UNIVERSIDAD POLITÉCNICA DE MADRID
ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA AGRONÓMICA,
ALIMENTARIA Y DE BIOSISTEMAS

**HYPERSPECTRAL IMAGE APPLIED TO DETERMINE
QUALITY PARAMETERS IN LEAFY VEGETABLES**

PhD THESIS

MIGUEL ÁNGEL LARA BLAS
MASTER IN AGRO-ENGINEERING
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**IMAGEN HIPERESPECTRAL APLICADA A LA
DETERMINACIÓN DE PARÁMETROS DE CALIDAD EN
HORTALIZA DE HOJA**

TESIS DOCTORAL

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*He nacido hoy de madrugada,
viví mi niñez esta mañana
y sobre el mediodía
ya transitaba mi adolescencia.
Y no es que me asuste
que el tiempo se me pase tan deprisa.
Solo me inquieta un poco pensar
que tal vez mañana
yo sea demasiado viejo
para hacer lo que he dejado pendiente.*

Jorge Bucay.

*I was born today at dawn,
I lived my childhood this morning
and around midday
I was living my adolescence.
And I'm not scared
that my time goes so fast.
Just I'm a little worried
because maybe tomorrow
I'll be too old
to do what I've left undone.*

Jorge Bucay

*Ser feliz es sentir la convicción
de estar en el camino correcto.*

*Dedicado a todas las personas
que están en mi camino
y me hacen estar convencido.
Y muy especialmente
a quien camina a mi lado...*

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ABREVIATIONS NOMENCLATURE

ABREVIATION	MEANING
1-MCP	1-Methyl cyclopropene
2D	Two dimensional
3D	Three dimensional
A&I	Active and intelligent packaging
AEW	Acidic electrolyzed water
ANN	Artificial neural network
ANOVA	Analysis of variance
AOTF	Acousto-optic tunable filters
ARI1	Anthocyanin reflectance index 1
ARI2	Anthocyanin reflectance index 2
ARVI	Atmospherically resistant vegetation index
B	Blue
BD	Band difference
BOPP	Biaxially-oriented polypropylene
BP-NN	Back propagation neural networks
BR	Band ratio
c	Units with values between m and M in microbiological analysis.
CA	Correlation analysis
CCD	Charged-coupled device
CCI	Citrus colour index
CEBAS-CSIC	Centro de edafología y biología aplicada del Segura – Consejo superior de investigaciones científicas
CEI-Moncloa	Campus de excelencia internacional Moncloa
Cfu	Colony forming unit
CIE	Commission internationale d'éclairage
CIELab	L*a*b* colour space by CIE
CMOS	Complementary metal-oxide-semiconductor
CRI1	Carotenoid reflectance index 1
CRI2	Carotenoid reflectance index 2
Ct	Control
DA	Discriminant analysis
DBP	Disinfection by-product
DLS	Dynamic light scattering
DWSI1	Disease water stress index 1
e.g.	Exempli gratiā

EC	Electrical conductivity
ED	Euclidean distance
EMSC	Extended multiplicative scatter correction
ENVI	Environment for visualizing images
et al.	et alii
E.T.S.I Agrónomos	Escuela técnica superior de ingenieros agrónomos
EU	European Union
E.U.I.T. Agrícola	Escuela universitaria de ingeniería técnica agrícola
EVA	Ethylene vinyl acetate
EVI	Enhanced vegetation index
EVOH	Ethylene-vinyl alcohol
F	Fluorescence
FDR	Fisher discriminant ratio
FEPEX	Federación española de asociaciones de productores exportadores de frutas, hortalizas, flores y plantas vivas.
FT	Fourier transform
GA	Genetic algorithm
GaAs	Gallium Arsenide
GIR	Green and indigo ratio
GML	Gaussian maximum likelihood
GRAS	Generally recognized as safe
HDPE	High density polyethylene
HgCdTe	Mercury cadmium tellurium
HPLC	High performance liquid chromatography
HPP	High pressure processing
HSI	Hue, saturation, lightness colour space
I	Interactance mode
IBDA	Iterative Bayesian discriminant analysis
ICA	Independent component analysis
InAs	Indium arsenide
InGaAs	Indium gallium arsenide
IR	Infrared
IRSTEA	Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture
kNN	K-nearest neighbour
LAB	Lactic acid bacteria
LAD	Leaf angle distribution
LAI	Leaf area index
LASSO	Least absolute shrinkage and selection operator

LCTF	Liquid crystal tunable filters
Ld	Loading of PCA
LDA	Linear discriminant analysis
LDPE	Low density polyethylene
LED	Light emitting diode
LEDI	Lettuce decay index
LEVE	Leafy vegetable evolution index
LLDPE	Linear low density polyethylene
LPF-TAGRALIA	Laboratorio de propiedades físicas y técnicas avanzadas en agroalimentación
LSI	Lettuce salinity index
LS-SVM	Least squares - Support vector machine
LV	Latent variable
m	limit of bacteria count; maximum c sample units can exceed this limit.
M	limit of bacteria count; none of the sample units must to exceed this limit.
MAP	Modified atmosphere packaging
MCARI	Modified chlorophyll absorption in reflectance index
MCT	Mercury cadmium tellurium
MC-UVE	Monte Carlo uninformative variable elimination
MICINN	Ministerio de Ciencia e Innovación
MLA	Mean leaf angle
MLP	Multi-layer perceptron
MLR	Multi linear regression
mNDVI ₇₀₅	Modified red edge normalized difference vegetation index
MNF	Minimum noise fraction
MS	Mass spectrometry
MSAVI	Improved soil adjusted vegetation index
MSC	Multiplicative scatter correction
MSI	Moisture stress index
MSPC	Multivariate statistical process control
mSR	Modified simple ratio
n	Units per sample in microbiological analysis.
NB	Naïve Bayes
NEW	Neutral electrolyzed water
NDII	Normalized difference infrared index
NDVI	Normalized difference vegetation index
NDVI ₇₀₅	Red edge normalized difference vegetation index
NDWI	Normalized difference water index
NIR	Near infrared

NPCI	Normalized difference pigment chlorophyll index
OSAVI	Optimized soil adjusted vegetation index
OSC	Orthogonal signal correction
OTR	Oxygen transmission rate
PC	Principal component
PCA	Principal component analysis
PCR	Principal component regression
PE	Polyethylene
PL	Pulsed light
PLA	Poly lactide
PLS	Partial least squares
PLSR	Partial least squares regression
PNN	Probabilistic neural network
PP	Polypropylene
PRI	Photochemical reflectance index
PRI2	Photochemical reflectance index 2
PSRI	Plant senescence reflectance index
PVC	Polyvinyl chloride
PVdC	Polyvinylidene chloride
r	Correlation coefficient
R	Reflectance mode
Rd	Red
R _p	Regression coefficient of prediction
R _λ	Reflectance at a given wavelength (λ)
RBF-SVR	Radial basis function support vector regression
RC	Radiometric correction
RE	Red edge
REP	Red edge position index
REPAsI	Derivative-based red edge position pseudo-absorbance where the value is the steepest slope
RGB	Red green blue colour space
RGRI	Red green ratio index
ROC	Receiver operating characteristic
ROI	Region of interest
RTU	Ready to use
S	Scattering mode
S _i	Saline treatment number i
SAM	Spectral angle mapper
SCM	Spectral correlation mapper

SD	Second derivative
SDA	Stepwise discriminant analysis
SECV	Standard error of the cross validation
SFS	Sequential forward selection
SG	Savitzky-Golay algorithm
SIG	Spectral information divergence
SIMCA	Soft independent modelling of class analogy
SIPI	Structure insensitive pigment index
SLDA	Stepwise linear discriminant analysis
SLS	Static light scattering
SNV	Standard normal variate
SPA	Successive projection algorithm
SR	Simple ratio
SRVI	Simple ratio vegetation index
SSC	Soluble solids content
STD	Standard deviation
SVM	Support vector machine
SVR	Support vector regression
SWIR	Short-wave infrared
SWR	Stepwise regression
SWSI1	Salinity and water stress index 1
SWSI2	Salinity and water stress index 2
SWSI3	Salinity and water stress index 3
T	Transmittance mode
TBARS	Thiobarbituric acid reactive substances
TCARI	Transformed chlorophyll absorption in reflectance index
TSP	Trisodium phosphate
TVC	Total viable count
ULDPE	Ultra low density polyethylene
UPM	Universidad politécnica de Madrid
US	Ultrasound
USA	United states of America
UV	Ultraviolet
UV-C	Short wave ultraviolet
VIS	Visible
VOG1	Vogelmann red edge index 1
VOG2	Vogelmann red edge index 2
VOG3	Vogelmann red edge index 3

WC	Water content
WHC	Water holding capacity
WI	Water index
WSRR	Wetland salinity reflectance ratio
WT	Wavelet transform

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ABSTRACT

Hyperspectral imaging system integrates both spectroscopic and imaging techniques into one system that can acquire a spatial map of spectral variation of the current sample. It has been widely studied and developed as a potential tool for non-destructive analysis and assessment for food quality and safety, resulting in many successful applications.

On the other side, minimally processed leafy vegetables have increased their popularity since years ago, because they are fresh and healthy products, easy to prepare and to use. However, this minimal processing procedure reduces their shelf-life in comparison to non-processed products, increasing the physiological, biochemical and microbiological deterioration processes. It is necessary to monitor and to control the quality and safety of these products by using fast and non-destructive techniques.

Along this work, a deep review of the most recent applications of hyperspectral imaging in quality determination of food products was done. However, there is not so much research focused on the application of imaging techniques for quality inspection of fresh-cut leafy vegetables. In this thesis the application of hyperspectral imaging is studied for monitoring the deterioration and aging processes of two different leafy vegetables: spinach and watercress. Moreover, as a different approach, hyperspectral imaging is applied to observe salinity effects in fresh-cut lettuce leaves, another leafy vegetable, because saline stress can affect the quality of the leaves as a ready-to-eat fresh product.

Hyperspectral images of spinach leaves (*Spinacia oleracea*) samples were acquired, through the packaging film, along 21 days of cold storage (4°C). In order to avoid the variation in transmittance of the plastic films during time, a radiometric correction of the hyperspectral images was proposed. Three different spectral pre-processing procedures (no pre-process, Savitzky-Golay algorithm and Standard Normal Variate normalization, combined with Principal Component Analysis) were applied to the spectra, to obtain different prediction models. The corresponding artificial images of scores were studied by means of Analysis of Variance to compare their ability to sense the aging of the leaves. All models were able to monitor the aging through storage period, with different degree of success. Moreover, radiometric correction seemed to work properly and could allow the monitoring of shelf-life in leafy vegetables directly through commercial transparent packaging films.

The same procedure was carried out using leaves of a different leafy vegetable variety (watercress, *Nasturtium officinale*), in order to obtain new prediction models and to compare with the models developed in the case of spinach leaves. The purpose of this comparison was to evaluate the ability of a common model for different species of leafy vegetables that may be present in several commercial products such as salads ready to eat. Some principal components retained analysing watercress leaves spectra showed loadings very similar to those retained in the case of spinach. However, in the case of watercress, there were more principal components apparently related to aging of the leaves. All those models, those developed using watercress leaves spectra and those developed for spinach leaves, were tested for monitoring the shelf-life of watercress leaves. The artificial images of scores obtained applying each model were compared by means of Analysis of Variance and Wilks- λ . All models were able to monitor the aging during the storage period. However, the models developed using watercress spectra were more suitable for monitoring the shelf-life of watercress leaves during time. There were some differences between the models applied for spinach and for watercress leaves. It seems necessary to study the spectral features of each leafy vegetable variety independently for developing prediction models abler to monitor the aging of the leaves.

Finally, hyperspectral images of lettuce (*Lactuca sativa*) leaves were acquired, from lettuce plants grown considering four different saline solutions (Ct=0, S1=50, S2=100 and S3=150 mM of NaCl). The mean spectra of the leaves were pre-processed by means of Savitzky-Golay and Standard Normal Variate Normalization algorithms. Principal component analysis was then performed with the resulting pre-processed spectra, yielding an initial model for salinity effect detection. A second model was later proposed based on an index computing an approximation to the second derivative at the red edge region. Both models were applied to the hyperspectral images of the leaves to obtain the corresponding artificial images of scores and of the index. Those virtual images were studied using Analysis of Variance in order to compare their ability for detecting salinity effects on the leaves. Both models showed significant differences between each salinity level, and the hyperspectral images allowed the detection of the distribution of the salinity effects on the leaf surfaces, which were more intense in the areas

distant from the veins. However, the index-based model was simpler and easier to apply in comparison to principal component because it was based solely on the reflectance at three different wavelengths, thus allowing for the implementation of less expensive multispectral devices.

In this research, the potential of hyperspectral imaging as a non-destructive tool for quality inspection in leafy vegetables was proved. The findings demonstrate that it is possible to evaluate the freshness of commercial ready-to-eat leafy vegetables directly through the final packaging films. This is a great advantage for the quality control of this kind of products, both in the industry and along the distribution chain. Moreover, the models developed for monitoring the shelf-life of these products can be applied to different leafy vegetables, allowing to be used directly on salads or mixes of leaves. On the other side, a three-wavelengths-based index was developed for detecting the effect of irrigation water salinity on lettuce leaves, allowing the detection before the leaves show visual symptoms. This index showed the best segregation performance of the lettuce leaves submitted to different saline levels than the rest of the reviewed indexes in the literature for salinity effect detection on plants.

RESUMEN

La imagen hiperespectral aúna la espectroscopia con las técnicas de análisis de imagen en un solo sistema, capaz de adquirir un mapa espacial de la variación espectral de la muestra. Ha sido ampliamente estudiada y perfeccionada en los últimos tiempos como una herramienta potencial para el análisis no destructivo y aseguramiento de la calidad de los alimentos, desarrollándose con éxito múltiples aplicaciones en diferentes productos alimentarios.

Por otro lado, los productos vegetales mínimamente procesados o listos para consumir, denominados comúnmente productos de cuarta gama, han ganado popularidad desde hace años, al tratarse de productos frescos y saludables, fáciles de preparar y de consumir. Sin embargo, ese mínimo procesado suele implicar operaciones que disminuyen la vida comercial del producto, al mismo tiempo que aumentan los procesos de degradación fisiológica, bioquímica y microbiológica. Se hace necesario la supervisión y control de la calidad y seguridad de estos productos mediante el uso de técnicas de análisis rápidas y no destructivas.

A lo largo de este trabajo se ha realizado una profunda revisión de las aplicaciones más recientes de imagen hiperespectral para la supervisión de la calidad en productos alimentarios. Sin embargo, existen pocos estudios de aplicación de técnicas espectrales para la inspección de la calidad en hortaliza de hoja y sus productos cuarta gama listos para consumir. En esta Tesis Doctoral se ha estudiado la aplicación de imagen hiperespectral para la supervisión de los procesos de deterioro y envejecimiento que tienen lugar a lo largo de la vida útil en dos especies diferentes de hortaliza de hoja: espinaca y berro. Además, en un enfoque diferente, se ha aplicado imagen hiperespectral para observar el efecto de la salinidad en una tercera especie de hortaliza de hoja, lechuga, puesto que la salinidad puede afectar la calidad de las hojas y, por tanto, su uso potencial como producto fresco listo para consumir.

Las imágenes hiperespectrales de muestras de hojas de espinaca (*Spinacia oleracea*) fueron adquiridas, a través del plástico de envasado, a lo largo de 21 días de almacenamiento en refrigeración (4°C) del producto. Para evitar el efecto de la variación de transmitancia del plástico de envasado a lo largo del tiempo en las imágenes hiperespectrales, se llevó a cabo un procedimiento de corrección radiométrica de las imágenes. Tres diferentes procedimientos de pre-procesado de espectros (sin pre-procesado, algoritmo Savitzky-Golay y normalización Standard Normal Variate, combinados con Análisis de Componentes Principales) fueron aplicados a los espectros de las hojas para obtener diferentes modelos de predicción. Las correspondientes imágenes virtuales de 'scores' obtenidas fueron estudiadas utilizando Análisis de Varianza, para comparar la efectividad de dichos modelos en la detección de la evolución de las hojas. Todos los modelos fueron capaces de detectar la evolución de las hojas a lo largo del tiempo, con diferente grado de efectividad. Además, la corrección radiométrica funcionó adecuadamente, pudiendo permitir la supervisión de la vida útil en hortaliza de hoja directamente a través de los plásticos de envasado comerciales del producto.

El mismo procedimiento se llevó a cabo utilizando una variedad de hortaliza de hoja diferente (berro, *Nasturtium officinale*), con el fin de obtener nuevos modelos de predicción para este caso y compararlos con los modelos desarrollados en el caso de espinaca. El propósito de esta comparación es evaluar la capacidad de un modelo común para diferentes especies de vegetales de hojas que pueden estar presentes en varios productos comerciales tales como ensaladas listas para comer. Algunos de los componentes principales seleccionados al analizar los espectros de hojas de berro mostraron 'loadings' muy similares a los seleccionados en el caso de espinaca. Sin embargo, en el caso de berro, hubo más componentes principales aparentemente relacionados con la evolución de las hojas a lo largo del tiempo. Todos los modelos seleccionados, los desarrollados a partir de espectros de berro y los desarrollados para espinaca, fueron aplicados a las imágenes hiperespectrales de hojas de berro para ver su capacidad para detectar la evolución de las hojas. Las imágenes virtuales de 'scores' obtenidas aplicando cada modelo fueron comparadas mediante Análisis de Varianza y Wilks- λ . Todos los modelos fueron capaces de detectar la evolución de las hojas a lo largo del periodo de ensayo. Sin embargo, los modelos desarrollados a partir de los espectros de berro dieron mejores resultados en la supervisión de la vida útil de las hojas de berro a lo largo del tiempo. Se vieron diferencias entre los modelos obtenidos tras el análisis de los espectros de berro y los obtenidos a partir de los espectros de espinaca. Parece necesario estudiar los espectros de cada especie de hortaliza de hoja independientemente para así

desarrollar modelos de predicción más adecuados para el estudio de la evolución de las hojas de cada especie vegetal.

Por último, se adquirieron imágenes hiperespectrales de hojas de lechuga (*Lactuca sativa*) procedentes de plantas cultivadas en cuatro soluciones salinas diferentes (Ct=0, S1=50, S2=100 y S3=150 mM de NaCl). Los espectros medios de las hojas fueron pre-procesados mediante los algoritmos Savitzky-Golay y el de normalización Standard Normal Variate. Posteriormente se realizó un Análisis de Componentes Principales sobre los espectros pre-procesados, obteniéndose un primer modelo de detección del efecto de la salinidad en las hojas. Un segundo modelo fue propuesto después basándose en un índice que computa una aproximación a la segunda derivada de los espectros en la zona del llamado “red edge”. Ambos modelos fueron aplicados sobre las imágenes hiperespectrales de las hojas para obtener las correspondientes imágenes virtuales de ‘scores’ y del índice. Esos modelos fueron comparados mediante Análisis de Varianza para conocer su capacidad de detectar los efectos de la salinidad en las hojas de lechuga. Ambos modelos mostraron diferencias significativas para cada nivel de salinidad. Las imágenes hiperespectrales permitieron observar la distribución de los efectos de la salinidad sobre la superficie de las hojas, siendo más intensos en las zonas lejanas a los nervios de la hoja. Sin embargo, el modelo basado en el índice fue más sencillo y fácil de aplicar, al estar basado solamente en el valor de reflectancia correspondiente a tres longitudes de onda diferentes, pudiendo aplicarse más fácilmente en dispositivos de imagen multispectral más económicos.

En este trabajo se demuestra el potencial de la imagen hiperespectral como método no destructivo de inspección de la calidad en hortalizas de hoja. Los resultados obtenidos demuestran que es posible evaluar la frescura en productos comerciales de hortaliza de hoja mínimamente procesada directamente a través de su envase final, proporcionando una gran ventaja para el control de calidad de este tipo de productos, tanto en la industria de procesado como en los puntos de distribución y venta. Además, los modelos generados para la supervisión de la vida útil del producto pueden ser aplicables a diferentes especies de hortaliza de hoja, abriendo la posibilidad de ser utilizados directamente en productos tipo ensalada o mezcla de hojas. Por otro lado, se ha desarrollado un índice multispectral, basado solamente en tres longitudes de onda, para la detección del efecto del riego con agua salina en hojas de lechuga, permitiendo su detección antes de que se muestren síntomas en la hoja apreciables de forma visual. Dicho índice ha mostrado una mejor segregación de las hojas de lechuga en función de su nivel de salinidad que el resto de índices encontrados en la bibliografía para la detección del efecto de la salinidad en plantas.

INTRODUCTION

This thesis was carried out in the frame of several research projects. The work described in Section I and Section II was funded by the Ministerio de Economía y Competitividad (MICINN) with the project *“Advanced techniques for the assessment of quality and safety of fresh and fresh-cut vegetables”* (MULTIHORT); and by the Universidad Politécnica de Madrid with the project *“Aplicación de nuevas tecnologías para el seguimiento de la vida útil de productos de 4ª gama: visión hiperespectral y sensores de gases”* (DURASFRUT II). The work exposed in Section III was funded by the MICINN with the projects MULTIHORT and *“Biomarcadores de calidad y seguridad de hortalizas de hoja”* (QUALITYLEAF). This work was addressed in the Laboratorio de Propiedades Físicas y Técnicas Avanzadas en Agroalimentación (LPF-TAGRALIA), in the Departamento de Ingeniería Agroforestal from E.T.S.I. Agronómica, Alimentaria y de Biosistemas (Universidad Politécnica de Madrid).

The objective of MULTIHORT was to explore physical techniques for the on-line assessment of the quality of fresh horticultural products, especially green vegetables: spectrophotometry, multispectral and hyperspectral vision based techniques. The aim was to establish their feasibility and to develop applications focused to the competitiveness of the industry. The proposed techniques were mainly developed for fruits, showing their technical and economical potential for the sector. Therefore, they should be explored for solving specific problems of fresh horticultural products, related to their commercial, organoleptic and health quality characteristics.

In the same line, the project DURASFRUT II had the objective to implement spectrophotometric techniques (multispectral and hyperspectral imaging) and gas sensors for developing applications in the detection of the quality and safety of minimally processed vegetables and fruits.

Meanwhile, QUALITYLEAF project was focused on searching biomarkers for the assessment of the quality and safety of leafy vegetables. Loss of quality in leafy vegetables is usually due to enzymatic browning, off-odours development, tissues softening and/or microbial growth. Stress factors, such as salinity, can also have a great impact on the quality of these products. The main objective of this project was the development of different biomarkers for the prediction and description of intrinsic and extrinsic factors related to the quality and safety of vegetables, with the aim of selecting the best genotypes that ensure the quality, processing and shelf-life of minimally processed leafy vegetables. In the frame of this project, an experimental design for evaluating the effect of salinity on the quality of lettuce leaves, for RTU purposes, was carried out, using hyperspectral imaging techniques.

PROBLEM STATEMENT

Fresh-cut or ready-to-use (RTU) vegetables are very popular food products nowadays. Their consumption has been increasing since 1980 (Mayen and Marshall, 2005), due to the interest of the consumers for acquiring healthier products (Ragaert et al., 2004). In 2012, the volume of RTU fruits and vegetables commercialized in Spain was more than 75.000 tons and 180 millions of euros, 96'3% was RTU vegetables (data from "Federación Española de Asociaciones de Productores Exportadores de Frutas, Hortalizas, Flores y Plantas Vivas FEPEX"). In 2015, the consumption of RTU fruits and vegetables in Spain was three kilograms per person and per year (data from www.interempresas.net). This kind of products shows several advantages in restaurants: decrease of time of preparation and costs of personnel and food processing (Watada et al., 1996).

However, minimal processing of RTU vegetables decreases their shelf-life, increasing the physiological, biochemical and microbiological deterioration processes. The main physiological effects of deterioration are ethylene production, increasing of respiration, loss of water, loss of chlorophyll, pigments degradations, loss of acidity, tissue softening, enzymatic browning, lipolysis and lipid oxidation. For this reason, most RTU products are sold within few weeks after packaging. It is necessary to enhance the shelf-life and quality of these products and, for that, much research has been conducted for developing treatments to minimize the deterioration (Ramos et al., 2013; Braidot et al., 2014; Costa et al., 2013; Gergoff-Grozeff et al., 2013); and for evaluating their organoleptic and nutritive quality properties (Agüero et al., 2011; Martínez-Sánchez et al., 2012; Siripatrawan et al., 2011).

Most of the analytical methods developed for quality and safety control in food products are time-consuming, expensive, laborious and, above all, destructive. Hyperspectral imaging has a great potential in many food applications as a non-destructive assessment tool for evaluating quality of food products. It produces spectral data, generally in the range of VIS-NIR, from each spatial position of the product studied. This technique generates a huge amount of information that can be related to many characteristics of the product, such as firmness (Mendoza et al., 2011a, Cen et al., 2012), soluble solids content (SSC) (Mendoza et al., 2011b; Leiva-Valenzuela et al., 2012), maturity (Lleó et al., 2011; Herrero-Langreo et al., 2011), external defects (Cho et al., 2013; Wang et al., 2012), faecal contamination (Lee et al., 2014; Kang et al., 2011), microbial or insect infestation (Gómez-Sanchis et al., 2013; Lu and Ariana, 2013), and so on. Hyperspectral imaging was widely employed for quality determination in fruits and vegetables, but there is not so much research focused on using this technology for quality inspection in RTU leafy vegetables.

The great success of RTU leafy vegetables, along with the problem of short shelf-life, makes necessary the development of new methodologies, such as hyperspectral imaging, for a fast and non-destructive quality assessment, able to be implemented on the industrial production lines.

On the other hand, salinity is the main abiotic factor that negatively affects the growth and yield of most of the crops (Allakhverdiev et al., 2000b), including leafy vegetables. The potential of leafy vegetables grown under salinity conditions to be employed as RTU products can be affected, changing their quality, organoleptic properties and shelf-life. Hyperspectral imaging was used for detecting salinity in soils and canopies, in both approaches, remote sensing and local acquisition of images. However, there is not so much research about the effect of salinity in leafy vegetables for RTU purposes.

Non-destructive detection of the effect of salinity in leafy vegetables can help to improve and ensure the quality of the corresponding RTU products.

LITERATURE REVIEW

SHELF-LIFE OF FRESH-CUT LEAFY VEGETABLES

1. INTRODUCTION

The quality decay of minimally processed leafy vegetables can be due to either physiological or microbial deterioration. The former is due to metabolic and enzymatic activity of living tissues of the plant. The latter is due to the proliferation and development of spoilage microorganisms in the product.

The industrial process of food products eliminates in many cases the natural protection of the epidermis of plants. The internal structure (plant cell walls) is destroyed and the enzymes and substrates are put in contact. As a result, physical damage is suffered by the leaf tissues making them more susceptible than intact products (Artés et al. 2007). Furthermore, the processing of these products causes a stress response that manifests itself in increased cell respiration and production of ethylene, which leads to a faster metabolic degradation (Watada et al., 1996; Saltveit, 1999; Saltveit & Kang, 2002). Damage on the plant tissue also causes greater exposure to air, desiccation, enzymatic and microbial activity, which produce a degradation of the quality (Klein, 1987; King & Bolin, 1989).

Both physiological processes of degradation and the appearance of nutrients on the surfaces of the cuts produced during processing allow the growth of microorganisms (Lund, 1992; Mercier & Lindow, 2000). The development of these organisms is directly related to the amount of sugars present in the leaf surface, especially in cuts (Babic et al., 1996). Some of these microorganisms can produce pectolytic enzymes which degrade the surface and the texture of the vegetables, producing more nutrients for microbial growth.

The intrinsic properties of the fresh product (such as pH and nutrient availability) determine the growth of microorganisms and the type of microorganisms developed, and consequently, the decay pattern. In products with lower pH, acids, moulds and yeasts are mainly developed. In most neutral pH products, like most leafy vegetables, bacterial growth is predominant. The extrinsic properties (eg. temperature storage or gas surrounding atmosphere) are also determinant factors in the deterioration of the product, influencing in the physiological deterioration (Ragaert et al., 2011).

2. MICROBIAL DETERIORATION

2.1 MICROORGANISMS ASSOCIATED TO DETERIORATION OF MINIMALLY PROCESSED VEGETABLES

Vegetable products are susceptible to contamination from various sources such as soil, irrigation water, animals, harvest processes, industrial processing and packaging. The bacterial population during storage and conservation of these products usually consist mainly of species of the genera *Pseudomonadaceae* and *Enterobacteriaceae*, plus some species of lactic acid bacteria (Lund, 1992; Vankerschaver et al., 1996; Bennik et al., 1998). In addition to bacteria, numerous species of yeasts have been identified in these products, primarily species of *Candida* sp., *Cryptococcus* sp., *Rhodotorula* sp., *Trichosporon* sp., *Pichia* sp., and *Torulaspora* sp. (Nguyen-the & Carlin 1994). Moulds are not common in the case of minimally processed vegetables because their intrinsic properties favour the development of bacteria and yeasts (Giménez et al., 2003).

