obtained suggest the importance of the optimization of the new generation NIRS instruments before their use for *in situ* and online analysis of spinach plants and summer squashes. The results also showed the complementary use of the MicroNIR$^{TM}$ 1700 and the Matrix-F instruments for global monitoring, allowing spinach plants to be analysed throughout the whole food supply chain. [*This conclusion was met in the research articles: 'Simultaneous detection of quality and safety in spinach plants using a new generation of NIRS sensors'. Postharvest Biology and Technology 160, 111026 (2020); 'Safety and quality issues in summer squashes using handheld portable NIRS sensors for real-time decision making and on-vine monitoring'. Journal of the Science of Food and Agriculture 99, 6768–6777 (2019)*].

8. Over the coming years, recalibrations may be required in order to enhance the precision and accuracy of the models here obtained, using a sufficiently large and highly representative sample database of the variability of horticultural products analysed. [*This conclusion is reached in the research articles: 'Monitoring texture and other quality parameters in spinach plants using NIR spectroscopy'. Computers and Electronics in Agriculture 155, 446–452 (2018)*; *'Simultaneous detection of quality and safety in spinach plants using a new generation of NIRS sensors'. Postharvest Biology and Technology 160, 111026 (2020); 'Safety and quality issues in summer squashes using handheld portable NIRS sensors for real-time decision making and on-vine monitoring'. Journal of the Science of Food and Agriculture 99, 6768–6777 (2019)*].

# Chapter 6

# Chapter 6. FINAL CONSIDERATIONS AND RECOMMENDATIONS FOR FUTURE R&D&I WORKS

The results obtained in this PhD dissertation show the feasibility of using NIRS sensors for the rapid and non-destructive quality assurance and safety of complex biological products such as vegetables, and the great potential of NIRS technology, both in the field and in the processing plants, for the preharvest monitoring of individual vegetables, their subsequent checking and postharvest evaluation. This technology enables real-time monitoring of a larger volume of product, thus representing a major step towards the improvement and innovation in the horticultural sector.

However, NIRS analysis of vegetables faces and difficulties, due to their high moisture content and considerable variability both in external appearance and in physical/chemical composition; as a result, NIRS analysis methodology needs to be optimized for the measurement of quality and safety parameters in the horticultural sector.

This chapter of the PhD dissertation is concerned with future research works that should be carried out for the optimization and consolidation of NIRS sensors for the quality and safety assessment of horticultural products and for their characterization, authentication and categorisation. These works should be mainly focused on:

- ✓ Intensification in the sampling sets used in this PhD dissertation in order to increase the robustness of the models developed for their routine use in the field and at industrial level.

- ✓ Faced with such a wide diversity in the characteristics and features of the portable NIRS sensors now available, there is a need for them to be previously evaluated in order to choose which is the most suitable for a certain application or a specific product. Additionally, transfer protocols between databases obtained with different NIRS instruments must be ready, so that spectral databases can be expanding and updating easily for the new instruments.

- ✓ Collaborative creation of a multiproduct database for horticultural products including vegetables grown in different seasons and under diverse cultural practices, facilitating the development of universal NIRS models in the horticultural sector.

- ✓ Evaluation of advanced chemometric strategies for increasing the robustness of predictive models, mainly for complex parameters such as physical features or parameters presented in low concentrations.

- ✓ Development of conformity tests and early warning systems using the NIRS fingerprint of products for the detection of product failing to meet quality and safety standards and the prediction of optimal postharvest shelf-life in horticultural products.

# Chapter 7

## Chapter 7. REFERENCES

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# Appendixes

# Appendix I. Participation in Congresses, Conferences and Symposia

## *I.1. IV Congreso Nacional de Ingenieros Agrónomos "Retos Tecnológicos, Innovación y Apuestas de Futuro en Ingeniería Agroalimentaria y Medio Rural" (Córdoba, España)*

# PREDICCIÓN *IN-SITU* DE LA CALIDAD DE FRUTAS MEDIANTE EL USO DE SENSORES NIRS



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# PREDICCIÓN *IN-SITU* DE LA CALIDAD DE FRUTAS MEDIANTE EL USO DE SENSORES NIRS

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## INTRODUCCIÓN

El sector frutícola demanda la incorporación de métodos de análisis no invasivos, rápidos, de gran exactitud y precisión, y económicos, destinados a facilitar el aseguramiento *in-situ* de la calidad de sus productos.