These products are often considered as relatively safe food from a microbiological point of view, compared with other animal products or ready-to-eat products. However, they can also be a vehicle for transmission of foodborne pathogens, it is enhanced by the fact that they are usually consumed fresh. Some of foodborne pathogens, which are associated with this sort of products, are: *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp, *Shigella* spp, *Bacillus cereus*, *Yersinia enterocolitica*, *Campylobacter*, *Clostridium botulinum*, viruses and parasites (Francis et al., 1999).

Microbial growth results in the production of enzymes and metabolites, which cause visual and textural defects as well as off odour. Microbiological degradation and physiological processes interact and are closely related. Product composition determines the type of deterioration.

The growth of yeasts and lactic bacteria in products which are rich in sugars cause degradation resulting in ethanol production, organic acids and odours (Jacxsens et al., 2003). In packaged salads or cut lettuce, packaged in a low O₂ and high CO₂ level atmosphere, fermentations occur resulting in the production of ethanol, acetaldehyde and ethyl acetate, and causing odours (López-Gálvez et al., 1997; Smyth et al., 1998).

In products with low concentration of sugars, such as lettuce, usually microbial spoilage is due to growth of bacteria *Pseudomonas* sp., resulting in visual defects and texture primarily, but may also generate volatile compounds responsible for off odours.

3. PHYSIOLOGICAL DETERIORATION

The cut or torn of the living tissues of plants produces a series of metabolic cascade reactions which result in changes in the texture of the product, acceleration of maturity and senescence, off odours, discoloration and other undesirable changes that make the product not merchantable (Baldwin & Bai, 2011). The handling and processing of these products cause an increase in the concentration of ethylene, gaseous hormone that accelerates ripening of plants, which promotes faster ripeness and senescence (Karakurt & Huber, 2007). Also, it produces respiration increases, degradation of sugars and acids that cause a shortening of the commercial life. Finally, the skin and the tissue protective membrane are removed, which encourages microbial growth and contamination, as it has been already mentioned.

Ethylene is a biologically gaseous hormone present in plants which is active at low concentrations (ppm to ppb) (Saltveit, 1999). Its production occurs in all tissues of plants in a minimum level since it is necessary for growth (Baldwin, 2004). It is also responsible for the onset of ripening in fruits. For that reason, it is commercialized to ripen climacteric fruits as those fruits can ripen off the mother plant in response to ethylene (Baldwin, 2004). Ethylene is also used to remove the green colour in citrus (Watada, 1986).

However, ethylene is produced in high concentrations in plants in response to stress or surface damage (Baldwin, 2004), which is the case of minimally processed fresh products. Ethylene causes a series of metabolic reactions that are triggered and they are involved in the production of browning enzymes, secondary metabolites, chlorophyllases, pectinases and more ethylene production (chain reaction). All aforementioned processes accelerate ripening and senescence of tissues, produce enzymatic browning, loss of chlorophyll and green, cell wall breakage and softening of texture and odour, shortening their commercial life.

Ethylene synthesis requires the presence of O₂, and it is inhibited by high levels of CO₂. Therefore, packaging of these products in modified atmospheres is very effective to increase their shelf life (Abeles et al., 1992).

Ripening, senescence and response to tissue damage require energy consumption by vegetables. Once harvested, fruits and vegetables have limited energy sources, especially fresh processed products. Sugars and acids are used as a substrate by the vegetables in the respiration process in order to obtain energy and it results in a shortening of their life (Tucker, 1983).

Different processing techniques have been developed in order to prevent the physiological degradation reactions of fresh products (Artés & Allende, 2005; Palumbo et al., 2007): pre-cooling to reduce respiration (Vigneault et al., 2008); modified atmospheres in the packaging to reduce respiration and ethylene production (Jacxsens et al., 2000; Reyes et al., 1995); the use of substances which minimize ethylene action (Toivonen, 2008a); surface treatments and coatings to reduce dehydration and browning (Olivas et al. 2008; Vargas et al., 2008); ethylene sorbents to retard ripening and senescence (Watada & Abe, 1991); or calcium salts to preserve product firmness (Martin-Diana et al., 2007). All of them have been applied with different rate of success but, in general, the use of modified atmosphere packaging of such products has been extended due to its effectiveness in lengthening the shelf-life.

4. SHELF-LIFE DETERMINATION

Each producer is responsible for establishing the life of their products. For those minimally processed vegetable products, the shelf-life is relatively short. The quality and safety of food are the basis to study and establish the shelf-life of these products.

These products are relatively safe because of their nutritional composition, refrigerated storage and conservation in modified atmospheres. This makes microbial growth slowdowns. Determining the shelf-life of these products from a microbiological point of view is simple, it is enough to keep microbial counts below the limits established by law (see Table 1). When these limits are exceeded the end of the shelf-life of the product is reached.

Microbiological limits (Regulation CE 1441/2007)					
Parameter	n	c	m	M	Stage of application
<i>Escherichia coli</i>	5	2	10 ² Cfug	10 ³ Cfug	During processing
<i>Salmonella</i>	5	0	Absence/25g		During shelf-life
<i>Listeria</i>	5	0	100 cfu/g		During shelf-life
<i>monocytogenes</i>	5	0	Absence/25g		Before launching

Table 1.- Microbiological limits of minimally processed fruits and vegetables (Regulation CE 2073/2005 modified by Regulation CE 1441/2007 of European Commission). n = analysis per sample. c = analysis with values between m and M. m = limit of bacteria count; maximum c sample analysis can exceed this limit. M = limit of bacteria count; none of the sample analysis must exceed this limit.

The shelf life estimation based on food quality is carried out by sensory analysis. The sensory panel is usually constituted by four to six trained members who evaluate the organoleptic properties of the product along the time. As a result of both analysis, the shelf life of the product will be the lowest date obtained.

Normally, the sensory quality is lost before reaching the required microbiological limits (Ragaert et al., 2011).

5. PACKAGING MATERIALS

Packaging of RTU vegetables is a key factor widely studied and improved since these products began to succeed. The goal of food packaging is to contain food in a cost-effective way, protecting the food products from outside influences and damage, satisfying industry requirements and consumer desires, maintaining food safety and minimizing environmental impact (Marsh and Bugusu, 2007).

Different polymeric films can be used as food packaging materials. The main characteristics of the most common used packaging films are summarized in Table 2. The major factors to be taken into account while selecting the packaging materials are (Mangaraj et al., 2009):

- Type of package: flexible, rigid or semi-rigid.
- Barrier properties needed.
- Physical properties of machinability, strength, clarity and durability.
- Integrity of closure (heat sealing), fogging of the film as a result of product respiration.
- Sealing reliability.
- Water vapour transmission rate.
- Resistance to chemical degradation.
- Nontoxic and chemically inert.
- Printability.
- Commercial suitability with economic feasibility.

Table 2.- Characteristics of most common packaging films used for RTU fruits and vegetables applications.
Adapted from Mangaraj et al. (2009)

TYPE OF FILM	MAIN ADVANTAGES	DISADVANTAGES
Low-density polyethylene (LDPE)	Soft, flexible and strong material. Light weight. Low cost Good moisture barrier. Resistance to chemicals Heat sealable and easy to seal. Relatively transparent High ratio of CO ₂ to O ₂ permeability. Can be laminated and coextruded	Slight haze or translucency Difficult to be recycled for films
Linear low-density polyethylene (LLDPE)	Soft, flexible and strong material. Light weight. Low cost Better impact strength, tear resistance and higher tensile strength and elongation. Better puncture resistance Good moisture barrier, grease resistance and inert Good low-temperature performance	Slight hazy appearance Difficult to be recycled for films Not suitable for applications involving significant exposure to heat
High-density polyethylene (HDPE)	Flexible, strong and tough. Light weight. Low cost Higher softening point than LDPE and barrier properties Resistance to chemicals and moisture. Permeable to gases Easy to process and easy to form	Slight haze or translucency Difficult to be recycled for films
Biaxially - oriented Polypropylene (BOPP)	Stronger, denser and more transparent than polyethylene Moderate gas barrier and good water vapour barrier Good resistance to chemicals. Excellent grease resistance Good response to heat sealing. Light weight. Low cost Higher clarity, strength and durability than LDPE	Difficult to be recycled for films
Polyesters	Excellent transparency and mechanical properties Good/adequate barrier to gases, moisture and odours Good resistance to chemicals, heat, solvents and acids Light weight. Glass-like transparency. Shatter resistance	Difficult to be recycled for films Inexpensive, but higher cost among plastics
Polyvinyl chloride (PVC)	Strong and transparent. High clarity. Largely used. Good gas barrier and moderated moisture barrier. Excellent resistance to chemicals and grease	Recyclable but it contains chlorine
Polyvinylidene chloride (PVdC)	High barrier to gases and moisture Heat sealable	Recyclable but it contains chlorine Excessive gas barrier Inexpensive, but higher cost among plastics
Polystyrene	High tensile strength. Excellent transparency Used as a "breathable" film	Poor barrier to moisture and gases
Polyamide	Strong. Mechanical and thermal properties similar to PET Excellent odour barrier. Reasonably good oxygen barrier Good chemical resistance. Excellent high-temp performance	Poor moisture barrier Relatively costly
Ethylene-vinyl alcohol (EVOH)	Excellent barrier to oxygen and odours Often used as oxygen barrier material	Low moisture barrier Moisture sensitive
Ethylene-vinyl acetate (EVA)	Excellent transparency. Excellent clarity Very good heat seal. Very good adhesive properties	Poor gas and moisture barrier
Poly(lactide) (PLA)	Biodegradable. Hydrolysable	Relatively expensive
Laminates / Coextrusions	Properties can be tailored for product needs Flexible in design and characteristics	Relatively expensive but cost-effective for purpose

In the beginning, producers used the packaging technologies commonly applied in other food products, employing single-layered plastic bags made of a combination of polyethylene (PE) and ethylene vinyl acetate (EVA). However, RTU vegetables show a different behaviour in comparison to other food products: they need a balanced oxygen/carbon dioxide atmosphere to preserve the freshness; so packaging films should be oxygen-permeable. The oxygen transmission rate (OTR) is a key property of the packaging films to improve the shelf-life of RTU vegetables. There are different ways to control the OTR: changing the film thickness; using different materials or manufacturing procedures; and employing micro-perforated films or micro-porous membranes (Gorny and Gil, 1997).

Firstly, manufacturers improved the OTR by changing the thickness and the proportion of EVA in those single-layered PE/EVA films, but products with high respiration rate could not be properly packed with films thin enough to allow the oxygen transfer. Moreover, these films have a foggy appearance and bad printing quality. Trying to solve those problems, multi-layered laminated films were developed. These films provide a better OTR control and allow a better printing quality, because the printed surface is commonly a polypropylene (PP) layer incorporated among the other layers, preventing wear during handling. However, they are expensive due to the manufacturing procedure. Co-extruded films were after developed to reduce the manufacturing cost of laminated films. Many layers are produced simultaneously and melted together without any adhesive. They are up to 25% cheaper than laminated films and better for high OTR purposes. However, sealing properties and printing quality are better in laminated films. A greater uniformity of layer thickness and density is reached using low density polyethylene (LDPE), linear low density polyethylene (LLDPE) and ultra-low density polyethylene (ULDPE) films, allowing a better control of OTR and better mechanical properties for flexible packaging applications. Last improvements in that way are metallocene films, produced by specific PE catalysis for a better density control. These polymers also present greater properties of transparency and sealing.

For high respiration rate products, OTR can be improved by using micro-perforated films, which are produced by mechanical or laser drilling of the film layers. The atmosphere inside the bag is determined by the total area of perforations. The commercial brand P-Plus® presents a micro-perforated film for products needing high carbon dioxide and moderate oxygen atmospheres. It is very effective to control the relative humidity in the bag, improving the shelf-life in products highly sensitive to dehydration, such as leafy vegetables (i.e spinach and watercress). Micro-porous membranes are another option to combine with flexible and non-permeable films. This membrane has very small porous to allow the gas exchange and it is very effective to create atmospheres with high carbon dioxide and moderate oxygen levels.

Recent innovations in packaging films are intelligent films and active films. Intelligent films are able to change the OTR depending on the external temperature. This prevents the formation of anaerobic atmospheres, which generate off-odours and product degradation. Active films can incorporate different molecules inside, such as antimicrobial compounds or ethylene adsorbents, to preserve the product shelf-life (Gorny and Gil, 1997).

6. METHODS TO EXTEND SHELF-LIFE AND ENHANCE SAFETY

There are variety of methods used to reduce microorganism's population and physiological degradation on whole and fresh-cut products. These methods can be commonly classified in chemical and physical methods, and they have been proved to be moderately efficient in reducing the indigenous microflora and also the contaminating pathogens (Parish et al. 2003).

6.1 CHEMICAL METHODS

Chemical methods of cleaning and sanitizing the surfaces of the products usually involve the application of mechanical washing in the presence of sanitizers, followed by rinsing with potable water (Wei et al., 2006). Several sanitizing agents may be used for fruit and vegetable washing with the intention of reducing the risk of microbial contamination, helping in the prevention of postharvest diseases and foodborne illness. Most commonly used chemical sanitizers are reviewed in Table 3.

Chlorine is the most commonly used sanitizer due to its effectiveness at low concentrations, although it is inactivated by organic material. It is commonly used as free chlorine, hypochlorite, chlorine dioxide or

acidified sodium chlorite, with different advantages and disadvantages. Chlorine is easily available and it is a low cost reagent, but it is corrosive and generates chlorine vapours and odours when it is used. Chlorine dioxide is a less corrosive alternative, with effectiveness less pH dependant. Moreover, it can delay ripening in fresh products due to the inhibition and elimination of ethylene. However, it is not permitted for cut products in USA and it is not regulated in EU yet. At permitted concentrations it is poor effective. Other halogen elements, such as bromine or iodine, are also used for reducing the microbial contents in fruits and vegetables, but they are not so common.

Salts like trisodium phosphate (TSP) or calcium are successful methods to reduce the microbial load in fruits and vegetables. TSP is effective in *Salmonella* inactivation on tomato surface (Beuchat, 1998). However, it is less promising in removing *L. monocytogenes* from shredded lettuce (Parish et al., 2003). Solutions containing more than 10% TSP damaged the sensory quality of lettuce. Calcium salts, on the contrary, are widely used for delicate fruits and products with high senescence index, as they maintain product firmness and quality, reducing microbial load. Calcium lactate is one of the most utilized compounds. In a study with fresh-cut lettuce and carrots, this compound demonstrated the same effectiveness as chlorine in reducing microbial load (Martin-Diana et al., 2005b).

Organic acids, such as lactic, citric, acetic or tartaric, have been described as strong antimicrobial agents due to environment or internal cellular pH reduction, disturbance of membrane transport and/or permeability and anions accumulation (Parish et al., 2003). Most commonly used organic acids in fruits and vegetables are citric and ascorbic.

Quaternary ammonium compounds are cationic surfactants with greater activity against fungi and gram-positive bacteria than gram-negative bacteria. Although they are not approved for direct food contact, they may have some limited usefulness on whole product, since the product must be peeled prior to consumption (Parish et al., 2003). Direct food contact with quaternary ammonium compounds would require regulatory approval and a demonstration that the product is safe for consumption.

Hydrogen peroxide (H_2O_2) possesses bacteriostatic and bactericidal activity due to its strong oxidizing power and also due to the generation of cytotoxic agents (hydroxyl radical). It is used as antimicrobial or bleaching agent in the range of 0.04 – 1.25% up to 80 ppm in product wash water (Akbas & Olmez, 2007; Alexandre et al., 2012a). Combining hydrogen peroxide with peracetic acid, peroxyacetic acid is obtained. It is a strong oxidant agent used to wash fruits and vegetables in concentrations up to 80 ppm, it is more effective on the inactivation of pathogenic microorganisms at lower concentrations than chlorine. Recently, a combination of peracetic acid and chlorine dioxide or sodium hypochlorite has been proven very active against novovirus in ready-to-eat fruits and vegetables (Girard et al., 2016).

Ozone is a strong antimicrobial agent with high reactivity and penetrability, generally more effective in gaseous form than in aqueous solution (Klockow & Keener, 2010). Shelf-life of fresh products can be enhanced and extended by using ozone as a sanitizer. Several studies are dealing with in-package gaseous ozone action. Results indicate that this treatment is very effective against *E. coli* O157:H7, also extending shelf life. However, there are some limitations when it is applied in fresh-cut products. Quality changes can be produced, such as physiological injuries in plant tissues or deterioration of flavour and colour. In some products like spinach, notable colour degradation can occur (Klockow & Keener, 2010).

There are two types of electrolyzed water with sanitizing properties: acidic electrolyzed water (AEW) and neutral electrolyzed water (NEW). These solutions are conventionally generated by electrolysis of aqueous sodium chloride (0.5 – 1% NaCl). An electrolyzed acidic solution (AEW) or an electrolyzed basic solution (NEW) is produced at the anode and cathode, respectively. The AEW has a strong bactericidal effect on pathogenic and spoilage microorganisms (Ramos et al., 2013). Electrolyzed water has demonstrated to be very effective for improving postharvest quality and shelf-life in mushrooms (Aday, 2016).

1-Methylcyclopropene (1-MCP) has been extensively used to control senescence of fresh and minimally processed vegetables (Toivonen, 2008; Watkins, 2008). 1-MCP has shown to reduce leaf yellowing of whole fresh Tsai Tai (Zhang et al., 2010) and it also extends postharvest life of detached spinach leaves (Grozoff et al., 2010). Even in the presence of ethylene, application of 1-MCP significantly increased shelf-life of minimally processed Tsai Tai (Able et al., 2003) and prevented yellowing of rocket (*Eruca sativa*) leaves (Koukounaras et al., 2006).

6.2 PHYSICAL METHODS

Most commonly used physical methods are reviewed in table 4, with an overview of the major advantages, limitations and effectiveness for each method.

Modified packaging is one of the most effective tools to preserve and enhance the shelf life of fresh fruits and vegetables. The simpler form is vacuum packaging, where the product is packed with permeable film; the air is evacuated and the package is sealed with low concentration of O₂ (Arvanitoyannis, 2012). Modified atmosphere packaging (MAP) involves the modification of the internal atmosphere composition of a package by reducing the amount of oxygen and replacing it by carbon dioxide and/or nitrogen. This process intends to extend the postharvest life of whole and pre-cut commodities by reducing their respiration rate and the production of ethylene, minimizing metabolic activity, delaying enzymatic browning and maintaining visual appearance (Cui et al., 2009). The postharvest quality of fresh-cut watercress can be significantly improved by using argon and nitrogen inert gas-enriched modified atmosphere packaging (Pinela et al., 2016).

Active and intelligent packaging (A&I) is one of the most innovative developments in the area of food packaging in the last decades. Active packaging refers to the incorporation of certain agents into packaging systems to improve food quality and safety and therefore extend their shelf life (Dainelli et al., 2008). The nature of the active agents that can be added is very diverse, as well as the nature of the materials into which they are included. Many studies can be found regarding applications of active packaging to food industry (Bolumar et al., 2011; Cruz-Romero et al., 2013). López-de-Dicastillo et al. (2012) produced active antioxidant food packaging films by the incorporation of ascorbic acid, ferulic acid, quercetin and green tea extract into an ethylene vinyl alcohol copolymer matrix. These films were tested by real packaging applications of brined sardines. The evolution of the peroxide index and malondialdehyde content showed that, in general, the films improved sardine stability. The purpose of intelligent packaging is to give indication on, and to monitor, the freshness of the food. Substances responsible for the active or intelligent function can be contained in a separate container, for instance in a small paper sachet or as substances directly incorporated in the packaging material (Arvanitoyannis, 2012).

The use of fillers with at least one nanoscale dimension (nanoparticles) produces nanocomposites. Recently, using nanocomposite materials improved fundamental characteristics of food-packaging materials such as strength, barrier properties, antimicrobial properties and stability to heat and cold (De Azeredo, 2009). Nanoscale technologies can be promising techniques to improve product packaging. However, more studies are required before their application in food industry. The storage time of mushrooms packaged with nanocomposite based packaging material can be extended to more than 14 days (Donglu et al., 2015).

Another common physical method is irradiation. Gamma-ray, X-ray and electron beams are ionizing radiations. Low-dose irradiation is applied to fresh fruits and vegetables to delay produce maturation, and it is very effective in reducing bacterial, parasitic and protozoan pathogens (Lu et al., 2005). Shelf-life of watercress was increased a day and a half by using gamma ray radiation (Martins et al., 2004). Gamma irradiation also preserve the antioxidant activity, total flavonoids, unsaturated fatty acids, tocopherols and total phenolics in fresh-cut watercress during cold storage (Pinela et al., 2016b). Despite the effectiveness, ionizing radiations are not permitted in Spain for food, except in the case of aromatic herbs. Ultraviolet radiation (UV) is also applied to fresh fruits and vegetables, since it acts directly or indirectly as an antimicrobial agent. UV from 100 to 280 nm (UV-C) can cause direct bacterial DNA damage or may induce resistance mechanisms against pathogens in different fruits and vegetables. Pulsed light is an alternative technology to UV light. It is a rapid and effective treatment on microbial inactivation since it kills microorganisms using short time high frequency pulses of an intense broad spectrum, rich UV-C light.

High pressure processing (HPP) is a method where food is subjected to elevated pressures (in the range of 100-1000 MPa) to achieve microbial and enzymatic inactivation, without the degradation in flavour and nutrients associated with traditional thermal processing. As shown in several studies, HPP can be suitable for fruit and vegetable processing. This treatment provides high quality food with high safety and extended shelf-life, while maintaining similar characteristics than fresh products (Guerrero-Beltrán et al., 2005).

Power ultrasound (US) has emerged as an alternative processing technology to food conventional thermal approaches. Ultrasound is used at frequencies in the range of 20-100 kHz and requires the presence of a liquid medium for power transmission. On its own, US is not significantly effective on decreasing high load microbial contamination (Alexandre et al., 2012b). Due to this reason, this treatment has been used in combination with aqueous sanitizers, showing better results (Cao et al., 2010). The US potential is to improve the aqueous sanitizer's effectiveness by enhancing the penetration of these solutions to inaccessible sites. Ultrasound application is valid for inactivating aerobic mesophylls, yeasts and moulds in watercress, parsley and strawberry, combined with chemical sanitizers. However, it causes some disadvantages such as darkening in watercress and parsley or loss of firmness in strawberry (Sao José and Vanetti, 2015).

An emerging antimicrobial technology for decontaminating infected surfaces is the use of non-thermal ionized gases (cold gas plasmas). Plasma is composed of gas molecules, which have been dissociated by an energy input. It is constituted by photons, electrons, positive and negative ions, atoms, free radicals and excited or non-excited molecules that, in combination, have the ability to inactivate microorganisms (Ramos et al., 2013). Most studies regarding cold plasma decontamination of plant product report the use of noble gases such as argon, or common diatomic gases such as nitrogen, oxygen and/or air for attaining the plasma state. However, it is also possible to induce cold plasmas in MAP gases for the treatment of fresh product (Misra et al., 2014). Studies on lettuce and strawberry surfaces, and potato tissues, had shown that cold plasma is highly effective on the removal of surface human pathogens, such as *E. coli* O157:H7 and *Salmonella* spp. (Fernandez et al., 2013). The application of cold plasma at atmospheric conditions on radicchio (red chicory) caused a significative reduction of *E. coli* and *L. monocytogenes*, maintaining the antioxidant capacity of the leaves intact (Pasquali et al., 2016).

6.3 BIOLOGICAL METHODS

In addition to chemical and physical methods to preserve the quality of fruits and vegetables, some biological methods can be also considered. In an attempt to prevent growth of pathogens and spoilage microorganisms on product, research on the application of biocontrol agents has been made. This biological method is known as biopreservation and consists on the extension of the shelf-life and the improvement of food safety using microorganisms and/or their metabolites (Ramos et al. 2013). Some particular microorganisms with an antagonistic effect on pathogens are the lactic acid bacteria (LAB). This bacterial group is naturally present in food products and different studies suggested that, when LAB are applied to surfaces of the product, they are strong competitors for physical space and nutrients and/or may produce a wide range of antimicrobial metabolites such as organic acids, hydrogen peroxide, diacetyl and bacteriocins that negatively affect pathogens (Sagong et al. 2011). Applying *Lactobacillus plantarum* to sliced apples and lamb's lettuce increased both the safety and shelf-life without detrimental effects on the organoleptic qualities (Siroli et al., 2015).

Bacteriocins are proteinic compounds biologically active, produced by microorganisms. They have an antimicrobial effect in other microorganisms. These bacteriocins are "generally recognized as safe" (GRAS) and have been commonly employed in combination with other food additives as protective agents in fresh-cut products (Rodgers, 2008). They are less effective against gram-negative bacteria and have a limited effect, because they only inhibit microorganisms similar to the producing microbial strain. There are several reviews about the use of bacteriocins in food preservation (Settanni & Corsetti, 2008; Deegan et al., 2009; García et al., 2010). Therefore, biopreservation is a promising innovative way of extending the shelf life of fresh fruits and vegetables, and reducing microbial hazards. The use of a bacteriocin in an alginate coating for minimally processed papaya inhibited microbial growth until 21 days (Narsaiah et al., 2015).

Table 3.- Chemical methods used for the preservation of minimally processed vegetables and fruits.

METHOD	ADVANTAGES	LIMITATIONS	EFFECTIVENES	REFERENCES
Chlorine (hypochlorite)	Low cost Easily available Long history of use	Liberation of chlorine vapors during production and formation of chlorinated by-products (DBPs) Efficacy is affected by the presence of organic matter Corrosive Activity pH dependant Sensitive to temperature, light and air Banned in some European countries	Very high concentrations may not eliminate all the pathogens on products Chlorine dosages (50-200 ppm) and contact times (1-2 min) used result in 1 to 2 log ₁₀ bacterial inactivation Some resistance by bacterial spores and protozoan oocysts	Baert et al. (2011) Brackett (1999) Burnett & Beuchat (2000) Butot et al. (2007) De Roever (1998) Harris et al. (2003)
Chlorine dioxide	<i>ClO₂ Aqueous</i> Higher antimicrobial efficacy at neutral pH than chlorine Effectiveness less pH dependant compared to chlorine Fewer potentially hazardous DBP formation than chlorine Less corrosive than chlorine and ozone Can delay ripening of produce due to ethylene elimination and inhibiting its production Aqueous ClO ₂ treatments (4, 6 and 8 mg/l) exhibited anti-browning effects in apple juices <i>ClO₂ gas</i> Effective in a wide pH range Great penetration ability	<i>ClO₂ Aqueous</i> Not efficient at permitted levels for fresh products Requires on-site generation Explosive Not permitted for cut products in USA and not regulated in EU Final water rinsing is required after treatment More iodinated DBP formation than chlorine if iodine exists in water Formation of specific by-products, chlorite and chlorate Requires monitoring in indoor applications <i>ClO₂ gas</i> Decomposes into chlorine and oxygen Must be produced in situ Activity affected by gas concentration, time of exposure, humidity, temperature	<i>ClO₂ Aqueous</i> Studies revealed that very high concentrations are needed to have a significant reduction in the microbial load of fruits and vegetables Reduction of a few logs reported when used at permitted concentrations <i>ClO₂ gas</i> Inactivates <i>E. coli</i> O157:H7 and <i>L. monocytogenes</i>	Baur et al. (2005) Beltrán et al. (2005b) Casteel et al. (2008) Sanz et al. (2002) Ukuku (2006) Vandekinderen et al. (2008) Wei et al. (2007)

Table 3.- Chemical methods used for the preservation of minimally processed vegetables and fruits (*continuation*).

METHOD	ADVANTAGES	LIMITATIONS	EFFECTIVENES	REFERENCES
Acidified sodium chlorite	Greater efficacy than hypochlorite due to low pH	Little information on production of chlorinated by-products Limited amount of research conducted	Inhibited enzymatic browning on fresh-cut products Usefulness for products needs further research	Allende et al. (2009) He et al. (2008) Luo et al. (2011)
Bromine	Possible synergy with chlorine compounds	Information lacking on production of brominated by-products and their potential health effects	Not widely used as a sanitizer	Beuchat (1998) Parish et al. (2003)
Iodine	Less corrosive than chlorine at low temperature Broad spectrum of microorganisms Iodophor less volatile than iodine	Stains commodities and equipment Corrosive above 50 °C Not approved for direct food contact	More active against vegetative cells Than against bacterial spores	Ayala-Zavala & Gustavo (2010)
Trisodium phosphate	Less corrosive than most other compounds	Not efficient against <i>Listeria</i> Has very high pH (11-12)	Concentrations between 1 and 15% yielded reductions in pathogen populations from 0 to 6 logs <i>Listeria</i> relatively resistant	Beuchat (1998) Weissinger et al. (2000) Zhuang and Beuchat (1996)
Quaternary ammonium compounds	Colorless, odorless Stable at high temperatures Non-corrosive Good penetrating ability Relatively stable to organic compounds	Limited usefulness at low pH (<6) Not compatible with soaps or anionic detergents Costly Not approved for direct food contact	Most effective against fungi and gram positive bacteria than gram negative bacteria, except <i>Salmonella</i> and <i>E. coli</i>	Parish et al. (2003) Reynolds (1991) Rossmore (2001)
Calcium based solutions	Can significantly increase calcium content of the final product Delays aging or ripening fruits and vegetables Reduces post-harvest decay	Bitterness and off-flavours associated with calcium chloride	Limited efficacy as antimicrobial agent Calcium lactate, as fresh-cut lettuce and carrots sanitizer, showed similar effectiveness than chlorine in reducing and keeping the microbial load	Kitis (2004) Ölmez & Kretschmar (2009)

Table 3.- Chemical methods used for the preservation of minimally processed vegetables and fruits (continuation).

METHOD	ADVANTAGES	LIMITATIONS	EFFECTIVENES	REFERENCES
Organic acids (lactic, citric, acetic, tartaric or ascorbic acid	Easy to use Economical, depending on type of acid and use No toxicity Allowed for organic products	Interferes with the sensory quality Relatively lower antimicrobial efficacy Low pH use only Antimicrobial effect dependent upon type of acid and strain of mcroorganism Disinfection with organic acids in fresh- cut industry has an impact on wastewater quality	The exposure times needed for a significant reduction in microbial load are very long (5-15 min)	Benarde et al. (1965) Francis & O'Beirne (2002) Fu et al. (2007) Singh et al. (2002) Zhong et al. (2006)
Hydrogen peroxide	No harmful DBP formation No residue production Not corrosive at permitted levels	Phytotoxicity against some products like lettuce and berries Negative impact on overall quality Low antimicrobial efficacy at permitted levels for vegetables Less effective against yeasts, fungi and viruses Not allowed for organic products Requires the removal of residual H ₂ O ₂ after processing	At low concentrations (1-2%) H ₂ O ₂ is not efficient in reducing the pathogenic bacterial load on the fresh produce At high concentrations (4-5%) it interferes with the overall quality of the produce	Akbas & Olmez (2007) Alexandre et al. (2012a) Hwang et al. (2001) Ölmez & Kretzschmar (2009)
Peroxyacetic acid	No harmful DBP formation Efficacy is not affected by water organic load Efficacy unaffected by temperature changes Good antimicrobial properties at low temperatures in the pH range (5-8) Not corrosive at permitted levels (<80 ppm) More efficient than chlorine or chlorine dioxide	Low antimicrobial efficacy at permitted levels for vegetables	At the highest concentration permitted (80 ppm), peroxyacetic acid in wash water is not sufficient to obtain a substantial reduction in the microbial load of fresh-cut fruits and vegetables	Sapers (2009) Sapers & Jones (2006) Vandekinderen et al. (2009) Girard et al. (2016)

Table 3.- Chemical methods used for the preservation of minimally processed vegetables and fruits (*continuation*).

METHOD	ADVANTAGES	LIMITATIONS	EFFECTIVENES	REFERENCES
Ozone	<p><i>Aqueous</i></p> <p>High antimicrobial activity</p> <p>Extends storage life of fresh non-cut products</p> <p>Effective at low concentrations and short contact time</p> <p>Broad spectrum of microorganisms</p> <p>Good penetration ability</p> <p>Effectiveness against protozoa</p> <p>Generally recognized as safe</p> <p>No hazardous DBP formation</p> <p>Decomposes to nontoxic products</p> <p>Does not leave hazardous residues on food</p> <p>Lower running cost</p> <p><i>Gaseous</i></p> <p>Higher antimicrobial activity than in solution</p> <p>Acceptable food quality</p> <p>Effective against spoilage and pathogenic microorganisms</p>	<p><i>Aqueous</i></p> <p>Possible deterioration of products flavour and colour.</p> <p>Can cause products physiological injury and loss of antioxidant constituents</p> <p>Unstable, very highly reactive</p> <p>Possible human toxic effects in processing facilities</p> <p>Corrosive to equipment</p> <p>Requires on-site generation</p> <p>Requires monitoring in indoor applications</p> <p>Higher initial investment cost</p> <p><i>Gaseous</i></p> <p>Ozone gas is hazardous so it must be contained and destroyed</p> <p>Toxic and reactive</p> <p>Can damage plant tissues</p> <p>Product quality changes may occur</p> <p>Control of O₃ leakage required</p>	<p><i>Aqueous</i></p> <p>Effective against a variety of postharvest pathogens reported on fruits and vegetables</p> <p><i>Gaseous</i></p> <p>More effective than the solution form</p>	<p>Alexandre et al. (2011a)</p> <p>Alexandre et al. (2011b)</p> <p>Anino et al. (2006)</p> <p>Conway et al. (1992)</p> <p>Martin-Diana et al. (2005b)</p> <p>Picchioni et al. (1996)</p>
Electrolyzed water	<p>Inactivates several pathogenic and spoilage microorganisms</p> <p>Neutralizes harmful substances such as cyanides and ammonium</p>	<p>Reduces quality on fresh-cut vegetables</p>	<p>Has strong bactericidal effect on pathogenic and spoilage microorganisms of minimally processed vegetables</p>	<p>Guzel-Seydim et al. (2004)</p> <p>Habibi and Haddad (2009)</p> <p>Karaca and Velioglu (2007)</p> <p>Aday (2016)</p>
1-Methyl cyclopropene (1-MCP)	<p>Control senescence of fresh and minimal processed vegetables</p> <p>Extend shelf-life of fresh-cut products</p> <p>No toxicity, low residue production</p>	<p>Very recent application</p> <p>Properties and limitations under study</p>	<p>Effective at very low concentrations</p> <p>Extend shelf-life of fresh-cut products 1-2 days more used with cold storage and optimal humidity conditions</p>	<p>Wang et al. (2014)</p> <p>Toivonen (2008)</p> <p>Watkins (2008)</p>

Table 4.- Physical methods used for the preservation of minimally processed vegetables and fruits.

METHOD	ADVANTAGES	LIMITATIONS	EFFECTIVENES	REFERENCES
Modified atmosphere packaging (MAP)	<p>Extends storage life of the fresh products by 50-400%.</p> <p>In general, fresh-cut products are more tolerant to higher CO₂ levels than the intact product</p> <p>Reduced economic losses</p> <p>Provides a high-quality product</p> <p>Odorless and convenient packages</p> <p>Sealed packages can act as barriers to further product recontamination</p> <p>Delay of ripening</p> <p>Improved presentation, clear view of product and all-around visibility</p>	<p>Often produces high levels of CO₂ with a consequent development of off-flavours and potential stimulation of pathogens growth</p> <p>Temperature control necessary</p> <p>Different gas formulations per product type and target microorganism</p> <p>Packaging material, and temperature per product and/or per target microorganism</p> <p>CO₂ dissolving to food could lead to package collapse and increased drip</p> <p>Plastic films may be environmentally undesirable</p>	<p>Effective on preserving the quality of fresh and processed vegetables and in reducing the postharvest disease incidence for several fruits and vegetables</p>	<p>Arvanitoyannis (2012)</p> <p>Cliffe-Byrnes & O'Beirne (2002)</p> <p>Cui et al. (2009)</p> <p>Graça et al. (2011)</p> <p>Kim et al. (2000)</p> <p>Rico et al. (2008)</p> <p>Salveit (2003)</p> <p>Ward (2016)</p> <p>Pinela et al. (2016)</p> <p>Oliveira et al. (2015)</p>
Active and intelligent packaging (A&I)	<p>Food quality and sensorial improvements</p> <p>Food safety improvement</p> <p>Delays oxidation</p> <p>Monitorization of the temperature along transport</p> <p>Control of respiration rate, microbial growth and moisture migration</p> <p>Can be used to check the effectiveness and integrity of active packaging systems</p>	<p>Difficult to evaluate its safety when compared to the traditional packaging</p> <p>Can occur migration of substances from the package to the food</p> <p>Incorrect use of the packaging due to the insufficient labeling</p> <p>Non-efficacious operation of the A&I packaging</p> <p>Regulation does not cover the use of this type of packaging</p> <p>Non-uniformity of international legislation. Labelling requirements</p>	<p>Efficient on product shelf-life improvement</p> <p>Monitors the integrity and safety of the packed product</p>	<p>Dainelli et al. (2008)</p> <p>Kerry et al. (2006)</p> <p>Yam et al. (2005)</p> <p>Lee et al. (2015)</p>
Ultrasound (US)	<p>Enhance the penetration of solutions to inaccessible sites</p> <p>Heat transfer rate increased</p> <p>Reduction of process times and temperature</p>	<p>Needs to be combined with other processes to be effective</p> <p>Complex due to difficulty to scale-up</p> <p>Changes on food structure and texture</p> <p>Penetration affected by solids and air</p>	<p>Effective against common food bacteria pathogens</p> <p>Also effective against vegetative cells, spores and enzymes</p>	<p>Alexandre et al. (2012b)</p> <p>Cao et al. (2010)</p> <p>Chemat et al. (2011)</p> <p>Mukhopadhyay et al. (2012)</p> <p>Sagong et al. (2011)</p>

Table 4.- Physical methods used for the preservation of minimally processed vegetables and fruits (*continuation*).

METHOD	ADVANTAGES	LIMITATIONS	EFFECTIVENES	REFERENCES
Nanocomposite packaging	Environment friendly Improvement of mechanical properties e.g. strength, modulus and dimensional stability Low permeability to gases, water and hydrocarbons Thermal stability Chemical resistance Electrical conductivity Optical clarity in comparison to conventionally filled polymers	Increased viscosity (limits process ability) May reduce impact performance Optical issues Scarce information on formulation/ structure/property relationships	Development of new food-packaging materials with improved characteristics	De Azeredo (2009) De Azeredo (2013) Donglu et al. (2015)
Irradiation	Can be performed at room temperature Can be conducted after packaging Delays ripening and senescence of climateric fruits Extends shelf-life of products Low energy costs	Acceptance of irradiation by consumers Product quality may be affected specially at high doses Product texture alterations Not permitted in Spain, except for aromatic herbs	Variable effectiveness against postharvest pathogens reported in literature High efficiency for eliminating pathogenic bacteria and parasites from the surface of fruits and vegetables Effective in reducing bacterial and molds of climateric fruits Gamma rays and X-rays have higher penetration ability than electron beam	Gergoff-Grozeff et al. (2013) Allende & Artés (2003) Barakat (2010) Bidawid et al. (2000) Foley et al. (2002) Goularte et al. (2004) Hagenmaier & Baker (1997) Hussain et al. (2008) Molins et al. (2001) Martins et al. (2004) Pinela et al. (2016b)
High pressure processing (HPP)	Microbial and enzymatic inactivation No degradation in flavor and nutrients No evidence of toxicity Positive consumer appeal Uniformity of treatment throughout food	Affects porous integrity Expensive equipment Foods should have approx. 40% free water for antimicrobial effect	Effective in inactivating most vegetative pathogenic and spoilage microorganisms at pressures above 200 Mpa	Chawla et al. (2011) Considine et al. (2008) Guerrero-Beltrán et al. (2005)

Table 4.- Physical methods used for the preservation of minimally processed vegetables and fruits (*continuation*).

METHOD	ADVANTAGES	LIMITATIONS	EFFECTIVENES	REFERENCES
Ultraviolet light (UV)	Absence of residual toxicity Equipment relatively inexpensive and easy to use Can reduce deterioration of the produce Exposure to UV also induces the synthesis of health-promoting compounds such as anthocyanins and stilbenoids	Pre-treatment normally necessary Difficulties in accurately measure the UV dose Increase produce stress and respiration rate, and induce a lignification-like process Low penetration depth Limited application on solid food and opaque surfaces Can cause off-flavours and colour changes	Effective in reducing microbiota growth in fruits and vegetables Germicidal at UV-C interval	Alexandre et al. (2012c) Neves et al. (2012) Ohlsson & Bengtsson (2002)
Pulsed light (PL)	Rapid and effective on microbial inactivation in solid and liquid foods Few residual compounds Medium cost Low energy input	Food composition affects the efficacy Efficacy decreases at high contamination levels Possible resistance in some microorganisms Possible adverse chemical effects	Inactivates spoilage and pathogenic microorganisms	Braidot et al. (2014) Costa et al. (2013) Choi et al. (2010) Gómez-López et al. (2007b) Guerrero-Beltrán et al. (2005) Oms-Oliu et al. (2010)
Cold plasma	High efficiency Low impact on the internal product matrix No residues Resources-efficient Can be used on vegetables tissues surfaces No "shadow effects" (all product is treated) Could be included as part of the packaging process	Scarce information about the mechanism of inactivation Physicochemical changes in the product may occur Inactivation is affected by type of microorganisms, inactivation medium, number of cells, operating gas mixture, gas flow and physiological state of cells Scarce information about interactions with the food or packaging materials Scarce information about the stability of the plasma for large-scale operation	Inactivation of <i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>S. aureus</i> and <i>L. monocytogenes</i> by 1.5-3.7 log CFU/cm ²	Bermúdez-Aguirre et al. (2013) Critzler & Doyle (2010) Fernández et al. (2013) Fernández & Thompson (2012) Knorr et al. (2011) Perni et al. (2008) Surowsky et al. (2013) Pasquali et al. (2016)

EFFECTS OF SALINITY IN PLANTS AND LEAFY VEGETABLES

1. INTRODUCTION

Salinity of soil and/or irrigation water is a decisive factor affecting the growth of most of the crops, causing lots of problems in agriculture, mainly in arid or semiarid regions (Lamsal et al., 1999; Shannon et al., 1994). Industrial development and population growth have increased the contamination and salinization of surface and underground water and thus of agricultural soils. Salinity is the major environmental factor limiting plant growth and productivity (Allakhverdiev et al., 2000b). The detrimental effects of high salinity on plants can be observed at the whole-plant level as the death of plants and/or decreases in productivity. During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, and energy and lipid metabolism are affected.

Stress biology and plant responses to high salinity level have been widely discussed in the last decade (Hasegawa et al., 2000; Zhu, 2002; Parida & Das, 2005). In this section, the main effects of soil or irrigation water salinity in plants and crops are exposed briefly, and more extensively in the case of lettuce as the leafy vegetable studied in the present research.

2. EFFECTS OF SALINITY ON PLANTS

Salinity of soil and water is caused by the presence of excessive amounts of salts. Most commonly, high Na^+ and Cl^- cause the salt stress, but there are also many other salts or ions such as sodium sulphate, sodium bicarbonate or boron (Bie et al. 2004; Eraslan et al., 2007).

Tolerance of crops to salinity is very variable, depending mainly on the variety cultivated (Shannon & Grieve, 1998), but it is also affected by many other agricultural, soil and environmental factors (Maas & Hoffman, 1977).

Salinity affects crop growth in two ways: a) by increasing the osmotic potential of the soil, making water less available to plants, and b) by creating excessive concentrations of specific elements (Yamaguchi and Blumwald, 2005). Salt stress, as said before, affects all the major processes such as growth, photosynthesis, protein synthesis, and energy and lipid metabolism (Parida & Das, 2005). Most important effects of salinity in crops are summarized in the scheme of Figure 1 and explained in the following paragraphs.

Salinity stress results in a clear stunting of plants (Takemura et al., 2000). The immediate response of salt stress is the reduction in the rate of leaf surface expansion (Wang & Nil, 2000). Salt stress also results in a considerable decrease in the fresh and dry weights of leaves, stems and roots. The yield, height, weight and leaf area index (LAI) of elephant grass growing under salinity conditions of 1.5 – 25 dS/m decreased about 50% (Wang et al. 2002). The same effect was detected in wheat leaves with increasing concentrations of NaCl and boron (up to 75 mM NaCl + 200 μM H_3BO_3) (Masood et al., 2012). However, in some species, fresh and dry weights of plants increase with a moderate increase in salinity, for example, in *Salicornia rubra* the optimal growth occurs at 200 mM NaCl and the growth declines with a further increase in salinity (Khan, 2001).

Water and osmotic potential of plants become more negative with an increase in salinity, whereas turgor pressure increases with increasing salinity (Meloni et al., 2001; Khan, 2001; Romero-Aranda et al., 2001).

EFFECTS OF SALINITY	Growth	Stunting of plants. Decrease of fresh and dry weights of leaves, stems and roots. Decrease of yield. Decrease of height, weight and leaf area index (LAI).
	Water content	Water potential becomes more negative. Osmotic potential becomes more negative. Turgor pressure increases.
	Leaf anatomy	Changes in epidermal thickness, mesophyll thickness, palisade cell length, palisade diameter and spongy cell diameter. Decrease of intercellular spaces. Reduction of stomatal density. Necrosis, chlorosis and tipburn.
	Photosynthetic pigments and proteins	Decrease of chlorophyll and carotenoids in general. In some species under moderate salinity levels, increase of chlorophyll and carotenoids. Decrease of soluble protein contents.
	Ion levels	Na ⁺ and Cl ⁻ accumulation. K ⁺ deficiencies. Imbalances in Ca ²⁺ , Mg ²⁺ or Fe ²⁺ .
	Antioxidative enzymes and antioxidants	Oxidative stress. Formation of superoxides and peroxy radicals. Increasing activity of antioxidative enzymes such as catalase, peroxidase and superoxide dismutase.
	Other effects	Changes in lipids concentrations. Decrease of nitrate reductase activity. Inhibition of nitrogen fixation in some species.

Figure 1.- Scheme summarizing the most common effects of salinity in plants.

Salinity causes changes in epidermal and mesophyll thickness, in length and diameter of palisade cell and diameter of spongy cell in many vegetable species. Parida et al. (2004) showed a significant decrease of mesophyll thickness and intercellular spaces in leaves of the mangrove *B. parviflora* treated with NaCl. Moreover, salt stress can cause: development of vacuolation and partial swelling of endoplasmic reticulum; decrease in mitochondrial cristae and swelling of mitochondria; vesiculation and fragmentation of tonoplast; and degradation of cytoplasm by the mixture of cytoplasmic and vacuolar matrices in sweet potato leaves (Mitsuya et al., 2000). In tomato plants, salinity causes reduction of plant leaf area and stomatal density (Romero-Aranda et al., 2001).

Most of herbaceous crops affected by salinity at moderate levels do not show visible damage in their leaves and appear to be normal, although their growth and yield decrease. They may have deep green and more succulent leaves with more density and thickness (Maas and Hoffman, 1977). However, when the excess of salinity induces imbalances in concentrations of certain mineral elements, such as Cl, Na, B or Ca, necrosis, chlorosis and tip burn can appear in the leaves (Bie et al., 2004; Eraslan et al., 2007; Carassay et al., 2012).

The chlorophyll and total carotenoid contents of leaves decrease in general under salt stress. The oldest leaves start to develop chlorosis and fall with prolonged period of salt stress (Hernandez et al., 1999; Agastian et al., 2000). However, Wang & Nil (2000) have reported that chlorophyll content increases under conditions of salinity (300 mM NaCl) in *Amaranthus*. Lim et al. (2012) noticed that phenolic compounds and carotenoids content in Buckwheat (*Fagopyrum esculentum* M.) sprouts increase with salinity stress (50 and 100 mM NaCl).

High salt (NaCl) uptake competes with the uptake of other nutrient ions, especially K^+ , leading to K^+ deficiency. Increased treatment of NaCl induces increase in Na^+ and Cl^- and decrease in Ca^{2+} , K^+ , and Mg^{2+} levels in several species of plants (Khan et al., 1999, 2000a; Khan, 2001).

Salt stress causes water deficit as a result of osmotic effects on a wide variety of metabolic activities of plants and this water deficit results in oxidative stress because of the formation of reactive oxygen species such as superoxides and hydroxy and peroxy radicals. These reactive oxygen species cause membrane disfunction and cell death (Bohnert & Jensen, 1996). The plants defend against them by the induction of activities of certain antioxidative enzymes such as catalase, peroxidase, glutathione reductase, and superoxide dismutase, which scavenge reactive oxygen species. There are several reports of increasing activity of antioxidative enzymes (Hernandez et al., 2000).

Wu et al. (1998) have analysed the changes in lipid composition by NaCl stress in root plasma membrane of salt marsh grass (*Spartina patens*) and reported that molar percentages of sterols (including free sterols) and phospholipid decreases with increasing salinity, but the sterol/phospholipids ratio is unaffected by NaCl.

Nitrate reductase activity of leaves decreases in many plants under salt stress (AbdElBaki et al., 2000). The cause of a reduction of this enzyme in the leaves is the presence of Cl^- salts in the external medium. The effect of Cl^- seems to produce a reduction in NO_3^- uptake, and consequently a lower NO_3^- concentration in the leaves. However, a direct effect of Cl^- on the activity of the enzyme cannot be discarded (Flores et al., 2000). These effects have been reported also in leaves of *B. parviflora* (Parida & Das, 2004). Soussi et al. (1999) have reported that salinity inhibits nitrogen fixation by reducing nodulation and nitrogenase activity in chickpea (*C. arietinum* L.).

3. EFFECTS OF SALINITY ON LETTUCE

Lettuce is a crop moderately sensitive to salinity, mainly during the early growth stages and flowering. Its growth is affected from 1.3 dS/m of soil electric conductivity (EC), decreasing its yield 13% per EC unit above that value (Ayers et al., 1951). The optimal growth conditions of lettuce, with respect to salinity, are soil EC lower than 1.3 dS/m and irrigation water EC lower than 0.9 dS/m. Water with less than 115 ppm (5 meq/l) of sodium and 100 ppm (3 meq/l) of chloride has no restrictions of use. However, values over 460 ppm (20 meq/l) of sodium and 350 ppm (10 meq/l) of chloride should not be used.

3.1 EFFECTS OF SALINITY ON LETTUCE GROWTH AND LEAF STRUCTURE

Several studies demonstrate that NaCl salt reduces the growth and yield in lettuce crop (Shannon & Grieve, 1999), and in both hydroponics and traditional culture. Selma et al. (2012) compared lettuce growth and characteristics in hydroponics and traditional cultures and observed that hydroponics can enhanced the visual and nutritional quality for fresh-cut product, but it depends on the variety. However, salinity can affect highly the characteristics of lettuce in hydroponics. Lettuce growing in a soilless system, with irrigation water EC between 0.80 and 4.72 dS/m, showed an increase of fresh mass and growth until EC = 2.0 dS/m but, above that level, the growth and yield of lettuce were significantly reduced (Andriolo et al., 2005). Al-Maskri et al. (2010) also investigated the effect of different salinity levels (0, 50 and 100 mM of NaCl), in a soilless system. They noticed that the number of leaves, plant fresh weight, shoot fresh weight, shoot dry weight, shoot dry matter percentage, root fresh weight, root dry weight, root dry weight percentage, leaf area and leaf area index (LAI) were significantly affected by salinity levels, while shoot and root water contents percentage, ratio of the shoot to root fresh weight and ratio of the shoot to root dry weight showed no significant effect in response to salinity. Changes in the visual appearance of lettuce grown at different NaCl levels are shown in Figure 2.

Garrido et al. (2014) observed physiological, phytochemical and structural changes of lettuce plants in response to different salt concentrations (50, 100 and 150 mM of NaCl). Fresh weight per plant, fresh weight per leaf, leaf area, water content, colour saturation, chlorophyll a and b and the area of the intercellular spaces decreased with increasing concentrations of salt. Solute concentration, elasticity, total and individual phenolic acids and the spaces occupied by the palisade and spongy parenchyma cells, increased when the concentration of salt was increased.

3.2 EFFECTS OF SALINITY ON IONIC BALANCE

Salinity affects the availability of some other nutrients to the plant. With high concentration of NaCl in soil or water, lettuce accumulates Na^+ and Cl^- in its tissues, mainly in baby leaves (Neocleous et al., 2014). This accumulation has effects on other ions such as calcium (Ca), potassium (K), nitrogen (N), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn) or cadmium (Cd). Ünlükara et al. (2008) observed the response of lettuce (*Lactuca sativa* var. *crispa*) to increasing salinity on irrigation water (0.75, 1.5, 2.5, 3.5, 5 and 7 dS/m EC). The concentration of Ca and Fe in the leaves decreased with salinity, whereas accumulation of K, N, Cu, Mn and Zn in plant leaves was unaffected. However, Neocleous et al. (2014), working with two different lettuce varieties (*green Paris island* and *red sanguine*) growing in different NaCl solutions (0, 5, 10 and 20 mM), noticed a reduction in the concentration of K in plants with increasing levels of salinity, while Zn and Cu was enhanced in baby leaves of both cultivars. Moreover, a decrease in Ca concentration was observed in green lettuce; and an increase in Fe, Mn and B concentrations in red lettuce was noticed. Soil salinity can also stimulate Cd uptake of lettuce plants, particularly when Cd bioavailability is poor. Increasing levels of NaCl (0 – 50 mM NaCl) cause increases in the phyto-availability of Cd in leaves of lettuce plants (Ozkutlu & Turan, 2013). The decrease in calcium content due to salinity can produce physiological disorders in lettuce leaves, like tipburn. Tipburn appears as a necrosis in the margins of young developing lettuce leaves under saline conditions (Carassay et al., 2012).

Figure 2. - Visual appearance of lettuce grown at different NaCl levels (Qin et al., 2012).

3.3 EFFECTS OF SALINITY ON PIGMENTS, ENZYMES AND ANTIOXIDANTS

Changes in ion levels can induce imbalances in other compounds of lettuce leaves, such as pigments, enzymes or antioxidants, which cause changes in visual and nutritional quality of lettuce leaves. Kim et al. (2008) observed the influence of salinity stress on the growth, appearance and nutritional compounds, especially phenolic compounds and carotenoids, of romaine lettuce. In plants long-term irrigated with 5 mM NaCl water, the total carotenoid content increased without colour change, and the contents of major carotenoids in romaine lettuce, lutein and P-carotene, increased up to 37 and 80% respectively. However, no differences were observed in lutein and P-carotene contents in short-term high-salinity treated lettuce. The phenolic content of the romaine lettuce declined with short-term high-salinity irrigation, whereas there were no significant differences among samples long-term irrigated with low salt concentration. They concluded that long-term irrigation with relatively low salt concentration, rather than short-term irrigation with high salt concentration, can increase carotenoid content in

romaine lettuce without causing a trade-off in yield or visual quality. Neocleous et al. (2014) discovered that the use of saline waters enhanced health-beneficial phenolic compounds in baby lettuce leaves in some instances, which may be of importance in a diet rich in antioxidants. Qin et al. (2012) noticed a significant reduction in the chlorophyll b content on lettuce leaves of plants growing under salinity conditions, while chlorophyll a content did not change. These changes in pigments and phenolic compounds due to NaCl salinity depend on the varieties of lettuce. Mahmoudi et al. (2012a) observed different responses to salinity in Verte and Romaine lettuce, with respect to the carotenoids, phenolic compounds and flavonoids contents.

Salinity also affects the antioxidative system in lettuce increasing the activity of antioxidant enzymes such as superoxide dismutase, catalase and ascorbate peroxidase. Increased antioxidant enzyme activities, and also accumulation of ascorbic acid and proline, are involved in order to overcome salinity-induced oxidative stress (Eraslan et al., 2007). But also, increasing levels of salinity reduced both polyphenol oxidase and peroxidase activities after cutting and throughout 7 days of storage in fresh-cut lettuce leaves, which is very useful to improve the shelf-life in these products (Chisari et al., 2010).

3.4 EFFECT OF OTHER SALINITIES

Most of the studies carried out about salinity effect on plants are focused on the effect of NaCl salinity, but there are other effects such as those caused by CaCl_2 , Na_2SO_3 or NaHCO_3 . Borghesi et al. (2013) studied the effect of different concentrations of CaCl_2 added to the nutrient solution to obtain EC from 0 to 6.3 dS/m. Growth and yield of lettuce plants were not significantly affected, but nitrate contents in leaves decreased with increasing levels of CaCl_2 . Phenolic compounds and antioxidant activity increased at moderate concentrations of CaCl_2 , while the content of chlorophyll and carotenoids did not change, which are good characteristics for the conservation of fresh-cut leafy vegetables. Bie et al. (2004) observed the effect of Na_2SO_3 and NaHCO_3 salinity stress on two lettuce cultivars. The concentrations applied to the nutrient solution were 0, 20, 40 and 60 mM Na_2SO_3 and 0, 2.5, 5 and 7.5 mM NaHCO_3 . Leaf area, shoot dry weight, leaf length and leaf width decreased with increasing concentration of Na_2SO_3 or NaHCO_3 . Photosynthetic rate and stomatal conductance also diminished. NaHCO_3 was more toxic to lettuce growth than Na_2SO_3 . Leaf necrosis appeared above 40 mM Na_2SO_3 solution and leaves were chlorotic at NaHCO_3 concentrations above 5 mM. K and Ca concentrations also decreased in both cases.