La Espectroscopía de Reflectancia en el Infrarrojo Cercano (NIRS) permite satisfacer la demanda de calidad de dicho sector al cumplir los citados requisitos.

## OBJETIVO

Establecer un sistema de apoyo a la decisión en el sector frutícola destinado a establecer el momento óptimo de cosecha, mediante la predicción *in-situ* de los parámetros de calidad (sólidos solubles totales y acidez titulable), utilizando un espectrofotómetro manual portátil basado en tecnología MEMS-NIR.

## MATERIAL y MÉTODOS

### Material vegetal

- 126 Naranjas.
- 256 Mandarinas.
- 189 Fresas.

### Análisis NIRS

Instrumento: Phazir 2400

- Tecnología MEMS.
- Reflectancia: 1600-2400 nm.
- Producto intacto.

### Análisis de referencia

- Contenido en sólidos solubles totales (%). Refractometría.
- Acidez titulable (% ácido cítrico). Titración.

### Desarrollo de calibraciones

- Método de regresión: MPLS.
- Corrección de radiación dispersa: SNV + DT y sin corregir (ninguna).
- Derivadas: 1,5,5,1; 1,10,5,1; 2,5,5,1; 2,10,5,1.
- Software: WINISI II, versión 1.5.

## RESULTADOS

**Tabla 1. Rango, media, desviación típica (DT) y coeficiente de variación (CV) del colectivo de calibración.**

| Parámetro | Fruta | Rango | Media | DT | CV (%) |
|---|---|---|---|---|---|
| Sólidos solubles totales (%) | Naranjas | 9,35 - 14,80 | 12,13 | 1,28 | 10,53 |
| | Mandarinas | 9,95 - 15,65 | 12,58 | 1,18 | 9,38 |
| | Fresas | 5,40 - 11,55 | 7,52 | 1,44 | 14,15 |
| Acidez titulable (% ácido cítrico) | Naranjas | 0,36 - 1,05 | 0,59 | 0,15 | 26,01 |
| | Mandarinas | 0,71 - 2,08 | 1,19 | 0,26 | 21,85 |
| | Fresas | 0,43 - 0,93 | 0,63 | 0,11 | 17,46 |

**Tabla 2. Estadísticos de los mejores modelos de calibración para la predicción NIR de parámetros de calidad en frutas.**

| Parámetro | Fruta | Tratamiento matemático | $r^2_{vc}$ | ETVC | RPD | CV (%) |
|---|---|---|---|---|---|---|
| Sólidos solubles totales (%) | Naranjas | 2,10,5,1 - SNV+DT | 0,62 | 0,79 | 1,61 | 6,47 |
| | Mandarinas | 1,5,5,1 - Ninguno | 0,55 | 0,76 | 1,49 | 6,06 |
| | Fresas | 1,10,5,1 - Ninguno | 0,83 | 0,61 | 2,39 | 8,14 |
| Acidez titulable (% ácido cítrico) | Naranjas | 1,5,5,1 - SNV+DT | 0,09 | 0,14 | 1,05 | 23,78 |
| | Mandarinas | 2,5,5,1 - SNV+DT | 0,64 | 0,14 | 1,68 | 11,93 |
| | Fresas | 2,10,5,1 - Ninguno | 0,53 | 0,07 | 1,43 | 11,47 |

## CONCLUSIONES

Los resultados obtenidos permiten confirmar la viabilidad de incorporar sensores NIRS para la determinación de parámetros de calidad destinados a establecer el momento óptimo de cosecha en frutas analizadas de forma intacta a partir de un único análisis y utilizando un instrumento portátil, apto para determinaciones de calidad *in situ*, en árbol o en la mata.

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## *I.2. 5th Food Integrity Conference 'Assuring the Integrity of the Food Chain: Delivering Real World Solutions'* (Nantes, France)

# *IN-SITU* APPLICATION OF NEAR- INFRARED SPECTROSCOPY IN BELL PEPPER QUALITY DETERMINATION

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## INTRODUCTION

Bell pepper (*Capsicum annuum L.*) is among the most grown vegetables in greenhouses worldwide. From quality determination, soluble solid content and acidity are of key importance. These parameters are routinely evaluated using methods that are time-consuming, destructive, costly and contaminant. Therefore, there is a clear need for fast, accurate and non-destructive analytical techniques that can be used both in the field and by the industry.[(1)]

Near-infrared Reflectance Spectroscopy meets these requirements. This technology coupled with chemometric techniques based on modified partial least squares regression is an appropriate non-destructive technology for the *in situ* determination of quality parameters in vegetables.[(2)]

## OBJECTIVE

To develop quantitative models for the prediction of two of the main quality parameters (soluble solid content (SSC) and titratable acidity (TA)) in intact bell peppers.

## MATERIAL AND METHODS

### Sampling

- 147 bell peppers (*Capsicum annum* L.) were harvested at commercial stage in Murcia (Spain).

### Reference analysis

- Soluble solid content (SSC) (%). Refractometry.
- Titratable acidity (TA) (% citric acid). Titration.

### NIR analysis

Instrument: Phazir 2400

- MEMS technology.
- Reflectance: 1600-2400 nm.
- Intact product.

### Calibration development

- Regression procedure: MPLS.
- Scatter correction: SNV + DT.
- Derivatives: 1,5,5,1; 2,5,5,1.
- Software: WINISI II, ver. 1.5.

## RESULTS

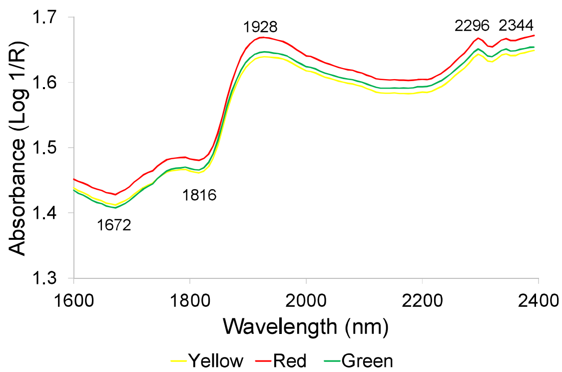


**Figure 1. Mean NIR spectra of bell peppers.**

**Table 1. MPLS regression statistics for NIR-based models for predicting internal quality parameters in intact bell peppers (N = 147).**

| | Mean | SD | SECV | $r^2_{cv}$ | RPD |
|---|---|---|---|---|---|
| SSC (%) | 6.58 | 1.33 | 0.89 | 0.55 | 1.49 |
| TA (% citric acid) | 0.21 | 0.06 | 0.04 | 0.54 | 1.50 |

## CONCLUSIONS

The results confirm that NIR spectroscopy using a portable manual instrument based on MEMS technology could be used as a fast and *in situ* preliminary screening technique for the classification of bell peppers by soluble solid content and titratable acidity.

## REFERENCES

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## ACKNOWLEDGEMENTS

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## *I.3. VI International Symposium on Applications of Modelling as an Innovative Technology in the Horticultural Supply Chain – Model IT (Molfetta, Italy)*

# APPLICATION OF NEAR INFRARED REFLECTANCE SPECTROSCOPY TO ASSESS ON-LINE QUALITY IN SUMMER SQUASHES

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## INTRODUCTION

In consideration of the increasing demand in term of quality assurance in the vegetable industry, there is a clear need for fast, accurate and non-destructive analytical techniques that can be used by the industry with a view to be assembled in the production line. Near Infrared (NIR) Spectroscopy meets these requirements.