3.5 BENEFITS OF SALINITY ON LETTUCE

Salinity not only causes negative effects on lettuce, it also can have a positive effect enhancing the quality and shelf-life of fresh-cut leaves. Enzymatic browning is the main problem encountered in minimal processing and further storage of leafy vegetables, leading to shorter shelf-life of products. Enzymatic browning is caused by the activity of some oxidative enzymes such as polyphenol oxidase and peroxidase. The content of both enzymes was reduced in fresh-cut lettuce leaves of plants cultivated under salinity conditions (2.8, 3.8 and 4.8 dS/m EC), and it also showed lowest change in colour and lowest reduction in total phenolic content and antioxidant capacity after 3 days of storage (Chisari et al., 2010). Scuderi et al. (2011), using the same salinity conditions, noticed an improvement of the properties of fresh-cut lettuce with increasing levels of NaCl salinity. The CO_2 production was reduced with a subsequent control of the decay process. Fresh-cut processing caused an activation of polyphenol oxidase and peroxidase; in all cases the product obtained by salinity treatments was less susceptible to oxidase activity and browning phenomena during storage. Increased salinity reduced the number of mesophilic bacteria, moulds and yeasts, assessed by plate counts on different culture media; in contrast, *Enterobacteriaceae* levels were unaffected. Garrido et al. (2014) affirmed that salt stress can have a positive impact on certain structural parameters, especially tissue elasticity, that can be closely linked to a higher number of cells of lower size and high leaf strength, explaining the postharvest longevity of lettuce. However, leaf growth and visual quality could be negatively affected by salt stress. It can be concluded that salinity, at moderate levels (up to 4.8 dS/m EC), can improve the shelf-life in fresh-cut lettuce leaves. Recently, Pérez-López et al. (2016) noticed an increase in nutritional value without yield reduction in lettuce cultivars (red and green) under salt stress conditions (0-200 mM NaCl).

3.6 TREATMENTS TO MINIMIZE THE NEGATIVE EFFECT OF SALINITY ON LETTUCE

In order to avoid or minimize the negative effect of salinity stress on lettuce plants several studies have been done. Blasco et al. (2013) applied iodate (20 and 40 mM) in lettuce plants under salinity stress (100 mM NaCl) and observed an increase on biomass, higher activity of antioxidative enzymes and lower activity of polyphenol oxidase. Another strategy to reduce salinity effect on lettuce plants is priming treatments on seeds. Priming is a form of seed preparation in which the seeds are pre-soaked before planting. Mahmoudi et al. (2012b) compared different priming treatments, such as water, potassium nitrate (KNO₃) and gibberelic acid, and concluded that hydropriming technique can be used as a simple commercial approach to alleviate the effects of NaCl induced stress in lettuce plants. Ouhibi et al. (2014) proposes UV-C radiation seed priming for a higher tolerance of lettuce plants to salinity stress conditions.

HYPERSPECTRAL IMAGING TECHNOLOGY

1. INTRODUCTION

Five kinds of interactions are given between electromagnetic radiation and matter (Clark, 1999):

- Reflection: process by which electromagnetic radiation is returned at the boundary between two media.
- Absorption: is the transformation of radiant energy to another type of energy, usually heat, by interaction with matter.
- Transmission: passage of electromagnetic radiation through a medium.
- Scattering: process of deflecting a unidirectional light beam into many directions.
- Refraction: the change in direction of a wave associated to a change in its wavelength when the wave travels from one medium to another with different refraction index.

Reflection, transmission and scattering leave the wavelength of the radiation unchanged, while refraction is used in spectral devices for the selective measurement of light at certain wavelengths.

Each interaction can disclose certain properties of the matter, reflection and absorption are generally associated with chemical properties of the sample, while scattering properties have been related to physical traits of the sample (Herrero-Langreo, 2010).

Figure 3 shows the electromagnetic spectrum, from short to long wavelengths, and the different kinds of radiation. Most of the spectroscopy applications are based on the interactions of ultraviolet radiation (10 – 380 nm), visible radiation (380 - 700 nm) or near infrared radiation (700 - 2500 nm), or the combinations of some of them, with the matter.

Figure 3.- Scheme of the electromagnetic spectrum.

Spectroscopy is “the study of light as a function of wavelength that is reflected or scattered from a certain material” (Clark, 1999). That reflected or scattered radiation is normally in the range of ultraviolet, visible and near infrared wavelengths.

There are three common sensing modes for spectral acquisition, namely reflectance, transmittance and interactance, as illustrated in Figure 4. Positions of light source and the optical detector are different for each acquisition mode. In reflectance mode, the detector captures the reflected light from the illuminated sample in a specific conformation to avoid specular reflection. External quality features are typically detected using reflectance mode, such as colour, surface texture and external defects. In transmittance mode, the detector is located in the opposite side of the light source and captures the transmitted light through the sample which carries more valuable internal information, but is often very weak. Transmittance mode is usually used to determine internal components concentration and to detect internal defects of relative transparent material such as fish, fruit and vegetables. Thickness of the sample is relevant in this mode. In interactance mode, both light source and the detector are located in the same side of sample and parallel to each other. On the basis of such setup, the interactance mode can detect deeper information into the sample and has less surface effects compared to reflectance mode. Meanwhile, the interactance mode reduces the influence of thickness, which is a practical advantage over transmission (Nicolai et al., 2007).

Figure 4.- Image sensing modes. From left to right: reflectance, transmittance and interactance (Wu and Sun, 2013a).

When a sample is exposed to light, some of the incident light is reflected at the outer surface (reflectance). The remaining incident energy is transmitted through the surface into the cellular structure of the sample. Then, it is scattered in a broad range of directions by the small interfaces within the tissue, or absorbed by cellular constituents. Both radiations, absorbed and scattered, are the two major physical processes that contribute to the visible appearance of most objects. Light absorption is related to certain chemical constituents in agro-food samples, such as sugar, acid, water, etc. Modern reflectance NIR spectrometers measure an aggregate amount of light reflected from a sample, from which light absorption may be estimated and then related to certain chemical constituents. However, scattering is a physical phenomenon that is dependent on the density, cell structures, and cellular matrices of fruit tissue (ElMasry and Sun, 2010). NIR does not provide quantitative information on light scattering in the sample (Lu, 2004; Peng & Lu, 2005). If both absorption and scattering are to be measured, more significant information about the chemical and physical/mechanical properties of food products could be gained (Lu, 2003b).

There are some applications nowadays of scattering spectroscopy, and commercial spectrometers based on static light scattering (SLS) and dynamic light scattering (DLS), for particle characterization of samples. SLS is an optical technique that measures the intensity of the scattered light in dependence of the scattering angle to obtain information on the scattering source. A typical application is the determination of the average molecular weight of a macromolecule, such as a polymer or a protein. DLS, also known as quasi-elastic light scattering, is one of the most popular light scattering techniques because it allows particle sizing down to 1 nm diameter. Typical applications are emulsions, micelles, polymers, proteins, nanoparticles or colloids. The basic principle is simple: the sample is illuminated by a

laser beam and the fluctuations of the scattered light are detected at a known scattering angle θ by a fast photon detector.

In addition, the absorbed light can also be re-emitted (fluorescence), usually at longer wavelengths than excitation. A number of compounds emit fluorescence in the VIS region of the spectrum when excited with UV radiation; these compounds are called fluorophores. Fluorophores are functional groups in the molecules that absorb energy of a specific wavelength range and re-emit energy at a different, specific longer wavelength range. The amount of the emitted energy and the wavelength at which the energy emits depend on both the fluorophore and the chemical environment of the fluorophore (ElMasry and Sun, 2010). The optical properties and fluorescence emission from the object are integrated functions of the angle and wavelength of the incident light and chemical and physical composition of the object (Chen et al., 2002). Fluorescence is a very sensitive technique able to detect very low concentrations of fluorophores. This fact represents the main advantage of fluorescence in comparison to transmittance or reflectance. The fluorescence technique has been used for investigating biological materials, detecting environmental, chemical, and biological stresses in plants, and monitoring food quality and safety (Noh & Lu, 2007).

When that interaction between light and matter is sensed and interpreted automatically for the whole surface of a sample, a spectral imaging system has been created.

2. SPECTRAL IMAGING SYSTEMS

The use of integrated devices to receive and interpret an image in a real scene automatically is usually called as machine vision system (Brosnan and Sun, 2004).

Machine vision systems normally used to determine food quality are based on monochrome, colour, multispectral, hyperspectral (Chen et al. 2002), and some authors also mention ultraspectral techniques (Wu & Sun, 2013a).

Colour vision systems acquire three digital values (colour coordinates) assigned to every pixel of a colour image. According to the tri-chromatic theory, colour can be discriminated by the combination of three elementary colour components (Zheng and Sun, 2008). Depending on the application, colour images can be defined in different colour spaces (RGB, CIE Lab, HSI, etc).

Spectral imaging combines spatial with spectral information of an object. A schematic representation of a hyperspectral imaging system is shown in figure 5. It acquires electromagnetic spectral information of each pixel, obtaining a set of images, each acquired at a narrow band of wavelengths. Normally, it would be considered a multispectral vision system rather than hyperspectral when less than 10 wavelengths are acquired (Abbott, 1999), whereas hyperspectral imaging acquires the whole spectral information, which can be processed as a continuous curve in a certain wavelength range, from each pixel of the surface of the object (Chen et al., 2002). There is no quantitative comparison between hyperspectral image and ultraspectral image. It is usually believed that ultraspectral imaging systems usually have a very fine spectral resolution (Wu & Sun, 2013a). Chung-Cheng et al. (2012) developed an ultraspectral imaging system able to acquire images with a spectral resolution of 0.3 nm.

Hyperspectral imaging is a powerful tool for the identification of key wavebands in the development of online automated multispectral imaging systems, significantly cheaper than hyperspectral technology (Gowen et al., 2007).

There are many other imaging techniques such as X-ray imaging, ultrasounds, electrical tomography and nuclear magnetic resonance imaging (Barreiro et al., 2008; Hernández-Sánchez et al., 2006; Hills et al., 2003). Although they have shown good results on the detection of diseases, defects and other internal traits in food products, their application is still limited due to the high technology cost and the low operational speed (Herrero-Langreo, 2010).

Figure 5.- (a) Basic configuration of a hyperspectral imaging for acquiring hyperspectral data structure “hypercube”. “Hypercube” could be displayed either as individual 2-D sub-images $I(x,y)$ at any given wavelength (b) or as spectra $I(\lambda)$ at any given pixels in the image (c) (ElMasry and Nakauchi, 2016).

2.1 SPECTRAL IMAGE ACQUISITION METHODS

There are four approaches to acquire 3D hyperspectral image cubes (x, y, λ) , which are point scanning, line scanning, area scanning and single shot method (fig. 6) (Wu and Sun, 2013a).

2.1.1 Point scanning or whiskbroom method

In this method, a single point is scanned at one pixel to provide the spectrum of this point. Other points are scanned by moving either the detector or the sample along two spatial dimensions (x and y). It needs a spectrophotometer equipped with a point detector to acquire a single spectrum for each pixel in the scene. The disadvantages of whiskbroom are the high time consumption for positioning the sample and the need of advanced repositioning hardware to ensure repeatability.

2.1.2 Line scanning or pushbroom method

This method is an extension of the point-scan method. Instead of scanning one point each time, this method simultaneously acquires a slit of spatial information, as well as full spectral information for each spatial point in the linear field of view. A special 2D image (y, λ) , with one spatial dimension (y) and one spectral dimension (λ), is taken at a time. A complete hyperspectral cube can be obtained as the line is scanned along the direction of x dimension. It uses imaging spectrographs. This is the most popular method of acquiring hyperspectral images for food quality and safety inspection. The main disadvantage

of this technique (also in point scanning method) is that the exposure time can be set at only one value for all wavelengths. Such exposure time has to be short enough to avoid saturation of spectrum at any wavelength, resulting in underexposure of other spectral bands and low accuracy of their spectral measurement.

2.1.3 Area scanning or band sequential method

Both mentioned before are spatial scanning methods, while the area scanning is a spectral scanning method. This approach keeps the image field of view fixed and acquires a 2D monochrome image (x, y) with full spatial information at a single wavelength at a time. A hypercube containing a stack of single-band images is built up as the scan is performed in the spectral domain. No relative movement between the sample and the detector is required for this method and a suitable exposure time can be set for each wavelength. A disadvantage is that it is not suitable for a moving sample or the inspection of real time delivery. Imaging systems using filters (filter wheels or electronically tunable filters) operate by this method.

2.1.4 Single shot method

The single shot method records both spatial and spectral information using a large area detector with one exposure to capture the images, making it very attractive when fast hyperspectral imaging is required. However, it is in the early stage of development and has limited resolutions for spatial dimensions and narrow ranges for spectral dimension.

Figure 6.- Spectral image acquisition methods. From left to right: point scan, line scan, area scan, single shot methods (Wu and Sun, 2013a).

2.2 COMPONENTS OF A HYPERSPECTRAL IMAGING SYSTEM

A hyperspectral imaging system generally consists of three fundamental parts (Wu & Sun, 2013a):

- A light source, to illuminate the sample.
- A wavelength dispersion device, to disperse the light signal received into different wavelengths.
- An area detector, to quantify the intensity of the acquired light.

2.2.1 Light sources

Light sources generate light to illuminate or excite the target, and they are an essential part of optical inspection systems. They can generally be classified into two categories: illumination sources, for reflectance, transmittance and interactance measurements; and excitation sources, for fluorescence measurements (Qin et al., 2013). Typical light sources used in hyperspectral imaging systems include halogen lamps, light emitting diodes, lasers and tunable light sources (Wu & Sun, 2013a).

Illumination sources

Broadband lights are generally used as the illumination sources for reflectance and transmittance imaging. The spectral constitution of the incident light changes after light-sample interactions. The measurement is performed based on the intensity changes at different wavelengths. Halogen lights are the most common broadband illumination sources, frequently used at spectral ranges visible (VIS) and near infrared (NIR) (Qin et al., 2013). Quartz tungsten halogen lamps generate a smooth spectrum without sharp peaks in the VIS-NIR region and have been used in many applications of hyperspectral reflectance measurements (Kim et al., 2007; Wu et al., 2012), and also in hyperspectral transmittance measurements (Ariana & Lu, 2008).

Broadband light-emitting diodes (LEDs) have started to find applications for food safety and quality inspection (Lawrence et al., 2007; Chao et al., 2008), owing their advantages over traditional lighting, such as long lifetime, low power consumption, low heat generation, small size, fast response, robustness and non-sensitivity to vibration (Qin et al., 2013). LEDs can produce not only broadband light, but also narrowband light at different wavelengths of ultraviolet, visible or infrared region, and can be used also as excitation sources. The disadvantages of LEDs include being sensitive to wide voltage fluctuations and junction temperature, and low light intensities as compared to halogen lights (Wu & Sun, 2013a).

Excitation sources

Narrowband lights are commonly used as excitation sources. When excited by high-intensity monochromatic light, some biological materials emit low-intensity light in a broad wavelength range. The energy change can cause fluorescence emission and/or Raman scattering, which carries information of the target that can be used for various inspection purposes. Lasers are powerful monochromatic sources widely used for excitation purposes (Qin et al., 2013). Monochromaticity, directionality and coherence are three unique properties of lasers. There are many applications of lasers as excitation sources for quality inspection of food, such as detection of fecal residues on poultry carcasses by hyperspectral fluorescence imaging (Cho et al., 2009) or detection of lycopene changes in tomatoes during postharvest ripening by Raman imaging (Qin et al., 2011).

Tunable light sources can be used as both illumination or excitation sources. When a broadband illumination source and a wavelength dispersion device are combined, a tunable light source is obtained. The tunable light sources allow scanning the area of interest obtaining both spatial and spectral information of the sample, by setting the wavelength dispersion device in the illumination light path. Because only narrowband light impacts on the object at a time, the intensity of the tunable light sources is relatively weak, which can reduce high irradiance and heat damage of sample (Wu & Sun, 2013a).

2.2.2 Wavelength dispersion devices

Dispersion devices are important for the hyperspectral imaging systems using broadband illuminating light sources. Their function is to disperse broadband light into different wavelengths and to project the dispersed light to the area of the detectors. Typical examples include filter wheels, imaging spectrographs, acoust-optic tunable filters, liquid crystal tunable filters, Fourier transform imaging spectrometers and single shot imagers (Wu & Sun, 2013a).

Filter wheels

A filter wheel carrying a set of discrete bandpass filters is the most basic and simple device for wavelength dispersion. The bandpass filters transmit the light at a particular wavelength efficiently while eliminating light at other wavelengths. There is a broad range of filters from ultraviolet, visible to near infrared wavelength ranges. Limitations include mechanical vibration from moving parts, slow wavelength switching and image unmatched, due to the filter movement.

Imaging spectrographs

An imaging spectrograph has the capability for dispersing incident broadband light into different wavelengths instantaneously and generating a spectrum for each point on the scanned line without the use of moving parts. Diffraction gratings are generally used in imaging spectrographs for wavelength dispersion. It is a collection of equally spaced reflecting or transmitting elements separated from one another by a distance that is in the order of magnitude of the wavelength of the light being studied.

Upon diffraction, an electromagnetic wave incident on a grating has its electric field amplitude, or phase, or both, modified in a predictable manner (Palmer, 2005).

Tunable filters

Acousto-optic tunable filter (AOTF) and liquid crystal tunable filter (LCTF) are both electronically tunable bandpass filters. By using acousto-optic interactions in a crystal, the AOTF can isolate light at a single wavelength from a broadband source through an applied acoustic field. A LCTF has electronically controlled liquid crystal cells inserted between two parallel polarizers to transmit light with a specific wavelength, while light energy out of the passband is rejected. Similar to a bandpass filter, tunable filters only disperse light at one particular wavelength at a time. Tunable filters can be flexibly controlled for different wavelengths by varying the frequency of the radio frequencies using a computer. They have moderate spectral resolution (5 - 20 nm) and broad wavelength range (about 400 – 2500 nm). In addition, because they have no moving parts, tunable filters have no problem of speed limitation, mechanical vibration, and image misregistration.

Fourier transform imaging spectrometers

Fourier transform imaging spectrometers employ an interferometer to self-interfere a broadband light, resulting in an interferogram that contains its spectral data. The generated interferogram is then calculated by an inverse Fourier transform to resolve the constitution of the frequencies (or wavelengths) of the broadband light. Michelson and Sagnac are two main interferometer designs for the current Fourier transform imaging spectrometers. Both designs have a beamsplitter and two flat mirrors. The difference of two designs is that one mirror and the beamsplitter are fixed in Michelson interferometer, while the other mirror moves to introduce optical path difference for generating interferogram. In Sagnac interferometer, two mirrors are fixed and the beamsplitter can be slightly rotated to create the interference fringes. In addition, two mirrors in Michelson interferometer are perpendicular to each other, while in the Sagnac spectrometer the two mirrors are not perpendicular but have a fixed angle ($<90^\circ$) between them. Because of no moving components, the Sagnac spectrometer has good mechanical stability and compactness, but relatively low resolution. On the contrary, the moving mirror in the Michelson spectrometer increases its sensitivity to vibrations. Although Fourier transform imaging spectrometers are now mainly used in bioanalytical chemistry and medicine, they are considered to have considerable potential impact in food science, due to its benefits of providing high spectral resolution down to a few micrometers (Wu & Sun, 2013a).

2.2.3 Area detectors

Area detectors have the function of quantifying the intensity of the acquired light by converting incident photons into electrons (Wu & Sun, 2013a). CCD (charge-coupled device) and CMOS (complementary metal-oxide-semiconductor) cameras are the two major types of solid state area detectors (Vila et al., 2005). Photodiodes made of light sensitive materials are the basic unit of both CCD and CMOS to convert radiation energy to electrical signal. These photodiodes (also called pixels) are tightly arranged by millions in rows forming an array. Silicon (Si), indium-gallium-arsenide (InGaAs) and mercury cadmium tellurium (MCT or HgCdTe) are three commonly used materials for hyperspectral systems.

CCD detector

During the measurement, the photodiodes array accumulates electric charges that must be moved out of the array to a place where the quantity of charges can be measured. There are generally four designs of CCD architectures for measuring two-dimensional region, namely full frame, frame transfer, interline transfer and frame interline transfer. Each one has its own properties, advantages and disadvantages. According to the light sensitive material used in the sensor, most commonly used CCD cameras can be classified in Silicon CCD cameras and InGaAs cameras.

Owing to its natural sensitivity to visible light, silicon is intensively used as sensor material for making cameras that work in the visible and short-wavelength near-infrared regions. For working in the entire NIR region, InGaAs, an alloy of indium arsenide (InAs) and gallium arsenide (GaAs), is the common substrate material of the image sensors. Standard InGaAs sensors cover the spectral region of 900-1700 nm. An extended wavelength range (1100-2600 nm) can be achieved by changing the percentages of InAs and GaAs for making the sensors.

CMOS detector

The main difference between CCD and CMOS sensors is that both photodetector and readout amplifier in each pixel are included within the CMOS image sensor (Litwiller, 2005) (Figure 7). After incident photon is converted to electron by photodiodes, a voltage signal is converted from the integrated charges by using optically insensitive transistors adjacent to the photodiode accordingly in CMOS, and then readout over the wires. Because the wires used in CMOS can transfer signal very fast, CMOS camera is especially suitable for the requirement of high-speed imaging for online industrial inspection. In CCD technology, blooming occurs when the charge in a pixel exceeds the saturation level and starts to fill adjacent pixels. However, because of the structure of photodetectors and readout amplifier one of the pixels, each pixel in CMOS array is independent of other pixels nearby, resulting in being immune to the blooming. Moreover, CMOS image sensors are smaller and cheaper than CCD, and they consume less power. The main disadvantage of CMOS cameras is the higher noise and dark current signal than the CCDs because of the on-chip circuits used to transfer and amplify signals, and as a result of lower dynamic range and sensitivity.

Figure 7.- Scheme of CCDs and CMOS functioning process. CCDs move photogenerated charge from pixel to pixel and convert it to voltage at an output node, CMOS imagers convert charge to voltage inside each pixel (Litwiller, 2005).

There are many examples of CCD cameras (Kim et al., 2001; Park et al., 2002; Lu, 2003; Nicolai et al., 2006) and CMOS cameras (Qiao et al., 2007) used in hyperspectral imaging systems for food inspection. Some examples will be summarized and described in the next section of this literature review.

2.3 ADVANTAGES AND DISADVANTAGES OF HYPERSPECTRAL IMAGING

Hyperspectral imaging is highly suitable for food quality and safety analysis and assessment. The major advantages can be summarized as follows (Wu and Sun, 2013a; Herrero-Langreo et al., 2010; Qin et al., 2013; Zhang et al., 2014):

- It can be directly applied to the surface of the sample and requires minimal sample preparation.
- It is a chemical-free assessment. Therefore, it saves labour, time, reagent cost and the cost of waste treatments. It is more economic than traditional methods.
- Hyperspectral imaging, like spectroscopy technique, is a non-invasive and non-destructive method that can be applied for both qualitative and quantitative analyses.
- Unlike traditional spectroscopy, hyperspectral imaging records a complete spectrum of every pixel within the scene. Therefore, it is able to delineate multiple distributions of different constituents within a sample, not just the bulk composition.
- It can determine the contents and distributions of several components simultaneously within the same sample. Such determination permits labelling and pricing of different entities in a sample simultaneously in sorting food products.

However, some disadvantages still need to be solved before industrial application and are summarized as follows:

- Hyperspectral images contain redundant data that pose considerable challenges for data mining, and hardware speed of the hyperspectral imaging system needs to be improved to satisfy the rapid acquisition and analysis of the huge hyperspectral data cube.
- Hyperspectral imaging is an indirect method as spectroscopy. Both of them need accurate reference calibration and robust model transfer algorithms and do not have low detection limits compared to chemical-based analytical methods.
- As spectroscopy, hyperspectral imaging also has the well-known problem of multicollinearity. Multivariate analysis, especially variable selection methods, are the way to reduce the effect of this problem.
- Hyperspectral imaging is not suitable when the region of interest within the surface of a sample is smaller than a pixel or the quality attributes have no characteristic spectral absorption.
- The analysis of liquids or homogenous samples does not need hyperspectral imaging but only spectroscopy, because the value of imaging lies in the ability to visualize spatial heterogeneities in samples.
- Hyperspectral imaging cannot detect the information of constituents deep inside the food sample. Most food products have very strong absorption of light, making them opaque over a distance of about several millimetres in visible and NIR region.
- The variation of temperature affects the water absorption spectrum. As water is a main component of food products, there is a potential heating effect for the measured hyperspectral images of food.
- Due to its long time data acquisition and analysis, hyperspectral imaging is not suggested for direct implementation in online application. A multispectral imaging system acquiring the spectral images only at several optimal wavelengths would be more suitable to meet the speed requirement of quality inspection. Such optimized multispectral imaging systems have much lower dimensionality than hyperspectral imaging systems, resulting in less data acquisition time. The optimal wavelengths can be determined through analysing the hyperspectral imaging data (Wu & Sun, 2013a).

3. HYPERSPECTRAL IMAGE ANALYSIS AND PROCESSING

After acquisition, image analysis process can be generally summarized in several steps, depending on the authors and according to the kind of images analysed (Brosnan and Sun, 2004):

- Image preprocessing.
- Image segmentation.
- Data processing.

3.1 IMAGE PREPROCESSING

The data volume of a hyperspectral image is usually very large. Raw spectral images contain noise, artifacts and useless signals due to aspects of the measurement environment and imperfect system components. The aim of preprocessing is to remove these effects and make the data independent of the imaging systems and the measurement conditions.

3.1.1 Reflectance calibration

The raw spectral image collected using hyperspectral imaging is actually detector signal intensity. Therefore, a reflectance calibration should be performed to calibrate the raw intensity image into reflectance or absorbance image with black and white references. In order to remove the effect of dark current on the camera sensor, the black image (about 0% reflectance) is acquired when the light source is completely turned off and the camera lens is completely covered with its non-reflective opaque cap. The white reference image is obtained under the same condition as the raw image using a white surface board which has a uniform, stable and high reflectance standard (about 99.9% reflectance). These two reference images are then used to correct the raw hyperspectral images by using the following equation:

$$\text{Eq. 1} \quad R = \frac{I_s - I_D}{I_W - I_D} \cdot 100$$

Where R is the corrected hyperspectral image; I_s is the raw hyperspectral image; I_D the dark image; and I_W the white reference image.

3.1.2 Image enhancement and spectral preprocessing

Image enhancement is an important process for improving the qualities of image. Some of image enhancement techniques are intended to make specified image characteristics more obvious, such as edge and contrast enhancement, magnifying, pseudo-coloring and sharpening. Others are used to reduce noise, such as convolution and spatial filtering, Fourier transform (FT) and wavelet transform (WT).

Spectral preprocessing algorithms are mainly used to improve mathematically the spectral data extracted from hyperspectral images. The goal of spectral preprocessing is to correct effects from random noise, length variation of light path and light scattering, resulting in producing a robust model with better predicting ability. The most widely used preprocessing algorithms include smoothing, derivatives, standard normal variate (SNV), multiplicative scatter correction (MSC), FT, WT and orthogonal signal correction (OSC). Smoothing (e.g. moving average, Savitzky-Golay, median filter and Gaussian filter) is used to reduce noise from the spectral data without reducing the number of spectral variables. Savitzky-Golay algorithm (Savitzky and Golay, 1964) is commonly used to perform smoothing simultaneously to differentiation. It fits a different polynomial to each point of the data surrounding this point. The results depend only on two parameters: the size of the moving window (increasing smoothing and decreasing accuracy) and the fit polynomial order (increasing accuracy but also noise). Derivatives (mainly first and second derivatives) are accomplished in correcting baseline effects in spectra. The 2nd derivative also has the function of resolving nearby peaks and sharpening spectral features. MSC is a transformation method used to compensate for additive and/or multiplicative effects in spectral data. SNV is a row-oriented transformation which centers and scales individual spectrum, performing the normalization dividing by the standard deviation of the spectrum (Barnes et al., 1993). Both MSC and SNV are competent to reduce the spectral variability due to scatter and baseline shifts. FT and WT separate noise from the spectra in the frequency domain. OSC filters the uninformative part for quality vector Y from the spectral matrix X based on constrained principal component analysis (PCA) or partial least square regression (PLSR) (Qin et al., 2013; Wu and Sun, 2013a).

3.2 IMAGE SEGMENTATION

The objective of image segmentation is to divide an image into isolated objects or regions and locate the regions of interest by means of masks for further spectral and textural feature extraction (ElMasry et al., 2009). "Manual segmentation can obtain accurate sectioned mask if the process is carefully executed, but the process is time-consuming, tedious and subjective, and therefore this method is not suitable to be extensively applied in industry applications. Image segmentation algorithms are more efficient than manual segmentation. The most used segmentation algorithms are thresholding (like global and adaptative thresholding), morphological processing (like erosion, dilation, open, close and watershed algorithms), edge-based segmentation (like gradient-based methods and Laplacian-based methods) and spectral image segmentation" (Wu & Sun, 2013a).

Figure 8.- Determination of the optimal threshold value using the image gray level histogram.