One fundamental issue in developing online NIRS applications is to make the correct choice of instrument to be used, which must be robust and stable when subjected to vibrations and thermal variations (Porep et al., 2015; Garrido et al., 2018).

## OBJECTIVE

To assess the feasibility of using a Fourier-Transform (FT)-NIR spectrophotometer for the online determination of the quality (dry matter and soluble solid content (SSC) in intact summer squashes in the processing industry.

## MATERIALS and METHODS

### Sampling

#### Summer squashes:

- A total of 222 summer squashes (*Cucurbita pepo* subsp. pepo morphotype zucchini cv. Mirza) grown in an open-air field in the district of La Montiela, Santaella (Córdoba, Spain), were weekly harvested at commercial maturity.

- Samples were stored under refrigerated conditions (5 °C and 85% RH) until the following day, when the analysis was performed. Prior to each measurement, the samples were left to reach room temperature.

### Reference analysis

- Dry matter (% fw): Desiccation at 105 ºC for 24 h (AOAC, 2000).
- Soluble solids content (ºBrix): Refractometry.

### NIRS analysis

- FT-NIR spectrophotometer:
  - Matrix-F (Bruker Optik GmbH, Ettlingen, Germany).
  - Reflectance. 834–2502.40 nm.
  - Equipped with a conveyor belt for the movement of the sample.
- Intact product.

### Calibration development

- Regression Procedure: MPLS.
- Scatter Correction: SNV + DT.
- Derivatives: 1,5,5,1; 2,5,5,1.
- Software: WINISI II, Version 1.5.

## RESULTS

Table 1. Statistical analysis of calibration and prediction sample sets, i.e., number of samples (N), data ranges, means, standard deviations (SD) and coefficients of variation (CV) for intact summer squashes

| Parameter | Set | N | Range | Mean | SD | CV (%) |
|---|---|---|---|---|---|---|
| Dry matter (% fw) | Calibration | 169 | 1.31–7.34 | 4.93 | 0.81 | 16,43 |
| | Validation | 53 | 3.67–6.22 | 4.79 | 0.71 | 14.82 |
| SSC (ºBrix) | Calibration | 169 | 2.80–5.63 | 4.29 | 0.49 | 11.42 |
| | Validation | 53 | 3.37–5.30 | 4.27 | 0.47 | 11.01 |

Table 2. Calibration statistics for internal quality parameters (dry matter and SSC) in summer squashes using MPLS regression

| Parameter | N | Mean | SD | SEC | $R^2_c$ | SECV | $R^2_{cv}$ | $RPD_{cv}$ |
|---|---|---|---|---|---|---|---|---|
| Dry matter (% fw) | 160 | 4.90 | 0.66 | 0.32 | 0.62 | 0.41 | 0.62 | 1.98 |
| SSC (ºBrix) | 162 | 4.30 | 0.45 | 0.27 | 0.57 | 0.30 | 0.57 | 1.63 |

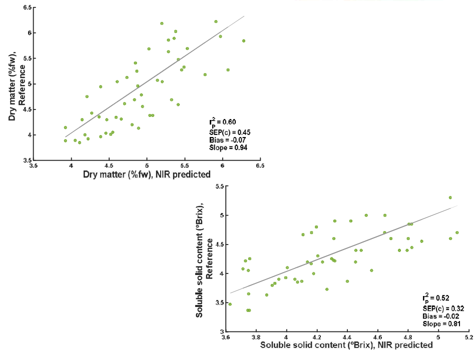


Figure 1. Reference measured data *versus* NIRS predicted data for the validation sets for dry matter (% fw) and SSC (ºBrix)

## CONCLUSIONS

The results showed that NIR technology is a very promising tool for the rapid, accurate and non-destructive on-line determination of dry matter and soluble solid content in the industry, with a view to guarantee the quality of the processed vegetables.

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