“Thresholding is a widely used image segmentation method due to its simplicity of implementation. Images containing the object with uniform graylevel and a background of unequal but also uniform graylevel would be appropriate for using thresholding (figure 8). Morphological processing is flexible and powerful for image segmentation. Erosion and dilation are two elementary operations to morphological processing from which all other morphological operations are based (figure 9). Erosion is the process of removing pixels on object boundaries in an image, while dilation is the process of adding pixels to the boundaries of objects. Edge-based segmentation is commonly used when pixels on edges/boundaries of objects have dramatic and discontinue graylevel changes. Spectral image segmentation is considered as a higher-level analysis compared to traditional segmentations that are regarded as low-level operations. Traditional segmentations operate on a monochrome image that has a scalar graylevel value of each pixel, while spectral image segmentation is a process of vector mining, because each pixel within hyperspectral image is a vector of intensity values. Spectral image segmentation integrates segmentation and classification into a single process” (ElMasry et al., 2011a).

3.3 DATA PROCESSING

As said previously, hyperspectral imaging contains huge amount of data. Multivariate analysis is required to efficiently decompose that massive quantity of features into useful information, and establish simple and easier understandable relationship between hyperspectral imaging data and the desired attributes of tested samples. Multivariate analysis can be classified into qualitative classification and quantitative regression (Wu and Sun, 2013a).

Figure 9.- Morphological image processing: erosion and dilation.

3.3.1 Qualitative classification

Qualitative classification (also called pattern recognition) includes unsupervised classification and supervised classification.

Unsupervised classification

Unsupervised classification is achieved according to the nature characteristics that can be correlation, distance, or combination of them, without a prior knowledge about the class information of the data. Typical unsupervised multivariate classification algorithms for the analysis of hyperspectral data include principal component analysis (PCA), k-means clustering and hierarchical clustering.

PCA is a useful tool that cannot only reduce the spectral dimension but also identify important bands. PCA decomposes the spectral data (containing possibly correlated variables) into several linearly uncorrelated principal components (PCs), to characterize the most important directions of variability in the high dimensional data space. The number of PCs is less (or equal) than the original variables, the first PC has the largest possible variance (that is, accounts for as much of the variability in the data as possible), and each succeeding component in turn has the highest variance possible under the constraint that it is orthogonal to the preceding components. The resulting vectors are an uncorrelated orthogonal basis set. The similar spectral signatures among samples and their class information can be evaluated by the first several PCs resulting from PCA.

K-means clustering classifies samples into k clusters in which each sample belongs to the cluster with the minimum distance to the cluster centroid. Hierarchical clustering is intended to build a hierarchy of clusters that is usually presented in a dendrogram. There are two types of hierarchical clustering: agglomerative and divisive. The hierarchical clustering is achieved by the use of a measure of distance between pairs of samples. It is not efficient for large data sets.

Supervised classification

Supervised classification is different from unsupervised classification by grouping new samples into predefined known classes according to their measured features. Typical supervised multivariate classification algorithms for the analysis of hyperspectral data include linear discrimination analysis (LDA), partial least square discriminant analysis (PLS-DA), k-nearest neighbour (kNN), artificial neural networks (ANN) and support vector machines (SVM). LDA finds an optimal linear projection of independent variables to classify the samples into separate classes. This method reaches maximal separation by maximizing the ratio of between-groups to within-groups variability. LDA includes class information of samples, while PCA only considers independent variables. PLS-DA is on the basis of partial least square regression (PLSR) approach (explained in 3.3.2) for the optimum separation of classes by encoding dependent variable of PLSR with dummy variables describing the classes. PLS-DA is then implemented in the usual way of PLSR. As a learning algorithm of instance-based, kNN is perhaps the simplest of all machine learning algorithms: an object is assigned to the class by a majority vote of its neighbours.

3.3.2 Quantitative regression

The goal of multivariate regression is to establish a relationship between the spectrum response of the tested sample and its target features with explanatory or predictive purposes. Multivariate regression can be linear or non-linear.

Linear regression

Multivariate linear regression methods in quantitative analysis of spectral data mainly include multiple linear regression (MLR), principle component regression (PCR) and PLSR. MLR establishes a relationship between spectrum and the desired attributes of tested sample in the form of a linear equation with features of being simple and easy to be interpreted. The regression coefficients of this equation are determined by the process of calculating the minimum error between reference and predicted values in a least squares sense. MLR fails when the number of variables is more than that of samples and is easy to be affected by the collinearity between the variables. In the case of analysing hyperspectral cubes, the effective variable selection or dimensionality reduction is required before MLR model establishment.

PCR is a regression method consisted of PCA and MLR. First, a PCA is carried out on spectral data. Instead of the original variables, the PCs are then used as independent variables in a MLR on the dependent variables. The advantages of PCR against MLR is that the PC calculation makes the independent variables uncorrelated and with less noise. The constraints of PCR are that its PC calculation does not consider the reference values of dependent variables, therefore the obtained PCs may be not informative for the dependent variables. Different from PCR, PLSR decomposes both the spectral (independent variables) and concentration (dependent variables) information simultaneously, resulting in extracting a set of orthogonal factors called latent variables (LVs). In the decomposition process, dependent variables are actively considered in estimating the LVs to ensure that the first several LVs are most related for predicting dependent variables. The building of the relationship between independent variables and dependent variables then becomes a simple task as to find out the optimal number of LVs which have the best predictive power in the relationship to dependent variables.

Non-linear regression

Sometimes, the relationship between the spectra of the tested sample and its quality may have the characteristics of non-linear, and the solutions of such analysis become better by using the non-linear regression techniques such as ANN and support vector regression (SVR). ANN simulates the behaviour of biological neural networks for learning and prediction purpose. The multilayer feed forward neural network is the most widely used ANN technique which has three layers of input, hidden and output to arrange the artificial neurons. Neurons are simple computational elements that process information using a connectionist approach to computation. Neurons are linked by weighted connections that are adjusted based on input and output information during the learning phase. The spectral features are introduced to the input layer with the results of the predicted values exported from the output layer. SVR is another powerful supervised learning methodology based on the statistical learning theory. The structural risk minimization principle is embodied instead of traditional empirical risk minimization principle, which is employed by conventional neural network to avoid overfitting and multidimensional problem. Especially, least squares support vector machine (LS-SVM), an optimized version of the standard SVM, is commonly used for spectral analysis. It employs nonlinear map function and maps the input features to a high dimensional space, thus to change the optimal problem into equality constraint condition. Lagrange multiplier is utilized to calculate the partial differentiation of each feature to obtain the optimal solution. ANN and SVM can be applied in both classification and regression tasks.

All of these spectral image processing routines can be developed using specific software packages (Environment for Visualizing Images ENVI, Hypercube, Hyperspec and MIA_Toolbox) or computer programming languages, such as MATLAB, C/C++, Visual Basic and LabVIEW.

HYPERSPECTRAL IMAGING APPLIED TO QUALITY CONTROL IN FOOD PRODUCTS

1. INTRODUCTION

Nowadays, quality and safety becomes a key factor for the modern food industry, because consumers always prefer food products with superior quality at an affordable price. It is a big concern to analyse and assess quality and safety attributes of food products in all processes of the food industry (Wu & Sun, 2013b). Recently, hyperspectral imaging technique has been investigated as a potential analytical tool for non-destructive analysis and assessment for food quality and safety. Several reviews and books on application of hyperspectral imaging in food quality assessment have been published in the last years (Gowen et al., 2007; Nicolai et al., 2007; Sun, 2010; Lorente et al., 2012; Qin et al., 2013; Wu & Sun, 2013b; Zhang et al., 2014). This review summarizes the recent developments and applications of hyperspectral image in different food products, such as meat, fish, grains, beverages... but focusing mainly in applications of quality control and inspection of fruits and vegetables.

2. HYPERSPECTRAL IMAGING APPLICATIONS IN FOOD PRODUCTS. GENERAL OVERVIEW

2.1 HYPERSPECTRAL IMAGING APPLIED TO QUALITY AND SAFETY CONTROL OF MEAT AND MEAT PRODUCTS

Meats, such as pork, beef, lamb and chicken, and meat products, play an important role in people's daily diet as they can provide good protein, vitamins and minerals to promote human health. Production of high quality meat and meat products is important for the industry. Superior quality of these products is always demanded by consumers and is considered as a key factor for success in today's highly competitive market (Xiong et al., 2014).

In recent years, the hyperspectral imaging technique has been extensively implemented for meat quality evaluation and detection. Table 5 summarizes recent applications of hyperspectral imaging techniques in detecting quality attributes of meat and meat products, showing the image acquisition mode and spectral range used, as well as the data processing methods applied.

2.1.1 *Measurements of chemical attributes*

It is known that meats are mainly composed of water, protein, fat, amino acids, fatty acids and so on. Chemical constituents of meats are intrinsic properties that affect meat quality. Water is the major component in food and agricultural products (Delgado & Sun, 2002), and it is a routine task to analyse water content or moisture content. The moisture content not only affects their quality but also their shelf-life, and any depletion of water will influence the economic profits of meats as they are usually sold by weight. A hyperspectral imaging system in the range of 900 – 1700 nm was applied to determine water content of lamb (Kamruzzaman et al., 2012b), pork (Barbin et al., 2012b), beef (ElMasry et al., 2013), pre-sliced turkey hams (Iqbal et al., 2013) and Spanish cooked ham (Talens et al., 2013). In these researches, prediction models were developed by using PLS regression, obtaining determination coefficients of $R_p = 0.89 - 0.88$ for water prediction in all cases.

Fat is an important quality criterium for the production controls from slaughter to final product in the meat industry. Fat and fatty acids contribute to the flavour when the meat is cooked. Hyperspectral imaging has been used for the prediction of fat content in meats. In the short-wave near infrared spectral region (760 – 1040 nm), interactance imaging technique was applied for on-line measurement of total fat content in inhomogeneous beef, developing a model with a correlation of 0.84 with the real fat content determined by classical chemical analysis (Wold et al., 2011). This technique has been also applied for determined total fat content in pork trimmings (O'Farrell et al., 2010). Also, in the range of 900 – 1700 nm, fat content has been determined in lamb meat (Pu et al., 2014) and ham (Talens et al., 2013). As a result of applying hierarchical variable selection method, based on clonal selection algorithm, the most segregative wavelengths for determining fat content in lamb meat were identified

as 1084, 1192 and 1212 nm (Pu et al., 2014). Kobayashi et al. (2010) not only determined fat content, but also total saturated fatty acids, total unsaturated fatty acids and the main fatty acids, such as myristic, palmitic, stearic, myristoleic, palmitoleic, oleic and linoleic, in beef cuts. In order to determine the fatty acid composition, which is linked to the properties of the fat, a NIR hyperspectral imaging system, working at wavelengths of 1000 – 2300 nm, was used. A correlation coefficient of 0.90 was obtained for total saturated and unsaturated fatty acids prediction.

Hyperspectral imaging is a powerful technique able to determine many different chemical attributes or properties of foods at the same image acquisition. Barbin et al. (2012b) used a NIR hyperspectral imaging technique for non-destructive determination of protein, fat and moisture content in both intact and minced pork samples. Results from traditional chemical methods were related with the spectral information by PLS regression models. These models showed that chemical composition could be predicted with coefficients of determination of 0.92, 0.95 and 0.87 for protein, fat and moisture, respectively. Moreover, spatially distributed visualisations of the sample composition can be yielded applying the models in each pixel of the hyperspectral images.

Collagen is a major component of connective tissues, and hydroxyproline is a feature amino-acid of collagen. Therefore, the presence of hydroxyproline makes contribution to forming collagen, which further affects the texture and the sensory characteristics of meat. Xiong et al. (2015b) studied the potential of hyperspectral imaging for predicting hydroxyproline content in chicken meat. Regression coefficients resulting from PLSR models was used to select eight wavelengths (419, 469, 541, 549, 575, 606, 632 and 896 nm), in the spectral range 400 - 1000 nm, as the most representative for hydroxyproline prediction. The model developed based on those eight optimal wavelengths showed a regression coefficient in prediction of $R_p = 0.874$ and was able to create distribution maps of hydroxyproline in the hyperspectral images of the meat.

2.1.2 Measurements of microbiological attributes

Spoilage in meats is caused by the growth and enzymatic activity of microorganisms, which could result in the decomposition of nutrition matter and the formation of metabolites. Contaminated meats can cause illness in humans. Hyperspectral imaging has a great potential for predicting microbiological attributes of chicken meat (Yoon et al., 2011), beef (Peng et al., 2011) and pork (Wang et al., 2011c). But not only microbial spoilage can be determined, also the total viable count (TVC) can be estimated (Feng & Sun, 2012; Peng et al., 2010), the psychrotrophic plate count (Barbin et al., 2012d) and some microorganisms, such as *Escherichia coli*, can be detected (Tao & Peng, 2014). For the TVC estimation, Feng & Sun (2012) acquired NIR reflectance hyperspectral images of raw chicken fillets, in the range of 900 – 1700 nm, and transformed the spectral information into absorbance spectra. Establishing full wavelength partial least regression models to correlate the absorbance spectra with the measured bacterial counts, they obtained correlation coefficients of 0.97 and 0.93 for calibration and cross validation, respectively.

Most of the studies published on predicting microbiological attributes of meats used linear regression methods for modelling. As an example, Wang and Zhang (2010a) used hyperspectral reflectance imaging for assessing the total plate count of chilled pork surface, and two prediction models were established using MLR and PLSR, giving encouraging results with $R = 0.886$ and 0.863 respectively. In addition to hyperspectral reflectance imaging, which is commonly used, hyperspectral scattering technique could be also a potential method in detecting microbiological spoilage, because the changes in scattering profiles are able to represent the changes in microbiological spoilage. For instance, a hyperspectral scattering technique has been applied in detecting *Escherichia coli* contamination of pork (Tao et al., 2012). In this study, the scattering profiles were fitted by Lorentzian distribution to give three parameters a (asymptotic value), b (peak value) and c (full width at $b/2$). The results showed that MLR models based on parameters a and " $a \& b \& c$ " gave high R of 0.877 and 0.841, respectively.

2.1.3 Measurements of sensory attributes

Colour is an important quality attribute in red meats, which can be determined using hyperspectral imaging (Wu et al., 2012b; Iqbal et al., 2013; Barbin et al. 2012c). Recently, Kamruzzaman et al. (2016c) have developed, using hyperspectral imaging, a model based on 6 wavelengths for detecting colour in red meats: pork, lamb and beef. This model is able to predict the L^* , a^* and b^* values with regression coefficients of $R_p = 0.97$, 0.84 and 0.82 , respectively.

Marbling, which is also called intramuscular fat, refers to the white flecks of fat present within the lean muscle in the meat. Meats with uniformly and finely distributed marbling are always considered as superior products and consumers pay higher prices for them (Xiong et al., 2014). In recent years, some researchers have applied hyperspectral imaging systems in marbling evaluation, obtaining reliable results (Qiao et al., 2007; Li et al., 2011). Li et al. (2011) used a hyperspectral scanning imaging system to collect hyperspectral images of beef in the spectral region of 400 – 1100 nm. The maximal ratio of gray value of fat and lean in each band was used to select characteristic bands. As a result, the images at 530 nm were used to differentiate beef-marbling. The prediction model established by MLR method gave a correlation coefficient of 0.92 for beef-marbling determination.

Tenderness is one of the most important factors in consumer perception of meat palatability or quality, and therefore its assessment has been investigated in some researches. Several researches show the great potential of hyperspectral imaging in the evaluation of tenderness (Naganathan et al., 2008; Kamruzzaman et al., 2012a; Tao et al., 2012; Cluff et al., 2008). For example, Naganathan et al. (2008) used a visible/near-infrared hyperspectral imaging system to assess tenderness of 14-day aged beef. Hyperspectral images of beef samples were acquired at 14-day post-mortem. Then, spatial and spectral features of the hyperspectral images were extracted using PCA and a co-occurrence matrix. On the basis of the extracted features, a discriminant model was established. Furthermore, with a leave-one-out cross-validation procedure, the model predicted three tenderness categories (tender, intermediate and tough) with an accuracy of 96.4%. Besides, some authors used hyperspectral scattering techniques to predict tenderness of red meats, because the changes in scattering profiles can represent the changes in tenderness. For instance, Tao et al. (2012) applied the hyperspectral scattering technique to predict tenderness of pork meat, and the final prediction model established with MLR methods gave high R^2 , ranging from 0.831 to 0.930.

2.1.4 Measurements of technological attributes

pH is a chemical parameter, which refers to the concentration of the hydrogen ion in aqueous solution, with a great influence on the storage and quality of meats by affecting their water holding capacity and colour. Nowadays, there is a potential to predict pH using hyperspectral imaging systems (Barbin et al., 2012c; Kamruzzaman et al., 2012c; Wu et al., 2012b; Iqbal et al., 2013). For example, Barbin et al. (2012c) were able to predict pH of pork meat using hyperspectral imaging. In this study, several spectral preprocessing methods, including SNV and MSC, were used to eliminate the influence of spectral variations. After extracting useful spectral information, a prediction model was then built up with a PLSR method. The results showed that pH could be well predicted using hyperspectral imaging systems with R^2 of 0.87.

Besides water content, water holding capacity (WHC), which is commonly measured as drip loss, is another water related attribute for food-processing industry. Hyperspectral imaging in the range of 900 – 1700 nm was applied to determine WHC / drip loss in beef, pork and lamb meats (Barbin et al., 2012b; ElMasry et al., 2011; Kamruzzaman et al., 2012c) (Figure 10). For instance, ElMasry et al. (2011) developed a near-infrared hyperspectral imaging system for non-destructive prediction of WHC in fresh beef. PCA were used to extract features of the spectra, leading to the selection of six important wavelengths (940, 997, 1144, 1214, 1342 and 1443 nm), related with the absorption bands of water, for establishing a prediction model with PLSR method. The model gave a reasonable accuracy to predict drip loss with R^2 of 0.87 and SECV of 0.28%.

Freshness of the meat was also predicted using hyperspectral imaging applications, related to the measurement of the content of the thiobarbituric acid reactive substances (TBARS). As a result of the lipid oxidation during time, malonaldehyde (MDA) is generated. TBARS value is an indicator of the MDA content of the sample. Wu et al. (2016) determined the distribution of TBARS in frozen-thawed pork applying NIR hyperspectral imaging technology, obtaining a correlation coefficient of prediction of 0.81. Xiong et al. (2015c) reached a $R_p = 0.944$ detecting TBARS content in chicken meat.

Figure 10.- Procedure for classification of meat pork. Extraction of sample spectral signature. (a) Masked hyperspectral image with zero background, (b) first and second PC score images, (c) PCA score plot of PC1 versus PC2 for all pixels, (d) using both score plot and score image together in an interactive mode to identify different clusters in the raw image which produce classification image and locating loin eye marked in blue and fat parts marked in red, and (e) mean spectra of marbling, loin eye and fat clusters identified in the classification image (Barbin et al. 2012a).

2.2 HYPERSPECTRAL IMAGING APPLIED TO QUALITY AND SAFETY CONTROL OF FISH

Fish products are of great dietary benefits for human health. Quality and safety inspection and documentation of fish products through the whole value chain are extremely important for both consumers and producers (Wu & Sun, 2013b). The first application of hyperspectral imaging for the quantitative measurement of fish quality was carried out in 2006 for moisture determination, and followed by determining fat distribution (ElMasry & Sun, 2010). Since 2010, more and more attributes of fish have been assessed by using hyperspectral imaging technology, including colour (Wu et al., 2012c), moisture (He et al., 2013), texture (Wu et al., 2012a), pH (He et al., 2012), water holding capacity (Wu & Sun, 2013c), ice fraction (Stevik et al., 2010), astaxanthin (Dissing et al., 2011) and microbial spoilage (Wu & Sun, 2013d). A review of the most recent applications of hyperspectral imaging for quality and safety analysis of fish is shown in table 6.

Candling and manual inspection are still used as the commercial way of quality inspection of fish, which are however inefficient, time-consuming, laborious and costly. Hyperspectral imaging was used for detecting fish ridge and nematodes in filets (ElMasry and Sun, 2010). Although many applications used hyperspectral imaging systems in reflectance mode, transmission mode was more popular in detecting nematode (Sivertsen et al., 2012; Sivertsen et al., 2011a). Recently, a system capable of automatic detection of nematodes in full size cod filets was presented at a belt speed of 25 mm/s (Sivertsen et al., 2011a). The performance of the system was comparable to manual detection on candling tables. However, the system could not process the filets with skin and had a slow speed. A new hyperspectral imaging system for automatic detection of nematodes in cod filets was further developed, operating at a conveyor belt speed of 400 mm/s to meet the industrial required speed (Sivertsen et al., 2012). The new system included a fiber optic light line on both sides of the imaging area and adopted the intertance mode that eliminates the effect of specular reflection and increase the signal received from inside the sample (Sivertsen et al., 2011b) (Figure 11). As a result, the Gaussian maximum likelihood

(GML) classifier detected 70'8% and 60'3% of the dark and pale nematodes, respectively, which is better than the previous work using a higher resolution instrument on a slow moving conveyor belt, and comparable or better to what is reported for manual inspection under industrial conditions (Sivertsen et al., 2012).

Figure 11.- A sketch showing the dimensions and position of the spectrometer and fiber lines of the hyperspectral interactance imaging system developed by Sivertsen et al. (2012) for automatic nematode inspection in cod fillets. Dimensions are in millimeters.

Recently, some research articles have appeared with different approaches to inspect freshness in fish and seafood. Dai et al. (2015) explored the potential use of VIS-NIR hyperspectral imaging to classify frozen and unfrozen prawns according to their freshness. This work has been expanded studying the application of this technology for predicting total volatile basic nitrogen contents, also working with prawns, which is one of the most effective techniques for freshness evaluation in seafood (Dai et al. 2016). Ivorra et al. (2016) applied hyperspectral imaging technology (scheme of the image acquisition system in Figure 12) for predicting shelf-life in smoked salmon, classifying the samples into five different groups (0, 10, 20, 40 and 60 days of shelf-life) with 87'2% prediction accuracy, employing only the seven most correlated wavelengths to develop the model. Xu et al. (2016) reached a correlation coefficient of 0'957 predicting TBARS value and pH in salmon fillets using NIR hyperspectral imaging technology.

Figure 12.- Hyperspectral imaging system for predicting shelf-life in smoked salmon. Up: spectral acquisition setup. A) RGB image of chilled smoked salmon. B) result of the fat tissue segmentation performed by the kNN model developed (Ivorra et al., 2016).

2.3 HYPERSPECTRAL IMAGING APPLIED TO QUALITY AND SAFETY CONTROL OF FRUIT, GRAINS AND VEGETABLES

External quality is an important and direct sensory quality attribute of agricultural products. In general terms, the external quality of fruits and vegetables is evaluated by considering their colour, texture, size, shape, and visual defects (Costa et al., 2011). Consumers would like to pay more for prime fruit and vegetables with superior quality and safety guaranteed. Hyperspectral imaging has proved its great capability for the quality and safety assessment of fruit and vegetables, such as contamination, bruises, surface defects, starch index, firmness, soluble solids content (SSC), sugar content, bitter pit and chilling injury / freeze damage (Lorente et al., 2012; Nicolai et al., 2007; Sun, 2010).

A deep review of the recent applications of hyperspectral imaging to the quality and safety analysis of fruit, grains and vegetables is summarized in table 8. Most of those applications are commented extensively later, in an independent section of this document.

2.4 OTHER APPLICATIONS

Besides the investigation of using hyperspectral imaging technique on the quality and safety analysis and assessment of meat, fish, fruits, grains and vegetables, there are many other food products whose quality parameters were successfully determined on the basis of hyperspectral imaging in recent years (table 7).

For inspecting egg quality, Abdel-Nour & Ngadi (2011) applied hyperspectral transmittance imaging (900 – 1700) to classify eggs into three types with different docosahexaenoic acid contents. Zhang et al. (2015), in that research line, predicted the internal quality of egg, including freshness, bubble formation and scattered yolk, by using transmittance hyperspectral imaging technology, achieving a correlation coefficient for freshness prediction of 0.87, and identification accuracies of 90.0 and 96.3% respectively for internal bubbles and scattered yolk. Figure 13 shows the score images obtained of bubble and intact eggs. Hyperspectral imaging has been used also for classifying many other popular food products, such as durum wheat pasta (Menesatti et al., 2004) or tea (Zhao et al., 2009).

Figure 13.- Internal bubbles detection in eggs using hyperspectral transmittance imaging and PCA analysis. (a and b) The first 2 principal component (PC1 and PC2) score images of bubble egg; (c and d) the first 2 principal component (PC1 and PC2) score images of the intact egg (Zhang et al., 2015).

Milk is another food product traditionally consumed around the world. Milk and dairy products have a great importance in human diet. Qin & Lu (2007) applied a hyperspectral imaging system to determine fat content in milk, which is very important technologically for standardizing milk and controlling dairy products processes. In 2010, Qin et al. detected melamine in milk using hyperspectral image. Melamine is a dangerous milk contaminant famous nowadays because of many tragic cases of dead and sick children in China.

Furthermore, not only fresh products have been analysed using hyperspectral imaging techniques, some other cooked products have been studied with many different purposes. Cooked potato is very popular in many countries. In order to predict the optimal boiling time, Nguyen et al. (2011) used a reflectance hyperspectral imaging system, combined with PLS-DA data analysis, obtaining less than 10% relative error. Pedreschi et al. (2010) obtained a good prediction results for fat ($r = 0.99$) and dry matter ($r = 0.97$) contents, and reasonable results ($r = 0.83$) for acrylamide content. Moreover, hyperspectral imaging has been applied in other cooked products such as shell-free cooked clam (Coelho et al., 2013) for detecting internal parasites. In this case, a hyperspectral imaging system in transmission mode, working in the range of 600 - 950 nm, was implemented. A specifically designed detector based on a reduced number of hyperspectral bands (624'1, 760'4 and 888'6 nm) was used. As a result, 85% detection accuracy for internal parasite in clam was obtained. Recently, Wold (2015) applied NIR hyperspectral imaging for on-line and non-contact detecting core temperature in fish cakes (at 10 mm depth) during industrial heat treatment, which is very relevant in food industries because it is a critical control parameter with respect to food quality and, in particular, to food safety.

Table 5.- Hyperspectral imaging applied to evaluate quality attributes of meat.

PRODUCT	WAVELENGTH	MODE	CLASSIFICATION	APPLICATION	REFERENCE
Beef	400 - 1000	R	PLS	Chicken adulteration	Kamruzamman et al. (2016)
	400 - 1000	R	PLS, LS-SVM, MLR	Moisture	Kamruzamman et al. (2016b)
	400 - 1000	R	MLR	Colour	Kamruzamman et al. (2016c)
	400 - 1100	S	PCR, PLS, BP-NN	Total viable count	Tao et al. (2015)
	328 - 1115	R	SPA, PLS	Total pigments	Xiong et al. (2015)
	900 - 1700	R	PLS	Water, protein and fat contents	El Masry et al. (2013)
	900 - 1700	R	PLS	Tenderness, colour and pH	El Masry et al. (2012)
	400 - 1100	R	MLR	Marbling level	Li et al. (2011)
	400 - 1100	S	MLR	Tenderness, colour and pH	Wu et al. (2012b)
	760 - 1040	I	PLS	Fat content	Wold et al. (2011)
	400 - 1000	S	MLR	Microbial spoilage detection	Peng et al. (2011)
	910 - 1700	R	PCA, PLS	Water holding capacity	El Masry et al. (2011b)
	400 - 1100	S	MLR	Tenderness, colour and pH	Wu et al. (2010)
	1000 - 2300	R	PLS	Fat and fatty acids	Kobayashi et al. (2010)
	400 - 1000	R	PCA	Tenderness	Naganathan et al. (2008)
Chicken	400 - 1000	R	PLS	Hydroxyproline content	Xiong et al. (2015b)
	328 - 1115	R	SPA, PLS	Thiobarbituric acid reactive substances (TBARS). Freshness	Xiong et al. (2015c)
	900 - 1700	R	PLS	<i>Pseudomonas</i>	Feng & Sun (2013)
	900 - 1700	R	PLS	Total viable count	Feng & Sun (2012)
	900 - 1700	R	PLS	Enterobacteriaceae	Feng et al. (2012)
	400 - 1000	R	BR	Contamination	Yoon et al. (2011)
	389 - 744	R	BR	Wholesome / unwholesome	Chao et al. (2010)
	389 - 744	R	BR	Wholesome / unwholesome	Yang et al. (2010)
	364 - 1024	T		Bone fragment detection	Yoon et al. (2008)
	430 - 850	R	Spectral angle mapper	Faecal and ingesta contaminants on surface	Park et al. (2010)
	430 - 850	R	PLS	Detection of faecal contamination	Lawrence et al. (2006)
	430 - 850	R	BR	Detection of faecal contamination	Park et al. (2006)
	425 - 710	F	Fuzzy algorithm	Detection of skin tumours	Kim et al. (2004)
	420 - 850	R	BR	Skin tumour detection	Chao et al. (2002)
Cured ham	900 - 1700	R	PCA, PLS	Moisture and NaCl	Garrido-Novell et al. (2015)

Table 5.- Hyperspectral imaging applied to evaluate quality attributes of meat (*continuation*).

PRODUCT	WAVELENGTH	MODE	CLASSIFICATION	APPLICATION	REFERENCE
Ham	900 - 1700	R	PLS	Water content, colour and pH	Iqbal et al. (2013)
	900 - 1700	R	PLS-DA	Water, protein and fat contents. Classification	Talens et al. (2013)
	900 - 1700	R	PCA, LDA	Quality grade	El Masry et al. (2011a)
Lamb	380 - 1028	R	PCA, ANN, SVM, LDA	Lamb muscle discrimination	Sanz et al. (2016)
	900 - 1700	R	Hierarchical variable selection	Fat, protein and water content	Pu et al. (2014)
	900 - 1700	R	PCA, PLS-DA	Classification	Foca et al. (2013)
	900 - 1700	R	PCA, PLS, MLR	Adulteration	Kamruzzaman et al. (2013)
	900 - 1700	R	PLS, MLR	Tenderness and sensory	Kamruzzaman et al. (2012a)
	900 - 1700	R	PLS	Water, protein and fat contents	Kamruzzaman et al. (2012b)
	900 - 1700	R	PLS	Water holding capacity, colour and pH	Kamruzzaman et al. (2012c)
	900 - 1700	R	PCA	Lamb type discrimination	Kamruzzaman et al. (2011)
Pork	400 - 1000	R	PLS	Moisture content	Ma et al. (2016)
	874 - 1734	R	SPA, PLS	TBARS distribution	Wu et al. (2016)
	900 - 2500	R	PLS	Quality of frozen pork without thawing	Xie et al. (2015)
	400 - 1100	R	MLR	Tenderness and <i>E.coli</i> contamination	Tao & Peng (2014)
	900 - 1700	R	PLS-DA	Fresh and frozen-thawed	Barbin et al. (2013)
	900 - 1700	R	PLS	Total viable count and Psychrotrophic plate count	Barbin et al. (2012d)
	900 - 1700	R	PLS	Sensory, colour and pH	Barbin et al. (2012c)
	900 - 1700	R	PLS	Water content, water holding capacity, protein and fat	Barbin et al. (2012b)
	900 - 1700	R	PCA	Quality grade, authentication	Barbin et al. (2012a)
	400 - 1000	S	MLR	Tenderness and microbial spoilage	Tao et al. (2012)
	400 - 1000	R	ANN	Microbial spoilage	Wang et al. (2011c)
	400 - 1000	R	MLR	Microbial spoilage	Tao et al. (2010)
	400 - 1000	R	PLS	Microbial spoilage	Wang et al. (2010b)
	400 - 1100	R	PLS	Total viable count	Wang & Zhang (2010a)
	400 - 1100	R	SVM	Total viable count	Wang & Zhang (2010b)
	400 - 1100	R	PLS	Total viable count	Peng et al. (2010)
	760 - 1040	I	PLS	Fat content	O'Farrell et al. (2010)
	430 - 1000	R	ANN	Quality and marbling level	Qiao et al. (2007)

Table 6.- Hyperspectral imaging applied to evaluate quality attributes of fish and seafoods.

PRODUCT	WAVELENGTH	MODE	CLASSIFICATION	APPLICATION	REFERENCE
Cod	400 - 1000	I	Gaussian maximum likelihood (GML)	Nematodes	Sivertsen et al. (2012)
	400 - 1000	I	PCA, PLS	Frozen-thawed	Sivertsen et al. (2011b)
	400 - 1000	T	Fisher discriminant ratio (FDR)	Nematodes	Sivertsen et al. (2011a)
	350 - 950	T	PLS	Detection of parasites	Heia et al. (2007)
Crab	760 - 1040	I	PLS	Detection of internal edible meat content	Wold et al. (2010)
Fish fillets	400 - 1000	R	SIMCA, LS-SVM, PNN, SPA	Discrimination fresh, cold-stored and frozen-thawed	Cheng et al. (2015)
	400 - 1000	R	PLS, LS-SVM, SPA	Chemical spoilage	Cheng et al. (2015b)
	400 - 1000	R	Local calibration method	Nematodes	Sivertsen et al. (2012)
	400 - 1000	R	Image segmentation	Ridge detection	Sivertsen et al. (2009)
	460 - 1040	R	MLR	Fat and water content	El Masry & Wold (2008)
Halibut	380 - 1030	R	PCA, SVM	Frozen-thawed	Zhu et al. (2013)
Prawn	400 - 1000	R	Wavelet analysis	Total volatile basic nitrogen content	Dai et al. (2016)
	400 - 1000	R	LS-SVM, Adaptive boosting, BP-NN	Freshness	Dai et al. (2015)
	900 - 1700	R	PCA, PLS, MLR	Colour	Wu et al. (2012c)
Rainbow trout	385 - 970	R	PCA, PLS	Astaxanthin	Dissing et al. (2011)
Salmon	400 - 1000	R	kNN	Shelf-life prediction	Ivorra et al. (2016)
	900 - 1700	R	PLS, Stepwise MLR	Oxidative degradation	Xu et al. (2016)
	400 - 1700	R	PLS	Moisture	He et al. (2013)
	400 - 1700	R	PLS, SVM	Total viable count	Wu & Sun (2013d)
	400 - 1700	R	PLS, SVM	Water holding capacity	Wu & Sun (2013c)
	400 - 1000	R	PLS-DA	Expired	Ivorra et al. (2013)
	400 - 1000	R	PLS	pH	He et al. (2012)
	400 - 1000	R	PLS	Texture	Wu et al. (2012a)
	400 - 1000	R	PLS	Colour distribution	Wu et al. (2012d)
	760 - 1040	I	PLS	Ice fraction	Stevik et al. (2010)
Sea bass	400 - 970	R	PLS	Classification	Costa et al. (2011)

Table 7.- Hyperspectral imaging applied to evaluate quality attributes in other food products.

PRODUCT	WAVELENGTH	MODE	CLASSIFICATION	APPLICATION	REFERENCE
Cheese	400 - 1000	R	PLS	Quality classification	Gowen et al. (2009)
Cooked clam	600 - 950	T	BR	Internal parasite detection	Coelho et al. (2013)
Cooked potato	400 - 1000	R	PLS-DA	Cooking time prediction	Nguyen do trong et al. (2011)
	400 - 1000	I	PLS	Fat, dry matter and acrylamide prediction	Pedreschi et al. (2010)
Egg	380 - 1010	T	SPA, SVR	Internal quality	Zhang et al. (2015)
	440 - 940	R	Classification algorithm	Quality. Dirty eggs classification	Lunadei et al. (2012)
	900 - 1700	T	K-means, PLS	Quality classification	Abdel-Nour & Ngadi (2011)
Fish cake	760 - 1040	R	PLS	Core temperature	Wold (2015)
Flour/bread	400 - 1000	R	Multivariate statistical process control (MSPC)	Adulterations with other grains	Verdu et al. (2016)
Herbal medicine	920 - 2514	R	PCA	Adulteration	Vermaak et al. (2013)
Milk	450 - 1000	R	Spectral similarity measures	Melamine detection	Qin et al. (2010)
	530 - 900	S	MLR	Fat content	Qin & Lu (2007)
Milk powder	990 - 1700	R	PLSR	Melamine detection	Lim et al. (2016)
Olive oil	900 - 1700	R	GA, SPA, LASSO	Acidity, moisture and peroxides	Martínez-Gila et al. (2015)
Pasta	400 - 1700	R	PLS	Quality	Menesatti et al. (2004)
Tea	874 - 1734	R	PLS	Moisture content	Deng et al. (2015)
	408 - 1117	R	MLR	Quality classification	Zhao et al. (2009)

3. HYPERSPECTRAL IMAGING APPLICATIONS IN FRUITS AND VEGETABLES

In the last years, hyperspectral imaging has been intensively explored for analysing physical, chemical and biological properties of a broad range of fruits and vegetables. Table 8 summarizes a deep review of the recent applications of hyperspectral imaging to the quality and safety analysis of fruits, grains and vegetables. Each research cited on table 8 shows the product analysed, the spectral range studied, the sensing mode employed for spectral acquisition and the methodologies applied for data processing. In the next paragraphs, some of those works are explained with more details.

Hyperspectral imaging has many different applications for quality assessment of fruits and vegetables. Due to the wide amount of works cited in this review and in order to classify all of them in a clear way, three different approaches have been considered: analysis of chemical properties, of physical properties and microbial contamination and insect infection.

3.1 CHEMICAL PROPERTIES INSPECTION

Maturity is one of the most important quality parameters for fruits and vegetables classification and it is related to different chemical (and also physical) components that can be sensed using hyperspectral imaging techniques. Maturity stage of fruit is important to determine the best harvest date and optimize the post-harvest treatment. Among other applications, hyperspectral imaging has been applied to determine the levels of maturity of peach (Herrero-Langreo et al., 2011), banana (Rajkumar et al., 2012), grape (Rodríguez-Pulido et al., 2013) and tomato (Polder et al., 2002), analysing chemical parameters such as soluble solids content, pigments content or moisture. A very interesting research was carried out by Rodríguez-Pulido et al. (2013) for predicting the stage of maturation of grapes by inspecting their seeds. Sugar content of grape pulp is crucial but, in wine grapes, the maturation of grape seeds is also critical. A NIR hyperspectral imaging (897-1752 nm) system, working in reflectance mode, was used to extract the spectral information of intact grape seeds. A PLSR model was developed and applied to predict the stage of maturation of the samples with correlation coefficients higher than 0.95.

The definition of wavelength ratios is a key target for the maturity evaluation, resulting in several optical indexes related to maturity stage. Lleó et al., 2011 proposed two optical indexes (Ind1 and Ind2), for determining the ripening stage of red skinned peach fruits. Those indexes are based on the combination of wavelengths close to the chlorophyll absorption peak at 680 nm. Both indexes reflected the ripening of the peaches. Moreover, Ind1 was able to avoid the convexity effect of the fruits.

Soluble solid content (SSC) is a quality attribute very used to determine optimal maturity stage and harvest date. The assessment of SSC is conducted mostly using the hyperspectral systems with the scatter mode for fruits, employing VIS-NIR range (400 – 1000 nm). Some different applications, among others, were developed in apple (Mendoza et al., 2011b), kiwifruit (Martinsen & Schaare, 1998), melon (Long et al., 2005), banana (Rajkumar et al., 2012), strawberries (ElMasry et al., 2007), blueberries (Leiva-Valenzuela et al., 2012), peach (Cen et al., 2012) and grapes (Baiano et al., 2012). As an example, Baiano et al. (2012), analysing hyperspectral images from seven cultivars of white and red grapes, obtained a coefficient of correlation $R = 0.94$ for SSC estimation, applying a PLSR model. Rajkumar et al. (2012) obtained a coefficient of correlation $R = 0.85$ for SSC prediction in banana.

Pigments contents in fruits and vegetables are also important factors due to their relationship with maturity or shelf-life. Chlorophylls, carotenoids and anthocyanins are the most important pigments in vegetables and their contents have been estimated using hyperspectral imaging systems. Chlorophyll is the major pigment in leafy vegetables and can be determined using this technique (Xue & Yang, 2009). Anthocyanins are also very important for grape quality in winery and can be determined using also hyperspectral imaging in the NIR region (900 – 1700 nm) (Chen et al., 2015). Fernandes et al. (2015) was able to determine not only anthocyanins content but also pH and °Brix, which are very important parameters for classifying grapes in winery or for predicting the harvest point.

In addition to Fernandes et al. (2015), Baiano et al. (2012) developed a hyperspectral imaging system, working in reflectance mode in the range 400 – 1000 nm, to detect acidity and pH, as well as sugar content, in grapes, which are the most important quality factors. Some other quality parameters and components determined by the use of hyperspectral images are moisture or fat and starch contents. Moisture and pH in strawberry were determined by ElMasry et al. (2007) using a reflectance mode

hyperspectral imaging system working in the VIS/NIR range (400 – 1000). The same technique was conducted by Qiao et al. (2005) to predict water content in potato, but using artificial neural network to analyse the spectral data. A correlation coefficient $R = 0.87$ was obtained by Rajkumar et al. (2012) for moisture prediction in banana fruits, using hyperspectral imaging technique in the VIS/NIR range (400 – 1000 nm). Two different approaches have been developed to determine oil content in corn: the first one, working in transmittance in the range 750 – 1090 nm (Cogdill et al., 2004); and the second one, working in reflectance mode in the NIR region (950 – 1700) (Weinstock et al., 2006). Finally, starch index in apple can be determined with a simple band ratio (Peirs et al., 2003).

3.2 PHYSICAL PROPERTIES INSPECTION

Firmness is related to maturity and it is an important physical quality attribute commonly used to determine harvest date. A lot of efforts have been made using hyperspectral imaging (mainly in the range between 500 – 1000 nm) for the determination of firmness of fruits and vegetables, including apple (Huang et al., 2012b; Wang et al., 2012; Mendoza et al., 2011a, 2011b) (figure 14), banana (Rajkumar et al., 2012), strawberries (Zhang et al., 2016; Tallada et al., 2006), blueberries (Hu et al., 2016; Leiva-Valenzuela et al., 2012), pear (Zhao et al., 2010b) and peach (Cen et al., 2012). Rajkumar et al. (2012), working in the range of 400 – 1000 nm, developed a multiple linear regression (MLR) model for predicting firmness in banana fruits, obtaining a correlation coefficient of $R = 0.91$. Zhang et al. (2016) used a hyperspectral imaging system covering two spectral ranges (380 – 1030 nm and 874 – 1734 nm) for evaluating strawberry ripeness according to its firmness. Support vector machine was applied to build a model able to classify strawberries into three classes (ripe, mid-ripe and unripe) with classification accuracy over 85%. Noh & Lu (2007) used a fluorescence hyperspectral imaging systems, working in the spectral range 500 – 1040, to determine, among others, firmness in apple. A hybrid method of combining principal component analysis and neural network modelling was used for developing prediction models, resulting in a relatively good correlation coefficient of 0.74 for firmness prediction.

Figure 14.- Hyperspectral imaging system for acquiring spectral scattering images from apple samples. Laboratory on-line system using an EMCCD camera for evaluation of “Jonagold” and “Red delicious” apples (Mendoza et al., 2011a).

The presence of surface defects influences the quality and price of fruits and vegetables, and early detection can prevent the infection of the whole batch. Hyperspectral imaging has been used for detection of surface defects on orange (Li et al., 2011), cherry (Guyer & Yang, 2000, Cho et al., 2013), jujube (Wu et al., 2016) and citrus (Nicolai et al., 2007). These works were mainly based on the identification of the spectral features of the defect and on the use of these spectral signatures to establish discriminant models. Hyperspectral imaging can be used as a tool for classification of fruits and vegetables in different quality levels according to the presence of defects. Working in reflectance mode, in the VIS/NIR range (400 – 1000 nm), Díaz et al. (2004) developed a hyperspectral imaging system to classify olives in function of their quality (presence of defects), with an accuracy of over 90% using artificial neural networks. Diezma et al. (2013), working also in reflectance mode (400 – 1000 nm) was able to classify spinach leaves according to their quality grade (deterioration stage). Razmjoooy et al. (2012) determined quality in potatoes and ElMasry et al. (2007) in strawberries. Other hyperspectral applications made use of fluorescence mode: for the quality inspection and classification of walnuts in the range 425 – 775 nm (Jiang et al., 2007).

Not only defects on the surface of the products can be detected. Ariana & Lu (2010), using a combined hyperspectral reflectance (400 – 740 nm) / transmittance (740 – 1000 nm) imaging system, were able to differentiate normal and defective cucumbers with a classification accuracy of 94.7%.

Bruise damage is the main cause for the quality loss and degradation of fruit, which usually occurs during the harvest or transport process. Apple is the main research object for the detection of bruises using hyperspectral imaging in the last decade (Zhang et al., 2014a; Luo et al., 2012; Baranowski et al., 2012; Xing et al., 2007). Four wavelengths (558, 678, 728 and 892 nm) have been selected to detect this injury on apples (Xing et al., 2005). The classification results were about 93% of the non-bruised apples recognised, and an accuracy of about 86% for detecting bruises. Besides apple, hyperspectral imaging has also been used for the bruise detection of pear (Zhao et al., 2010), cucumber (Ariana et al., 2006), mushroom (Esquerre et al., 2012; Gowen et al., 2008), strawberry (Nagata et al., 2006) and kiwifruit (Lü et al., 2011). Zhao et al. (2010), using PCA and spectral angle mapper classification algorithm, obtained a detection accuracy of 95% for bruise detection on pears.

Chilling injury is a common defect occurring during storage and transportation at low temperatures. Liu et al. (2006) developed a hyperspectral computer vision system to acquire VIS/NIR image in 700 – 850 nm to detect the chilling injury in cucumber by using band ratios and PCA methods. ElMasry et al. (2009) applied artificial neural network (ANN) on apples. The same procedure was used by Pan et al. (2016) to detect cold injury in peach. Menesatti et al. (2005) used hyperspectral imaging systems to detect chilling injury in citrus fruit.

Mealiness is a negative texture attribute in fruits characterized by abnormal softness of internal tissues and lack of juiciness. Several works have been conducted on the detection of mealiness in apple using hyperspectral images (Huang & Lu, 2010a; Huang & Zhu, 2011; Huang et al., 2012b; Wang et al., 2011a). All of these works employed the scatter mode in the spectral range of 600 – 1000 nm.

Some other hyperspectral imaging applications have been developed with the purpose to detect internal defects in fruits and vegetables, such as damage in almond (Nakariyakul & Casasent, 2011) or internal pits in cherry (Qyn & Lu, 2005). Zhu et al. (2007), using fluorescence hyperspectral imaging technology, and a combination of independent component analysis with k nearest neighbour classifier for models computation, was able to differentiate walnut shells from walnut meat, with a detection rate of 90.6%. Delwiche et al., (2013) employed a hyperspectral imaging system in the NIR range (1000 – 1700 nm) to classify wheat kernels according to their milling quality. A different approach was done by Calvini et al. (2015), using NIR hyperspectral imaging to classify Arabica and Robusta coffee species from the green coffee grains.

3.3 MICROBIAL CONTAMINATION AND INSECT INSPECTION

Decay is another main problem affecting the post-harvest storage and transport, and it is usually due to fungal or microbial infections. Hyperspectral imaging has been used for early detection of fungal infections in post-harvest of citrus fruits, instead of the manual detection with ultraviolet lightning. Lorente et al. (2013a, 2013b) used a VIS/NIR hyperspectral imaging system (400 – 1100 nm) for detecting decay in mandarin after infection with fungi *Penicillium*. Classification of the mandarins with and without decay was achieved with an average success rate up to 89.1% using seven optimal spectral features: reflectance at 550, 670, 690, 720 and 950 nm, normalized difference vegetation index (NDVI) and citrus colour index (CCI) (Jimenez-Cuesta et al., 1981). Decay is usually caused by two different fungi: *Penicillium digitatum* and *Penicillium italicum*. The first one is the main responsible of rottenness in citrus fruits; it has been studied and detected in mandarin by Gómez-Sanchis et al. (2013), using hyperspectral imaging (350 – 2500 nm). They used a methodology based on hyperspectral imaging and advanced machine-learning techniques (artificial neural networks and classification and regression trees) for the segmentation and classification of images of citrus free of damage and affected by both moulds, obtaining an accuracy of 93% in the classification.

Detection of faecal contamination is very important as feces are the source of a lot of pathogens. Many works suggested that hyperspectral fluorescence imaging has better capability of detection of traces of faecal spots than hyperspectral reflectance imaging, although the cost and stability of the excitation laser for fluorescence emission remain the questions for practical implementation (Liu et al., 2007). Faecal contamination has been detected in many fruits and vegetables using hyperspectral imaging systems (mainly in the range of 320 – 800 nm), such in apple (Kim et al., 2007; Yang et al., 2012a and 2012b), leafy vegetables (Lee et al., 2014; Everard et al., 2014), melon (Vargas et al., 2005), onion (Wang et al., 2012) and tomato (Yang et al., 2012c).

Citrus canker is a serious disease affecting most commercial citrus species that is caused by the bacterium *Xanthomonas axonopodis*. In recent years, more efforts were made on selecting critical bands of the hyperspectral image data for developing multispectral methods to inspect citrus canker (Qin et al., 2012; Zhao et al., 2010a). Qin et al. (2012) proposed a two-band ratio (R_{834}/R_{729}), selected by correlation analysis (CA), for detecting citrus canker in real-time. A one-line commercial fruit sorting machine was developed, with a speed of five fruits per second, and an overall classification accuracy of 95.3% was achieved testing a sample of 360 fruits.

Toxigenic fungi grown in grain are toxic for humans and animals. Hyperspectral imaging systems in visible and short-wave near infrared region (400 – 1000 nm) or long-wave near infrared region (962 – 1662 nm and 1000 – 2498 nm) has been successfully applied to indirectly detect *Fusarium* damage in maize (Williams et al., 2010) and wheat (Barbedo et al., 2016; Shahin & Symons, 2011); and also to investigate the fungal development in maize (Williams et al., 2012) and peanuts (Jiang et al., 2016). Barbedo et al. (2016) acquired reflectance hyperspectral images (528 – 1785 nm) of wheat kernels and developed an algorithm for automatic detection of *Fusarium*. This algorithm reached a classification accuracy of 91% and was robust to factors such as shape, orientation, shadowing and clustering of kernels. Jiang et al. (2016) employed a NIR hyperspectral imaging system (970 – 2570 nm) to acquire reflectance images of both mouldy and healthy peanuts. Applying PCA, eight spectral bands (1100, 1207, 1302, 1509, 1634, 1722, 1847 and 1922 nm) were selected as the most mould-sensitive bands. The proposed method was able to identify mouldy peanuts in the validation samples with an accuracy of 98.73%.

Besides *Fusarium*, the damage of other toxigenic fungi, such as *Aspergillus flavus*, *Aspergillus parasiticus* or *Aspergillus niger*, on maize (Del Fiore et al., 2010) and wheat (Singh et al., 2012) has also been detected by analysing the hyperspectral images in 400 – 1000 nm or the combination of hyperspectral (700 – 1000 nm) and colour images. The detection of mycotoxin contamination is another issue for the grain industry. Hyperspectral imaging (720 – 940 nm) has been evaluated for predicting milled maize fumonisin contamination produced by *Fusarium* spp. (Firrao et al., 2010), and estimating aflatoxin concentration in corn kernels inoculated with *A. flavus* spores (Wang et al., 2015 and 2015b; Yao et al., 2013). Kandpal et al. (2015) utilized a short-wave infrared (SWIR) hyperspectral imaging technique (1100 – 1700 nm), working in reflectance mode, to detect aflatoxin contamination on corn kernels. The PLS-DA model developed reached an overall classification accuracy of 96.9%. Yellow rust is another important

defect in wheat, caused by the fungi *Puccinia striiformis*, and also it can be detected using hyperspectral imaging (400-1700 nm) (Zhang et al., 2014).

Detection of insect damages in fruit and vegetables is another challenge as they can cause serious economic loss. Hyperspectral imaging has been applied to detect not only external insect infections such as in jujube fruit (Wang et al., 2011b), mandarin (Saranwong et al., 2011), cucumber (Lu & Ariana, 2013) and mung bean (Kaliramesh et al., 2013), but also internal infections such as in vegetable soybeans (Huang et al., 2012a). All of the above researches considered the spectral range 400 – 1000 nm for the acquisition of hyperspectral images, except for jujube fruit where the images were acquired within the visible range (400 – 720 nm) and for mung bean (1000 – 1600 nm). Reflectance mode was used in the jujube fruit and mung bean applications, and transmittance mode in the work on vegetable soybeans. For cucumbers, both reflectance (450 – 740 nm) and transmittance (740 – 1000 nm) modes were integrated into a system for the image acquisition, in order to compare which is the best mode for detecting this kind of damage in cucumber. In this case, better results were obtained in transmittance mode.

4. HYPERSPECTRAL IMAGING APPLICATIONS IN LEAFY VEGETABLES

In recent decades, there has been a substantial increase in the consumption of fresh-cut and minimally processed fruits and vegetables, especially as a result of changes in the lifestyles of the consumers. Because consumers usually purchase fresh-cut products based on their visual appearance, colour is extremely important. Ready-to-eat vegetables can undergo colour changes due to different biochemical processes involving plant pigments that are important for leaf function. For this reason, variations in pigment content (e.g., carotene and chlorophyll degradation and oxidation of phenolic compounds) may provide information concerning the physiological state of leaves (Xue and Yang., 2009).

Chlorophylls, the most abundant pigments in green plants, are gaining increasing importance in the human diet, not only as food colorants, but also as healthy food ingredients (Fernandes et al., 2007). Leafy vegetables are an important source of chlorophylls and other pigments (Znidarcic et al., 2011). Hyperspectral imaging can be used for determining these compounds. Reflectance at visible region of the electromagnetic spectrum is mainly influenced by chlorophyll pigments. For this, most of the image applications for pigments content determination in leafy vegetables are based on reflectance mode.

Many spectral indexes have been developed to estimate chlorophyll, carotenoids or anthocyanins contents. Xue & Yang (2009) applied most of them to the reflectance spectra (350 – 2500 nm) measured in lettuce, spinach and pakchoy leaves, and developed some others. Strong correlations between these indexes and leaf chlorophyll content were obtained in most of the cases.

Leafy vegetables can become contaminated with pathogens during growth, harvesting, packaging or food preparation (Heaton & Jones, 2008). These food products are consumed fresh and have an enhanced risk of causing foodborne illnesses from contamination via faecal. Unsanitary growing and packaging conditions can cause *Salmonella* or *Escherichia coli* contaminations, which can produce illness and, in extreme cases, death. Field irrigation using contaminated water, inadequate hygiene practices of workers during food processing, pest infestation in packaging facilities and inadequate cleaning of process equipment can be the origin of faecal contamination. The detection of this contamination using hyperspectral imaging techniques has been investigated in recent years.

Siripatrawan et al. (2011) developed a rapid method for detection of *Escherichia coli* contamination in fresh spinach leaves using hyperspectral reflectance imaging working in the range of 400 – 1000 nm. Spinach leaves were inoculated with different concentrations of *E. coli* solution. The reflectance spectra of the leaves were pretreated using Savitzky-Golay algorithm and analysed using PCA and ANN. The generated prediction map was able to detect *E. coli* contamination in fresh spinach leaves.

Everard et al. (2014a) compared reflectance and fluorescence hyperspectral images for detection of faecal contamination on spinach leaves (figure 15). Three hyperspectral configurations coupled with two multivariate image analysis techniques were compared. Fluorescence imaging in the visible region with ultraviolet (UV) and violet excitation sources, and reflectance imaging in the visible to near-infrared regions were investigated. Partial least squares discriminant analysis (PLS-DA) and two band ratio (BR) (R678/R848) were used. Hyperspectral fluorescence showed superior accuracy than visible/near

infrared reflectance hyperspectral imaging for detecting faecal contaminants on spinach leaves. In further investigations, key wavelengths were identified and corresponding algorithms were developed for a contaminant screening optical imaging device (Everard et al., 2014b).

Other researches corroborate that fluorescence hyperspectral imaging is more suitable for detecting contaminants in leafy vegetables than reflectance hyperspectral imaging. Yang et al. (2013) developed a multispectral algorithm, using a fluorescence hyperspectral line-scan imaging system, to detect faecal contamination on baby spinach leaves. For this, the hyperspectral fluorescence images obtained were analysed and two different wavebands were selected (666 and 688 nm) and used as a band ratio to distinguish faecal spots on leaves surfaces. Another algorithm using different wavelengths (660 and 692 nm) was developed by Kang et al. (2011), allowing the detection of faecal contamination spots on the surface of romaine lettuce and baby spinach leaves. Recently, this algorithm has been modified and separated into two independent band ratios very focused and specialized on each application: romaine lettuce leaves (R_{666}/R_{680}) and baby spinach leaves (R_{661}/R_{680}) (Lee et al., 2014).

Figure 15.- Examples of fecal contamination detection on spinach leaves for three hyperspectral image configurations (UV, ultra violet; V, violet; Vis/NIR, visible/near infrared) coupled with partial least squares discriminant analysis (PLS-DA) and two band ratio (R_{680}/R_{688}) analysis (Everard et al., 2014a).

Multispectral imaging systems have been used to detect and monitor quality in leafy vegetables. Lunadei et al. (2012), using a 3-CCD camera centred at the infrared (IR), red (Rd) and blue (B) wavelengths, were able to classify spinach leaves into two different deterioration reference classes (A and B). The spinach leaves were stored under two different conditions of temperature and time, and images were taken in four different dates (0, 7, 14 and 21 days) during the storage period. Non supervised classifications, based on the B/Rd and Rd-B/Rd+B virtual images of the leaves, were able to segregate between the storage periods; in both cases, almost 93% of Class A was comprised of samples

measured at time zero and after seven days of storage, and almost 98% of Class B was comprised of samples analysed on the 14th and the 21st days.

Packaging of green leafy vegetables can postpone senescence and yellowing, but a drawback is the risk of anaerobic respiration leading to a loss of tissue integrity and development of an olive-brown colour. Lokke et al. (2013) monitored these quality parameters of colour and texture in packaged wild rocket (*Diplotaxis tenuifolia* L.) leaves using a multispectral imaging system retaining 18 different wavelengths in the range between 405 and 970 nm. The results were correlated with sensory analysis. The combination of information from both VIS and NIR wavelengths holds the information of colour and texture, respectively, and thus the ability to quantify changes in product quality during storage.

However, hyperspectral imaging has not been widely employed in leafy vegetables for monitoring shelf-life during storage. Diezma et al. (2013) applied hyperspectral imaging to supervise the quality deterioration in packed spinach leaves during storage. Two different storage conditions were considered and hyperspectral images were taken in several times along each condition. Spectra from the leaves were extracted according to three different stages of degradation (from optimal quality to maximum deterioration) and reference average spectra were defined for each class. Three different models were proposed for segregating leaves into the three levels of degradation, calculating spectral angle mapper distance (SAM), partial least squares discriminant analysis (PLS-DA) and a non-linear index (called Leafy Vegetable Evolution LEVE) combining five wavelengths. Those three models were able to show the degradation of the leaves during storage. Simko et al. (2015) applied hyperspectral imaging and chlorophyll fluorescence imaging to predict decay in fresh-cut lettuce, and developed two different lettuce decay indices (LEDI) that can be used to detect decay of leaf tissue.

Table 8.- Hyperspectral imaging applied to inspect quality attributes of fruits, grains and vegetables.

PRODUCT	WAVELENGTH	MODE	CLASSIFICATION	APPLICATION	REFERENCE
Almond nuts	700 - 1400	T	Support vector machine (SVM)	Internal damage detection	Nakariyakul & Casasent (2011)
Apple	400 - 1700	R	Minimum noise fraction (MNF)	Bruise detection	Zhang et al. (2014a)
	680 - 980	R	Partial least squares (PLS)	Firmness	Huang et al. (2012a)
	380 - 1000	R	PLS - Discriminant analysis (DA)	Bruise detection	Luo et al. (2012)
	400 - 2500	R	Principal components analysis (PCA)	Bruise detection	Baranowski et al. (2012)
	676 - 779	F	Band ratio (BR)	Defects	Yang et al. (2012b)
	480 - 780	F	BR	Contamination	Yang et al. (2012a)
	600 - 1000	S	PLS-DA, SVM	Mealiness	Huang et al. (2012b)
	500 - 1000	S	PLS	Firmness	Wang et al. (2012)
	600 - 1000	S	PLS-DA, SVM	Mealiness	Huang & Zhu (2011)
	600 - 1000	S	PLS-DA	Mealiness	Wang et al. (2011a)
	460 - 1100	S	PLS, Wavelet transform (WT)	Firmness and soluble solid content (SSC)	Mendoza et al. (2011b)
	480 - 780	F	BR	Contamination	Yang et al. (2011)
	500 - 1000	R		Firmness	Mendoza et al. (2011a)
	600 - 1000	S	PLS-DA	Mealiness	Huang & Lu (2010a)
	400 - 1000	R	Artificial neural network (ANN)	Chilling injury detection	El Masry et al. (2009)
	447 - 951	R	BR	Surface defects and contamination	Liu et al. (2007)
	500 - 950	R	PCA	Bruise detection	Xing et al. (2007)
	400 - 1000	F	Asymetric second difference method	Defects and faecal contamination	Kim et al. (2007)
	500 - 1040	F	Hybrid PC-ANN	Colour & firmness	Noh & Lu (2007)
	650 - 1000	S	Multi-linear regression (MLR)	Firmness	Peng & Lu (2006)
	954 - 1350	R	PLS	Detection of bitter pit defect	Nicolaï et al. (2006)
	500 - 950	R	PCA	Bruise detection	Xing et al. (2007)
	500 - 950	R	PCA	Bruise detection	Xing et al. (2005)
	430 - 900	R	Band difference (BD)	Surface defects and contamination	Mehl et al. (2004)
	868 - 1769	R	BR	Starch index	Peirs et al. (2003)
	900 - 1700	R	MLR	Bruise detection	Lu et al. (2003)
Banana	400 - 1100	R	Two-step k-means	Hand and finger segmentation	Hu et al. (2014)
	400 - 1000	R	MLR, PLS	Quality, moisture and maturity	Rajkumar et al. (2012)

Table 8.- Hyperspectral imaging applied to inspect quality attributes of fruits, grains and vegetables (continuation).

PRODUCT	WAVELENGTH	MODE	CLASSIFICATION	APPLICATION	REFERENCE
Blueberry	675 - 1000	I	LS-SVM, MC-UVE	Mechanical properties	Hu et al. (2016)
	500 - 1000	R	PLS	Firmness and soluble solids content (SSC)	Leiva-Valenzuela et al. (2012)
Cherry	450 - 1000	T	ANN	Detection of internal pits	Qin & Lu (2005)
	680 - 1280	R	ANN	Defects	Guyer & Yang (2000)
Citrus fruit	320 - 1020	R	Multilayer perceptron (MLP)	Decay	Lorente et al. (2013a)
	460 - 1020	R	Receiver operating characteristic (ROC)	Decay	Lorente et al. (2013b)
	730 - 830	R	BR	Canker detection	Qin et al. (2012)
	450 - 930	R	PCA	Canker detection	Qin et al. (2011)
	450 - 930	R	BR	Canker detection	Zhao et al. (2010a)
	450 - 930	R	BR	Canker detection	Qin et al. (2009)
	400 - 970	R	PLS	Identification of chilling damage	Menesatti et al. (2005)
Coffee beans	955 - 1700	R	PCA, kNN, PLS-DA	Classification	Calvini et al. (2015)
Corn	1100 - 1700	R	PLS-DA	Aflatoxin B ₁ detection	Kandpal et al. (2015)
	1000 - 2500	R	PCA	Aflatoxin B ₁ detection	Wang et al. (2015)
	1000 - 2500	R	PCA	Aflatoxin B ₁ detection	Wang et al. (2015b)
	400 - 600	F	Linear discriminant analysis (LDA)	Aflatoxin detection	Yao et al. (2013)
	950 - 1700	R	PLS	Oil and oleic acid content	Weinstock et al. (2006)
	750 - 1090	T	PLS	Oil and moisture content	Cogdill et al. (2004)
Cucumber	500 - 1000	R, T	SVM, kNN	Chilling injury detection	Cen et al. (2016)
	400 - 1700	R	PLS	Fly infestation detection	Lu & Ariana (2013)
	400 - 1000	R	PCA	Colour	Ariana & Lu (2010b)
	400 - 1000	T	Image thresholding	Quality evaluation	Ariana & Lu (2008)
	450 - 950	T	Image thresholding	Internal damage	Ariana & Lu (2006)
	900 - 1700	R	BR	Bruise detection	Ariana et al. (2006)
	447 - 951	R	BR	Identification of chilling damage	Liu et al. (2005)
	447 - 951	R	Integrated PCA - FDA	Identification of chilling damage	Cheng et al. (2004)

Table 8.- Hyperspectral imaging applied to inspect quality attributes of fruits, grains and vegetables (*continuation*) .

PRODUCT	WAVELENGTH	MODE	CLASSIFICATION	APPLICATION	REFERENCE
Grape	900 - 1700	R	PLS, SVM	Anthocyanin content	Chen et al. (2015)
	900 - 1700	R	Stepwise linear discriminant analysis (SLDA)	Identification of variety	Nogales-Bueno et al. (2015)
	380 - 1028	R	PCA, ANN	⁹ Brix, pH, anthocyanin content	Fernandes et al. (2015)
	914 - 1715	R	PCA, LDA, PLS	Maturity	Rodríguez-Pulido et al. (2013)
	400 - 1000	R	PLS	Acidity, pH, SSC	Baiano et al. (2012)
Jujube	918 - 1678	R	PCA, SVM, SIMCA	Common defects	Wu et al. (2016)
	400 - 1100	R	Stepwise discriminant analysis (SDA)	Insect infestation	Wang et al. (2011b)
Kiwifruit	408 - 1117	R	SVM	Bruise detection	Lü et al. (2011)
	650 - 1100	R	MLR	SSC distribution	Martinsen & Schaare (1998)
Leafy vegetables	464 - 800	F	PLS-DA	Contaminants	Everard et al. (2014b)
	400 - 1000	R	BR	Chlorophyll content	Xue & Yang (2009)
Lettuce	380 - 1012	F	BR	Decay	Simko et al. (2015)
	464 - 800	F	BR	Faecal contamination	Lee et al. (2014)
	320 - 400	F	Image segmentation	Fecal contamination	Kang et al. (2011)
Lychee	400 - 1000	R	PLS, SPA, BP-NN, RBF-SVM	Browning detection	Yang et al. (2015)
	400 - 1000	R	SPA, SWR, RBF-SVM	Anthocyanin content	Yang et al. (2015b)
Mandarin	400 - 1000	R	Iterative bayesian discriminant analysis (IBDA)	Insect damage	Saranwong et al. (2011)
	460 - 1020	R	ANN	Rottenness detection	Gómez-Sanchis et al. (2008b)
	350 - 2500	F	ANN	Detection of <i>Penicillium digitatum</i>	Gómez-Sanchis et al. (2004)
Mango	400 - 1000	R	PLS	Moisture distribution	Pu et al. (2015)
Melon	830 - 930	R	MLR	SSC distribution	Long et al. (2005)
	425 - 774	F	PCA	Detection of faecal contamination	Vargas et al. (2005)
Mung bean	1000 - 1600	R	PCA, LDA	Insect damage	Kaliramesh et al. (2013)

Table 8.- Hyperspectral imaging applied to inspect quality attributes of fruits, grains and vegetables (*continuation*).

PRODUCT	WAVELENGTH	MODE	CLASSIFICATION	APPLICATION	REFERENCE
Mushroom	880 - 1720	R	PLS-DA	Bruise detection	Esquerre et al. (2012)
	400 - 1000	R	PLS-DA	Colour, enzymatic browning	Taghizadeh et al. (2011)
	400 - 1000	R	PCA	Bruise detection	Gowen et al. (2008)
Oat	1006 - 1650	R	PCA, PLS-DA	Classification	Serranti et al. (2013)
Olive	400 - 1100	R	ANN - PLS	Quality classification	Díaz et al. (2004)
Onion	400 - 1000	R	SVM	Sour skin detection	Wang et al. (2012)
Orange	400 - 1000	R	PCA, BR	Defects	Li et al. (2011)
Peach	400 - 1000	R	PCA	Skin defects detection	Li et al. (2016)
	400 - 1000	R	ANN	Cold injury	Pan et al. (2016)
	400 - 1000	R	MC-UVE, MNF	Common defects	Zhang et al. (2015)
	515 - 1000	S	PLS, SVM	Firmness and SSC	Cen et al. (2012)
	680 - 800	R	BR	Maturity	Herrero-Langreo et al. (2011)
	400 - 1000	R	BR	Maturity	Lleó et al. (2011)
	500 - 1000	S	MLR	Firmness	Lu & Peng (2006)
Peanuts	970 - 2570	R	PCA	Molds inspection	Jiang et al. (2016)
Pear	400 - 1000	R	PCA	Bruise detection	Zhao et al. (2010b)
Potato	400 - 1100	R	SVM	Quality	Razmjooy et al. (2012)
	400 - 1000	R	ANN	Water content and weight	Qiao et al. (2005)
Soybean	400 - 1000	T	SVM	Insect damage	Huang et al. (2012a)

APPLICATIONS OF HYPERSPECTRAL IMAGE TO DETECT SALINITY EFFECTS ON PLANTS

1. INTRODUCTION

Salinity is one of the main environmental factors that adversely affect plant growth and development, reducing its yield. Therefore, it is essential to track salinity stress symptoms and provide relevant information such as location and intensity of the negative effects. Hyperspectral imaging has the potential to provide site-specific information about crop status due to its fine spectral and spatial resolution (Rud et al., 2013).

Salinity effect on crops has been studied in two different scales. First, the effect of salt stress can be determined using hyperspectral imaging at leaf scale, with local acquisition of hyperspectral images of the plants. Second, crops salinity can be measured directly at canopy scale, using hyperspectral imaging remotely sensed data (Knippling, 1970).

Spectral reflectance at visible (380-760 nm) and infrared (760-2500 nm) wavelengths is modified under stress conditions. Plants can be stressed by many environmental factors such as drought, nutritive deficiencies or salinity. All these stress factors usually cause ion imbalances and losses of pigments, among other effects, resulting in specific spectral responses. Thus, spectral reflectance would be a good tool for measuring plant response to environmental stress such as salinity.

Hyperspectral imaging has been widely employed to develop indexes correlated with stress factors affecting plant growth. Many of them have been used to detect salinity effects on plants. In this section, hyperspectral imaging applications to detect salinity effects on plants are reviewed.

2. HYPERSPECTRAL IMAGING FOR DETECTING PLANT STRESS AT CANOPY SCALE

A review of different studies carried out for detecting salinity and other stress conditions in crops using remote sensing hyperspectral imaging is presented in table 9.

Especially, the visible and the near infrared part of the spectra have been used in a number of studies for linking spectral reflectance and plant stress (Gao et al., 2011). Typical changes in spectra when leaves become senescent are shown in figure 16. Under stress conditions of any type, plants have several typical changes in their spectral response. An increase of reflectance in the visible part of the electromagnetic spectrum usually occurs due to the loss of chlorophyll and other pigments (Ustin et al., 2009). However, Hamzeh et al. (2013) observed a decrease of reflectance in the visible range, in sugarcane fields under salinity stress, in remote sensing.

In NIR region, a decrease of reflectance is normally observed, attributed to changes and damages in cell structure (Mutanga & Skidmore, 2007). This usually occurs in plants with nutritive deficiencies causing stress. However, under drought stress, plants frequently show the opposite trend. An increase in NIR reflectance is observed in many crops under water stress (Clevers et al., 2010; Harris et al., 2006). Under salinity stress, Zhang et al. (2011) observed an initial increase in NIR reflectance but a decrease when saline concentrations were higher.

Regarding the red edge position, a displacement to shorter wavelengths is observed in plants under stress conditions due to the loss of chlorophyll (Ustin et al., 2009). This can be also detected in plants under salinity stress conditions. Poss et al. (2010), working in a range of soil salinity from 2 to 22 dS/m, observed a shift to shorter wavelengths of the red edge in bluegrass canopy. Some vegetation indexes also vary in plants under salinity stress conditions. It was observed a decrease in NDVI values as a result of increasing saline concentrations (Poss et al., 2010; Peñuelas et al., 1997a).

There are important differences between remote sensing and local acquisition of hyperspectral images. In remote sensing there are a variety of vegetation properties of interest, and many of these have direct effects on canopy reflectance measurements. It is necessary to consider some parameters such as leaf area index (LAI), leaf angle distribution (LAD), absorption coefficient, scattering coefficient and

background reflectance, which influence decisively. Moreover, solar radiation is not constant in all the spectral range and climatic conditions affect the signal received by the sensors (Knipling, 1970).

The LAI is the green leaf area per unit ground area, which represents the total amount of green vegetation present in the canopy. The LAI is an important property of vegetation, and it has the strongest effect on overall canopy reflectance. The LAI of plants is an important indicator of productivity, photosynthetic capacity and degree of stress, and it can be estimated by using different hyperspectral vegetation indices (Liang et al., 2015). The LAD describes the overall variety of directions in which the leaves are oriented, but it is often simplified by specifying the mean leaf angle (MLA) and making assumptions about the actual distribution. The MLA is the average of the angle between each leaf in a canopy and the horizontal (Asner, 1998).

Under stress conditions, there is a variation of LAI and MLA. LAI decreases as a result of decreasing plant growth and development, which causes a decrease in NIR reflectance. MLA, however, increases because of the leaves decay in plants, which causes a global decrease of canopy reflectance, mainly in the infrared region (Asner, 1998). Both parameters affect the canopy/background ratio and also it is necessary to consider the scattering coefficient of the canopy, due to the leaves tissues, which is different according to the wavelength (Yoder & Pettigrew-Crosby., 1995). It is possible to obtain different remote sensing reflectance from two canopies with the same leaf reflectance, due to the effect of the LAI, MLA and scattering coefficient (Blackburn, 1998).

Figure 16.- Typical spectra of a vital and a senescent leaf (Behmann et al., 2014).

Table 9.- Spectral response of different plants to stress conditions at canopy scale

PLANT	STRESS	SPECTRAL OBSERVATIONS	REFERENCE
Sugarcane	Salinity stress Soil salinity 1'9 - 9'2 dS/m	Decreasing of VIS reflectance Decreasing of NIR reflectance	Hamzeh et al. (2013)
In general	Salinity stress Soil salinity 1 - 37 dS/m	In general, increasing of VIS reflectance and decreasing of NIR reflectance, as a response to the loss of chlorophyll and cell structural damage in stress conditions. It varies a lot depending on the plant. In salt-tolerant plants, NIR reflectance increases in a first moment but then decreases	Zhang et al. (2011)
Bluegrass	Salinity stress Soil salinity 2 - 22 dS/m	NDVI decreases. Red edge shifts to short wavelengths	Poss et al. (2010)
Barley	Salinity stress Soil salinity 0'8 - 1'9 dS/m	Increasing of VIS reflectance. Decreasing of NIR reflectance. Decreasing of NDVI index, which is related to the crop yield and biomass. Proposal of water index (WI), related to the water content of the plants	Peñuelas et al. (1997a)
Canopy	Drought stress	Increasing of NIR reflectance, mainly at 970 nm	Clevers et al. (2010)
Sphagnum	Drought stress	Increasing of VIS reflectance. Increasing of NIR reflectance	Harris et al. (2006)
Canopy	Drought stress	Increasing of NIR reflectance. Range 1550-1750 better for detecting water in remote sensing	Tucker (1980)
Canopy	Nutritive stress N deficiencies	Decreasing of NIR reflectance	Mutanga & Skidmore (2007)
Wheat canopy	Nutritive stress Stress conditions in general	Decreasing of NIR reflectance, due to cell structural changes Decreasing of chlorophyll absorption	Liu et al. (2004)
In general	Stress conditions in general Loss of chlorophyll	Increasing of VIS reflectance, due to loss of chlorophyll Red edge displacement to shorter wavelengths	Ustin et al. (2009)
Canopy	Stress conditions in general	Variation of Leaf Area Index (LAI) and Mean Leaf Angle (MLA) in stress conditions Increasing LAI causes increasing of NIR reflectance Increasing MLA causes decreasing of VIS and mainly NIR reflectances	Asner (1998)
In general	Stress conditions in general Drought stress	Decreasing of NIR reflectance, due to decreasing LAI Drought stress causes increasing NIR reflectance Loss of chlorophyll causes increasing VIS reflectance	Knipling et al. (1970)

3. HYPERSPECTRAL IMAGING FOR DETECTING PLANT STRESS AT LEAF SCALE

As it has been previously said, there are strong differences between remote sensing and local acquisition of hyperspectral images, and conclusions obtained from canopy scale not always can be applied at leaf scale without adaptations. Parameters such as LAI, LAD, climatic conditions or background reflectance are not significant when the images are obtained directly from the leaves in controlled conditions. Knipling (1970) observed changes in remote sensing mainly in NIR region, not so evident in laboratory experiments. In fact, Li et al. (2010), working at leaf scale, said that NIR reflectance is not appropriate to detect salinity stress. However, NIR reflectance is a commonly used indicator of stress conditions in remote sensing, mainly due to variations of LAI.

Anyway, several studies show variations in NIR reflectance, also at leaf scale, when crops suffer stress conditions (Wang et al., 2002a; Adams et al., 1999). However, the trend is not always the same. NIR reflectance varies depending on the type of stress suffered by plants (Knipling, 1970). Wang et al. (2002a) observed the effect of soil salinity (1.5 – 25 dS/m) on the reflectance of elephant grass. NIR reflectance decreases when soil salinity increases, due to structural changes in the leaves. The same effect was corroborated in soybean irrigated with saline water (4 dS/m) (Wang et al., 2002b).

More recently, Behmann et al. (2014) has detected a decrease in NIR reflectance in barley under stress conditions, because of changes in cell structure of leaves.

However, under nutritive stress conditions, several authors reported the opposite effect (Adams et al., 1999). In barley, sunflower, wheat and maize crops, under deficiencies of Fe, S, Mn and Mg, an increase of global reflectance was observed (Masoni et al., 1996). In drought conditions, plants show also an increase in NIR reflectance. It was confirmed in wheat and peanut leaves by Peñuelas & Inoue (1999). Gitelson et al. (2003), working with beech, maple, wild vine and chestnut leaves, observed the same effect when there is a loss of chlorophyll in leaves, under stress conditions, which causes an increase in density and width of leaves.

But most of the reflectance changes observed in plants under stress conditions, at leaf scale, occur in the visible part of the spectrum, due to changes in the pigment content, loss of chlorophyll and carotenoids (Behmann et al., 2014). Loss of chlorophyll a and b caused an increase in VIS reflectance in maize and sunflower with nutritive stress, iron deficiencies (Mariotti et al., 1996). The same effect was observed also in wheat and barley under Fe, S, Mg and Mn deficiencies (Masoni et al., 1996). N, P and K deficiencies caused also an increase of VIS reflectance in maize (Al-Abbasi et al., 1974). Carter (1993) studied the spectral response of many plants to different stress conditions. VIS reflectance increased in all the plants independently of the type of stress. NIR reflectance, however, was less sensitive.

VIS reflectance usually increases at green and yellow bands in plants under stress conditions (Milton et al., 1991). However, under salinity conditions, the response of plants can be variable depending on the level of stress. Li et al. (2010) studied the effect of different saline concentrations (0, 100, 200 and 300 mM NaCl) in castor bean seedlings growth and spectral response. They noticed that VIS reflectance decreased in a first moment (100 mM NaCl) but then increased (200 and 300 mM NaCl), and this fact corresponded with the variation of chlorophyll a and b content. Thus, loss of chlorophyll a and b causes an increase of VIS reflectance.

Bands at 550 and 680 nm were determined as the most sensitive to detect loss of chlorophyll due to stress conditions (Merzlyak et al., 2003). The slope in the range from 550 to 650 nm decreases when plants suffer stress conditions, and it can be used as an indicator. Wang et al. (2002b) suggests that the most sensitive bands to detect salinity stress are 660 and 830 nm in soybean, and they propose the “simple ratio vegetation index” (SRVI) and the “normalized difference vegetation index” (NDVI) indexes to detect salinity. On the other hand, Rud et al. (2013) propose the “green and indigo ratio” (GIR) index to detect salinity stress, which is based on the reflectance at 436 and 554 nm. This index increases when salinity effect is more intense.

As at canopy scale, an effect in the red edge position has been also registered in many studies at leaf scale when stress conditions affect plants. A displacement to shorter wavelengths of the red edge can be observed due to the loss of chlorophyll (Milton et al., 1989, 1991; Gitelson et al., 2003). This fact has been described in plants under all types of stress: salinity (Li et al., 2010), drought (Gitelson et al., 2003) or nutritive (Masoni et al., 1996; Mariotti et al., 1996).

Figure 17.- Leaf hyperspectral reflectance of castor bean seedlings at four salt levels (CK=0, 100, 200 and 300 mM NaCl) (Li et al., 2010).

This displacement is very sensitive to the chlorophyll content and it can be detected also to the opposite direction, depending on the level of salinity stress. Li et al. (2010) saw a displacement first to longer wavelengths, in castor bean seedlings under low saline concentrations (100 mM NaCl), because chlorophyll a and b contents also increase. But then, under moderate and high saline concentrations (200 and 300 mM NaCl, respectively), the displacement was to shorter wavelengths because of the senescence and loss of pigments in the seedlings (Figure 17).

Not only there is a displacement in the red edge position (around 700 nm), also a displacement to shorter wavelengths in the green edge (around 530 nm) (Gitelson et al., 2003) and blue edge position (around 520 nm) (Li et al., 2010) has been noticed when plants are stressed. Richardson et al. (2002) observed the red edge displacement in paper birch under stress conditions, due to the loss of chlorophyll, and suggested the first derivative as indicator to see this movement.

It is not easy to find research about the effect of salinity stress in leafy vegetables. It is more complicated to find research about spectral response of leafy vegetables under salinity stress. The general considerations of most of the plants could be applied but it is necessary to know the specific effect of salinity on these vegetables. Pacumbaba & Beyl (2011) studied the effect of nutritive deficiencies (N, P, K, Ca and Mg) on the spectral response of lettuce leaves. Stress caused an increase in red, NIR and IR reflectances, as well as the displacement of red edge to shorter wavelengths according with the loss of chlorophyll content. Additionally, it was observed the increase of reflectance in the yellow region and the decrease in the green region.

A review of different studies carried out for detecting salinity and other stress conditions in crops using hyperspectral imaging and spectroscopy at leaf scale is done in table 10.

Table 10.- Spectral response of different plants to stress conditions. Local acquisition hyperspectral imaging.

PLANT	STRESS	SPECTRAL OBSERVATIONS	REFERENCE
Cauliflower Kohlrabi Aubergine	Salinity stress 30 - 150 mM NaCl	GIR index (R436 / R554) increases	Rud et al. (2013)
Castor bean seedlings	Salinity stress 0, 100, 200 and 300 mM NaCl	VIS reflectance first decreases and then increases, according to chlorophyll a and b contents Loss of chlorophyll causes increasing VIS reflectance NIR reflectance is not appropriate to detect salinity stress Red edge and blue edge displacement, first to longer wavelengths and then to shorter	Li et al. (2010)
Elephant grass	Salinity stress Soil salinity 1'5 - 25 dS/m	NIR reflectance decreases, due to leaf structural changes. R660 increases.	Wang et al. (2002a)
Soybean canopy	Salinity stress Irrigation water 4 dS/m	NIR reflectance decreases, due to increasing leaf specific weight SRVI and NDVI to detect salinity. Most sensitive bands to salinity 660 and 830 nm	Wang et al. (2002b)
Lettuce	Nutritive stress N, P, K, Ca and Mg deficiencies	Red, NIR and IR reflectance increases	Pacumbaba & Beyl (2011)
Canopy	Nutritive stress	VIS reflectance increases (380-720 nm)	Adams et al. (1999)
Maize	Stress conditions in general	NIR reflectance increases (720-1500 nm)	Mariotti et al. (1996)
Sunflower	Nutritive stress Fe deficiencies. 0 - 4 mg/l	Loss of chlorophyll a and b Red edge shifts to shorter wavelengths	
Barley Sunflower Wheat, maize	Nutritive stress Fe, S, Mn and Mg deficiencies	Global reflectance increases Red edge shifts to shorter wavelengths Loss of chlorophyll	Masoni et al. (1996)
In general	Nutritive stress Stress conditions in general	VIS reflectance increases. NIR less sensitive Similar VIS changes for all species and types of stress studied	Carter (1993)
Soybean leaves	Nutritive stress Mn deficiency	Red edge shifts to shorter wavelengths	Adams et al. (1993)

Table 10.- Spectral response of different plants to stress conditions. Local acquisition hyperspectral imaging. (continuation)

PLANT	STRESS	SPECTRAL OBSERVATIONS	REFERENCE
Soybean	Nutritive stress P deficiency	VIS reflectance increases at green and yellow bands Red edge shifts to shorter wavelengths	Milton et al. (1991)
Soybean	Nutritive stress As and Se effect	VIS reflectance increases (550-650 nm). Red edge shifts to shorter wavelengths Selenium causes the opposite	Milton et al. (1989)
Maize	Nutritive stress N, P, K, Ca, S and Mg deficiencies	VIS reflectance increases, due to loss of chlorophyll	Al-Abbas et al. (1974)
Wheat Peanut	Drought stress	VIS reflectance increases. NIR reflectance increases WI or WI/NDVI as water content indicator. SIPI as pigment content indicator	Peñuelas & Inoue (1999)
Grapevine	Drought stress	VIS reflectance (peak at 550 nm) decreases. NIR reflectance (1500 nm) increases.	Rapaport et al. (2015)
Barley	Stress conditions in general	Increasing of VIS reflectance, due to decrease of pigments under senescence Decreasing of NIR reflectance, due to changes in cell structure	Behmann et al. (2014)
Hypericum	Stress conditions in general	Comparisson of different models to relate stress conditions to leaf reflectance	Temizel et al. (2014)
Beech, Maple Wild Vine Chestnut	Stress conditions in general Loss of chlorophyll	Red edge and green edge shift to shorter wavelengths NIR reflectance increases, due to higher density and width of leaves VIS reflectance increases, due to loss of pigments	Gitelson et al. (2003)
Apple tree	Stress conditions in general Loss of chlorophyll	R550 and R680 increases. Slope in the range 550 - 650 decreases	Merzlyak et al. (2003)
Paper birch	Stress conditions in general Loss of chlorophyll	VIS reflectance increases (500 - 700 nm) Red edge shifts to shorter wavelengths. First derivative to see RE displacements	Richardson et al. (2002)
In general	Stress conditions in general Loss of chlorophyll	Changes in VIS reflectance, mainly, due to loss of chlorophyll NIR reflectance varies depending on type of stress	Knipling et al. (1970)

4. INDEXES USED TO DETECT SALINITY STRESS ON PLANTS

Several hyperspectral vegetation indexes have been used for determining salinity at both, leaf and canopy scales. Table 11 summarizes vegetation indexes commonly used to detect stress in plants. Among others, the normalized difference vegetation index (NDVI), the normalized difference water index (NDWI) and the water index (WI), have been widely applied. These indexes were found to be sensitive to salinity and they are good indicators for detecting soil salinity (Poss et al., 2006; Zhang et al., 2011).

As said previously, there is a decrease of pigments in plants under senescence resulting in a higher reflectance in the pigment-related visible part of the spectrum. That is why many of the indexes used to detect pigment content have been used to supervise salinity effects in plants. NDVI is an example but there are many other indexes such as normalized difference pigment chlorophyll index (NPCl) or photochemical reflectance index (PRI). The PRI, which is sensitive to xanthophylls, was shown to be able to detect salinity in several studies (Poss et al., 2006; Tilley et al., 2007; Song et al., 2011). Naumann et al. (2007) used PRI to detect salinity stress on *Myrica cerifera* and *Iva frutescens*, obtaining correlation coefficients of $R^2 = 0.79$ and 0.72 , respectively.

As it was also shown before, red edge is the most used indicator to detect chlorophyll losses (Ustin et al., 2009). Many indexes are based on red edge displacements to detect stress in plants. Some of them, such as the red edge position (REP) and the red edge normalized difference vegetation index (NDVI₇₀₅), have also been used to delineate the effects of salinity (Thorhaug et al., 2006; Tilley et al., 2007). Blum (1988) noted that crops under salinity stress typically show the same symptoms as under water stress conditions. Water stress related indexes, such as NDWI or WI have been used for salinity detection purposes (Rud et al., 2013). Peñuelas et al. (1997a) studied the effects of a soil salinity gradient ($0.8 - 1.9$ dS/m EC) on spectral reflectance of 10 genotypes of barley (*Hordeum vulgare* L.), applying NDVI and WI. Both indexes showed significant differences between the salinity treatments applied, being, therefore, useful for measuring the agronomic response of barley to salinity.

Some other indexes have been developed specifically for salinity detection. Hamzeh et al. (2013) proposed the salinity and water stress indexes (SWSI1, SWSI2 and SWSI3) for soil salinity detection, based on sugarcane canopy reflectance, with moderate results. They tested 24 different vegetation indexes (among others, NDVI, REP, NDWI, WI, PRI...), showing a wide range of correlations with soil salinity, R^2 varied from 0.105 to 0.692 . The best results were obtained for the indexes proposed, SWSI1, SWSI2 and SWSI3, with correlations with soil salinity of $R^2 = 0.68$, 0.65 and 0.67 respectively, and the optimized soil-adjusted vegetation index (OSAVI), $R^2 = 0.692$.

Rud et al. (2011) introduced the green and indigo ratio (GIR), based on the reflectance changes of cauliflower and eggplant in the visible part due to carotenoids and chlorophylls contents. They measured the spectral reflectance in the range of $350 - 2500$ nm and compared the GIR with other existing vegetation indexes such as Vogelmann ratio (VOG), NPCl, structure insensitive pigment index (SIPI), transformed chlorophyll absorption in reflectance index (TCARI), carotenoid reflectance index (CRI) and simple ratio vegetation index (SRVI). The conclusion was the need to integrate different indexes to decrease the uncertainty of detecting salinity effects. Poss et al. (2006) studied different variations of NDVI, focused on green (green region NDVI) and red (far red region NDVI) bands, to improve its correlation to salinity; and also the displacement of red edge position due to salinity effect, through the derivative-based red edge position pseudo-absorbance where the value is the steepest slope (REPAsl).

As previously said in the chapter “Effects of salinity in plants and leafy vegetables”, in this literature review, salinity can have some benefits enhancing the quality and shelf-life of fresh-cut leaves. Ready-to-eat products from leafy vegetables grown under salinity conditions can be more succulent and long-lived. However, there are no specific indexes developed to detect the effect of salinity on leafy vegetables. That index would be a great advantage for quality control purposes and for better understanding of such benefits.

Table 11.- Vegetation indexes commonly used to detect stress in plants.

NAME	RELATED TO	EQUATION	REFERENCE
Normalized difference vegetation index (NDVI)	Chlorophyll	$(R800-R670) / (R800+R670)$	Tucker (1979)
Red edge normalized difference vegetation index (NDVI ₇₀₅)	Chlorophyll	$(R750-R705) / (R750+R705)$	Gitelson & Merzlyak (1994)
Simple ratio (SR)	Chlorophyll	$R750 / R705$	Rouse et al. (1973)
Enhanced vegetation index (EVI)	Chlorophyll	$2.5(R800-R670) / (R800+6R670-7.5R490+1)$	Huete et al. (1997)
Atmospherically resistant vegetation index (ARVI)	Chlorophyll	$(R800-2(R670-R490)) / (R800+2(R670-R490))$	Kaufman & Tanré (1996)
Vogelmann red edge index 1 (VOG1)	Chlorophyll	$R740 / R720$	Vogelmann et al. (1993)
Vogelmann red edge index 2 (VOG2)	Chlorophyll	$(R734-R747) / (R715+R726)$	Vogelmann et al. (1993)
Vogelmann red edge index 3 (VOG3)	Chlorophyll	$(R734-R747) / (R715+R720)$	Vogelmann et al. (1993)
Red edge position (REP)	Chlorophyll	$700+40\{[(R670+R780)/2-R700] / (R740-R700)\}$	Clevers et al. (2002)
Modified red edge normalized difference vegetation index m(NDVI ₇₀₅)	Chlorophyll Pigments	$(R750-R705) / (R750+R705-2R445)$	Sims & Gamon (2002)
Modified SR (mSR)	Chlorophyll Pigments	$(R750-R445) / (R705-R445)$	Sims & Gamon (2002)
Modified chlorophyll absorption in reflectance index (MCARI)	Chlorophyll Pigments	$[(R700-R670)-0.2(R700-R550)] \cdot (R700 / R670)$	Daughtry et al. (2000)
Normalized difference pigment chlorophyll index (NPCl)	Chlorophyll Pigments	$(R680-R430) / (R680+R430)$	Aparicio et al. (1994)
Transformed chlorophyll absorption in reflectance index (TCARI)	Chlorophyll Pigments	$3[(R700-R670)-0.2(R700-R550)] \cdot (R700 / R670)$	Haboudane et al. (2002)
Anthocyanin reflectance index 1 (ARI1)	Chlorophyll Anthocyanins	$(1/R550)-(1/R700)$	Gitelson et al. (2001)
Anthocyanin reflectance index 2 (ARI2)	Chlorophyll Anthocyanins	$R800 \cdot (1/R550)-(1/R700)$	Gitelson et al. (2001)
Red green ratio index (RGRI)	Anthocyanins Chlorophyll	$\text{Mean } R(500-600) / \text{Mean } R(600-700)$	Gamon & Surfus (1999)
Improved soil adjusted vegetation index (MSAVI)	Chlorophyll	$1/2 \cdot [2R800+1-\sqrt{(2R800+1)^2-8(R800-R670)}]$	Qi et al. (1994)
Optimized soil adjusted vegetation index (OSAVI)	Chlorophyll	$(1+0.16)(R800-R670) / (R800+R670+0.16)$	Rondeaux et al. (1996)
Photochemical reflectance index (PRI)	Xanthophyll Stress	$(R531-R570) / (R531+R570)$	Gamon et al. (1992)
Photochemical reflectance index 2 (PRI2)	Xanthophyll Stress	$(R570-R539) / (R570+R539)$	Filella et al. (1996)

Table 11.- Vegetation indexes commonly used to detect stress in plants. (*continuation*)

NAME	RELATED TO	EQUATION	REFERENCE
Carotenoid reflectance index 1 (CRI1)	Carotenoids	$(1/R510)-(1/R550)$	Gitelson et al. (2002)
Carotenoid reflectance index 2 (CRI2)	Carotenoids	$(1/R510)-(1/R700)$	Gitelson et al. (2002)
Structure insensitive pigment index (SIPI)	Carotenoids Chlorophyll	$(R800-R445) / (R800+R680)$	Peñuelas et al. (1995)
Plant senescence reflectance index (PSRI)	Stress Senescence	$(R680-R500) / R750$	Merzlyak et al. (1999)
Moisture stress index (MSI)	Water content	$(R1599 / R819)$	Hunt & Rock (1989)
Normalized difference infrared index (NDII)	Water content	$(R819-R1649) / (R819+R1649)$	Jackson et al. (2004)
Water index (WI)	Water content	$R900 / R970$	Peñuelas et al. (1997b)
Normalized difference water index (NDWI)	Water content	$(R860-R1240) / (R860+R1240)$	Gao (1996)
NDWI-hyperion (NDWI-Hyp)	Water content	$(R1070-R1200) / (R1070+R1200)$	Ustin et al. (2002)
Disease-water stress index 1 (DSWI1)	Water content	$R800 / R1660$	Apan et al. (2004)
Salinity and water stress index 1 (SWSI1)	Water content Chlorophyll	$(R803-R681) / \sqrt{(R905+R972)}$	Hamzeh et al. (2003)
Salinity and water stress index 2 (SWSI2)	Water content Chlorophyll	$(R803-R681) / \sqrt{(R1326+R1507)}$	Hamzeh et al. (2003)
Salinity and water stress index 3 (SWSI3)	Water content Chlorophyll	$(R803-R681) / \sqrt{(R972+R1174)}$	Hamzeh et al. (2003)
Wetland salinity reflectance ratio (WSRR)	Salinity	$R990 / R933$	Tilley et al. (2007)
Green region NDVI	Salinity	$(R550-R670) / (R550+R670)$	Poss et al. (2006)
Far red region NDVI	Salinity	$(R710-R670) / (R710+R670)$	Poss et al. (2006)
Simple ratio vegetation index (SRVI)	Salinity	$R830 / R660$	Wang et al. (2002b)
Green and indigo ratio (GIR)	Salinity	$R436 / R554$	Rud et al. (2011)
Derivative-based red-edge position pseudo-absorbance where the value is the steepest slope (REPAsI)	Salinity	$\max(dRE)$	Poss et al. (2006)
Red edge position (REP)	Salinity	Wavelength (position) of $\max(dRE)$	Curran et al. (1990)

HYPOTHESIS AND OBJECTIVES

As seen in the literature review, a lot of efforts have been made to develop new methodologies and techniques to preserve the quality and to improve the shelf-life of RTU vegetables, because this is the main disadvantage of these products. Hyperspectral imaging has been successfully applied in many applications to assess and control quality parameters of a wide variety of food products. Physiological and chemical changes in the leaves due to aging or senescence processes, such as loss of pigments (chlorophyll, carotenoids, anthocyanins), loss of water and turgor, nutritive imbalances..., have a spectral response that can be sensed and located using hyperspectral imaging.

The first hypothesis is that the spectral response of the leaves can be analysed and compared along the shelf-life, using hyperspectral imaging, allowing the development of models for monitoring the shelf-life of RTU leafy vegetables.

Also the effect of non-biotic stresses (such as soil or water salinity) on the growth and, finally, the quality of the leafy vegetables, can be analysed using hyperspectral imaging. Understanding the effect of salinity in the structure, physiology and composition of the leaves is important for the quality and safety assessment, when they are used as RTU products. The detection of the effect of NaCl salinity on the tissues of leafy vegetables can be used as a quality biomarker for selecting optimal varieties that ensure the best quality/yield relationship in determined applications.

The second hypothesis is that the effect of salinity in the growing conditions of leafy vegetables can be sensed by hyperspectral imaging of the leaves, allowing classify them according to the degree of saline effect, which can be relevant for quality classification of leafy vegetables.

According to these hypotheses, several main objectives were established for this work:

- To monitor the evolution of leafy vegetables during the shelf-life, identifying the relevant spectral bands where the main changes occur, and developing models able to monitor the aging of leafy vegetables during storage.
- To extract useful spectral information from hyperspectral images acquired directly through different commercial packaging films, allowing the non-destructive inspection of the final commercial RTU product.
- To develop common models for monitoring the shelf-life of leafy vegetables able to be applied to different species of leaves simultaneously, which would be a great advantage for inspecting combined products of different leaves or mixed salads.
- To develop models, based on hyperspectral imaging, to evaluate the salinity effect on leafy vegetables, understanding the spectral changes that occur and allowing classify the leaves according to the degree of affectation.

EXPERIMENTAL WORK

1. DOCUMENT SECTIONS

The experimental work carried out in the frame of this thesis is comprised of three different sections, according to three independent experimental tests and data sets obtained by application of hyperspectral imaging to determine quality parameters on three different leafy vegetables: spinach, watercress and lettuce.

SECTION I: MONITORING SPINACH SHELF-LIFE WITH HYPERSPECTRAL IMAGE THROUGH PLASTIC FILMS

As it was shown in the literature review, in recent years, hyperspectral imaging technique has been regarded as a tool for assess quality evaluation of food products in research, control and industries. The hyperspectral imaging system allows integrating spectroscopic and imaging techniques to enable direct identification of different components or quality characteristics and their spatial distribution in the tested sample.

The objective of this work was to develop hyperspectral image processing methods for the supervision through plastic films of changes related to quality deterioration in packed ready-to-use spinach leaves during shelf life. The experimental work and data processing of this section was carried out from July, 2011 to November, 2013.

SECTION II: USING SPINACH RESULTS IN THE CASE OF A DIFFERENT LEAFY VEGETABLE: WATERCRESS

Aging of RTU leafy vegetables can induce different spectral changes depending on the species analysed. Understanding the similarities and differences between the spectral responses given by each leafy vegetable can help to develop common prediction models able to monitor the evolution of different vegetable species. It would be a great advantage, simplifying the technology required for industrial applications and allowing the inspection of RTU mixed salads or combined products.

The aim of this work was to evaluate the models developed from spinach leaves spectra applying them directly to the leaves of other leafy vegetable: watercress. A comparison with models developed from watercress leaves was done, analysing differences and similarities. The acquisition of watercress leaves hyperspectral images was done in August 2011. The data processing of this section was carried out during 2012.

SECTION III: HYPERSPECTRAL IMAGING TO EVALUATE THE EFFECT OF IRRIGATION WATER SALINITY ON LETTUCE

Salinity is one of the most important stress factors affecting growth and productivity of vegetables. Lettuce is a very popular leafy vegetable, commonly consumed as a ready-to-eat product, and implanted in arid regions of the southeast of Spain, with saline soils and low quality water. High levels of salinity cause losses of productivity and can induce necrosis and other undesirable effects on the leaves. However, many studies show that saline concentration in the leaves can enhance postharvest shelf-life after harvest, decreasing enzymatic activities and microbial decay, which is very positive for ready-to-eat leafy vegetables.

The aim of the work carried out in this Section was to evaluate the feasibility of hyperspectral imaging to identify the influence of salt stress in fresh harvested baby lettuce leaves. Hyperspectral images of lettuce leaves were acquired at Centro de Edafología y Biología Aplicada del Segura – Consejo Superior de Investigaciones Científicas (CEBAS-CSIC) on 2011.

SECTION I

MONITORING SPINACH SHELF-LIFE WITH HYPERSENSPECTRAL IMAGE THROUGH PACKAGING FILMS

MONITORING SPINACH SHELF-LIFE WITH HYPERSENSPECTRAL IMAGE THROUGH PACKAGING FILMS

ABSTRACT

Different procedures for monitoring the evolution of leafy vegetables, under plastic covers during cold storage, have been studied. Fifteen spinach leaves were put inside Petri dishes covered with three different plastic films and stored at 4°C for 21 days. Hyperspectral images were taken during this storage. A radiometric correction is proposed in order to avoid the variation in transmittance of the plastic films during time in the hyperspectral images. Afterwards, three spectral pre-processing procedures (no pre-process, Savitzky-Golay and Standard Normal Variate normalization, combined with Principal Component Analysis) were applied to obtain different models. The corresponding artificial images of scores were studied by means of Analysis of Variance to compare their ability to sense the aging of the leaves. All models were able to monitor the aging through storage. Radiometric correction seemed to work properly and could allow the supervision of shelf-life in leafy vegetables through commercial transparent films.

Keywords: Image analysis, non-destructive assessment, radiometric correction, ready to use leafy vegetables, shelf-life, transparent plastic films, hyperspectral.

1. INTRODUCTION

The consumption of fresh ready-to-eat products and minimally processed foods has increased in recent years (Artes et al. 2009). Ready-to-eat vegetables have a shelf-life between 7 and 14 days (Garcia Gimeno & Zurera Cosano, 1997), depending on the type of vegetable, due to microbiology degradations and the loss of physical and organoleptic properties (Toivonen & Brummell, 2008).

The industry demands objective, non-destructive and low cost methods to sense the evolution of this product during storage and to evaluate new systems that have been introduced in the ready-to-use processes as UV-C radiations, O₃ treatments...

Modified atmosphere packaging (MAP) is an essential technology for the success of fresh-cut produce. Polymeric films are a key element in MAP. The most commonly used packaging films are a few plastic polymers: polyolefins (polyethylene and polypropylene), vinyl compound polymers (polystyrene and polyvinyl chloride), and polyethylene terephthalate (polyester). For fresh fruits and vegetables, the films need to be “breathable” because the fresh produce inside is still “alive” and “breathing”. Micro perforated films are a good solution for this kind of fresh food.

Hyperspectral imaging allows the acquisition of the spectrum of each pixel, in a specific wavelength range, and generates a spatial map of spectral variations. It is a fast, simple and non-destructive technology (ElMasry & Sun, 2010). However, the images obtained are very complex to manage directly and need to be pre-processed and then processed (Fernández Pierna et al., 2009). Hyperspectral imaging has been used on different applications for the characterization of food quality (Cubero et al., 2011; Du & Sun, 2006; Gowen et al., 2007; Sun et al., 2010) or food safety (Del Fiore et al., 2010; Yao et al., 2008). Regarding vegetables Siripatrawan et al. (2011) used hyperspectral imaging for the detection of *Escherichia coli* contamination in packaged fresh spinach.

Previous research has been done to compare pre-processing methods for scatter correction in spectra: multiplicative scatter correction (MSC), inverse MSC, extended MSC (EMSC), de-trending, standard normal variate (SNV), normalization and spectral derivatives (Fearn et al., 2009; Laxalde et al., 2011; Rinnan et al., 2009; Zeaiter et al., 2005; Zeaiter & Rutledge, 2009). Geometric pre-processing methods are widely carried out to correct spectral data from drift in baseline, non-linearity, curvilinearity, as well as additive and multiplicative effects. All pre-processing techniques have the goal of reducing the unmodelled variability in the data in order to enhance the performance of the model of prediction. However, the application of a wrong or too severe type of pre-processing, can remove valuable information. Pre-processing should maintain or decrease the effective model complexity and enhance the model performance.

The objective of the present research was to compare different pre-processing procedures applied to hyperspectral images, regarding their ability to monitor the evolution and spoilage of leafy vegetables under plastic covers during storage. First, the effect of the variation in transmittance of the plastic films that cover the leaves in the measurements was established and corrected. Second, three pre-processing procedures: a) no pre-process, b) Savitzky Golay (SG), and c) Standard Normal Variate (SNV) combined with principal component analysis (PCA), were applied and compared.

2. MATERIALS AND METHODS

Three groups of spinach (*Spinacia oleracea*) leaves (n=5 leaves per group) were randomly selected from bags acquired in a local market. Three micro perforated plastic films (polypropylene PPLUS® 160, P1; polypropylene PPLUS® 190, P2; and biaxially oriented polypropylene BOPP 30 µm, P3) commonly used in the packing industry of vegetables, were selected. Each group of leaves was assigned one type of plastic film. Each leaf was individually left inside a Petri dish with a piece of grey plastic and covered by the corresponding plastic film. The samples were stored at 4 °C during the whole measurement period. Hyperspectral images were acquired 7 times through 21 days: at 0, 4, 7, 11, 14, 18 and 21 days.

Hyperspectral vision system consisted of a CCD camera with a VNIR spectrometer (Headwall Photonics Hyperspec™) working between 400 and 1000 nm. It was equipped with a progressive line-by-line scan spectrograph with a slit of 25 µm. The selected spectral resolution was 3.2 nm (189 wavelengths), and the spatial resolution was 0.26 mm/pixel. One halogen lamp was used for the illumination. Specific

software, Headwall HyperSpec™, was used to control the equipment. Images were acquired according to the conditions shown in Table I.1. Samples were scanned acquiring the whole surface of the leaf. A hypercube dataset was obtained from each image.

Table I.1.- Configuration of the image acquisition conditions

Spectral binning	4 (189 wavelengths)
Frames to average	5
Scan length	140 mm
Slit	25 μm
Exposition time	80 ms

Relative reflectance hyperspectral images were computed simultaneously to the acquisition, by the software of the camera. White reference (barium sulphate) and dark current signal (acquired with the objective of the camera covered by a black tap) were acquired before each batch of images. Then, each line of the image was corrected pixel by pixel subtracting the dark current and dividing this result by the white reference minus the dark current.

Aiming to obtain a calibration data set completely independent on the validation data set, the 15 leaves were separated into two different groups: a calibration set with three leaves of each plastic, and a validation set with the remaining two leaves of each plastic. The average spectrum of each leaf of the calibration set was computed from the hyperspectral images. Each average spectrum was computed considering all the pixels belonging to the leaf; depending on the size of the leaf the number of spectra range from $n = 7293$ to $n = 85702$ (original images without reduction of the spatial resolution). Calibration data set was formed by 63 average spectra (3 leaves $\times$ 3 films $\times$ 7 dates). Models were generated with the calibration data set. Two types of validations were performed: a) one applying the models to all the pixels of the leaves of the validation set and b) a second one considering also all the pixels of the leaves of the calibration set.

2.1 PREPROCESSING PROCEDURES

All the processes described in this section were applied to the calibration set of spectra and also to all the pixels of the hyperspectral images.

2.1.1 Radiometric correction

Radiometric correction (RC) is currently employed in remote sensing for overcoming changes in the measured light due to the atmospheric transmittance and the position of the sun (Sims & Gamon, 2002; Song et al., 2001). In the present study, this correction was used to correct the measured signal for variation in plastic film transmission. A piece of grey plastic of 25 x 25 mm, having a global reflectance similar to a leaf, was inserted in each Petri dish, beside the spinach leaf.

Let be:

- two pixels of grey plastic (position a) and of leaf (position b),
- $S_0(a,t)$ and $S_0(b,t)$ the measured signal from the two pixels a and b on the white reference before the measurement made at date t
- $F(a,t)$ and $F(b,t)$ the internal gain factor of pixels a and b
- $I_0(a,t)$ and $I_0(b,t)$ the intensity of the input light for the two positions a and b at date t
- $P(t)$ the transmission of the plastic film, at date t, supposed independent of the position
- G the reflectance of the grey plastic supposed independent of the position and the date
- $R(b,t)$ the reflectance of the leaf at pixel b and date t

Figure I.1 illustrates the light path for the two pixels during the measurement. The signals finally collected by the camera are given by:

$$\text{Eq. I.1} \quad S(a,t) = I_0(a,t) \cdot P(t)^2 \cdot G \cdot F(a,t) \quad \text{and} \quad S(b,t) = I_0(b,t) \cdot P(t)^2 \cdot R(b,t) \cdot F(b,t)$$

However, assuming a unitary reflectance for the white reference, we have:

$$\text{Eq. I.2} \quad S_0(a,t) = I_0(a,t) \cdot F(a,t) \quad \text{and} \quad S_0(b,t) = I_0(b,t) \cdot F(b,t)$$

Then, the values collected for the two pixels are:

$$\text{Eq. I.3} \quad X(a,t) = S(a,t) / S_0(a,t) = P(t)^2 \cdot G \quad \text{and} \quad X(b,t) = S(b,t) / S_0(b,t) = P(t)^2 \cdot R(b,t)$$

The average value of $X(a,t)$ was computed over all the pixels of the grey plastic and then divided by the value of G measured before the experiment, yielding an estimation of $P(t)^2$. Each pixel b of the leaf was then divided by this quantity, giving an estimation of the true reflectance $R(b,t)$.

Figure I.1.- Light modifications between source and camera detector for the two pixels (a = reference, b = leaf) considering date t . $I_0(a,t)$ and $I_0(b,t)$ = Intensity of the input light for the two positions a and b , at date t . $P(t)$ = transmission of the plastic film, at date t . $F(a,t)$ and $F(b,t)$ = internal gain factor of pixels a and b . G = reflectance of the reference. $R(b,t)$ = reflectance of the leaf at pixel b and date t .

2.1.2 Multiplicative and additive effect

Savitzky-Golay smoothing and differentiation algorithm (SG) was applied to the spectra: a polynomial of order three was fitted to 21 wavelengths width and the second derivative function was applied to the smoothed spectra.

After SG algorithm, the multiplicative effect was corrected by Standard Normal Variate scaling (SNV). SNV subtracts to each wavelength of the spectrum (λ_i) the mean value of this spectrum (λ_m) and divides it by the standard deviation of this spectrum (STD). Resulting spectra have a unit standard deviation and a mean equal to zero.

$$\text{Eq. I.4} \quad \lambda_{i_SNV} = (\lambda_i - \lambda_m) / \text{STD}$$

2.2 DATA PROCESSING

Several Principal Component Analyses (PCA) were computed on the calibration set of average spectra of the selected leaves ($n=63$), in which the following pre-processes were successively performed: a) ~~only~~ radiometric correction; b) radiometric correction and SG algorithm and c) radiometric correction, SG algorithm and SNV. The resulting loadings of the PCA obtained from the spectra processed with these three procedures (a, b and c) were used for the projection of the corresponding corrected hyperspectral images with the aim to monitor the evolution of the surface of each leaf during time. Three virtual images of scores were obtained from such projections for each leaf, one per each different pre-processing method. The projections were carried out on images with a reduction of the spatial resolution (from 0.26 mm/pixel to 1.04 mm/pixel), in order to reduce the computation time.

The scores of the artificial images were studied by means of Analysis of Variance in order to compare the pre-processing procedures for the detection of the evolution of the leaves and the influence of each plastic film in the preservation of the quality and freshness.

In order to test the best pre-processing procedure for sensing the aging of the leaves, the pixels of the leaves were pooled together for the first day (first group) and for the last day (second group). ANOVA's were carried out for each of the three pre-processing procedures comparing these two groups. For an accurate comparison of the ANOVA results, the same number of pixels, randomly selected per group was considered for a) calibration set ($n=31.000$ per group), b) validation set ($n=17.000$ per group) and c) calibration plus validation set ($n=48.000$ per group). Consequently, nine ANOVA's were calculated (3 sets of data $\times$ 3 pre-processing procedures).

In order to evaluate the ability of the plastic films to preserve the quality of the leaves, additional ANOVA's were calculated for hyperspectral images in which only RC had been applied. For each plastic film separately, pixels of the leaf images were pooled together for the analysis, categorized in two extreme groups: leaves from the first day and leaves from the last day. The analyses were carried out on the calibration set and on the validation set. Same number of pixels, randomly selected, were considered on each plastic for an accurate comparison of ANOVA results (Calibration set $n=7.400$ per group, Validation set $n=3.900$ per group).

Finally, in order to analyse the evolution rates of the leaves, ANOVA's were computed on each individual leaf from calibration and validation sets, comparing the same number of pixels on each leaf, ($n=800$ per group, randomly selected). Only hyperspectral images with RC were considered in these ANOVA's.

3. RESULTS

3.1 RADIOMETRIC CORRECTION

The effect of the radiometric correction can be seen in Figure I.2, where all the average spectra of the leaves were plotted ($n=105$), before correction (upper left) and after RC (lower left). Without correction, a strange trend in some spectra could be observed: five spectra showed higher level of intensity at NIR range (higher than 700 nm) compared with the rest of the average spectra. A PCA was calculated and the corresponding scores were analysed (Figure I.2, upper right); the same five samples appeared separated from the rest of the population. These five outlier spectra corresponded to the five leaves of plastic 1 on the last date. The hypothesis is that plastics suffered changes on their structure and composition through the 21 days. After RC, these five spectra were completely corrected and did not appear anymore as outliers in the PCA plot (Figure I.2, lower right). In addition, the average RC spectra at NIR region was more scattered than in non RC spectra.

Figure I.2.- Upper left: raw mean spectra of the leaves (n=105) without radiometric correction. Upper right: scores of the PC1 vs. PC2 from PCA on raw mean spectra. Lower left: mean spectra of the leaves after the radiometric correction. Lower right: scores of the PC1 vs. PC2 from PCA on mean spectra after radiometric correction.

3.2 SPECTROSCOPY COMMENTS

3.2.1 Reflectance spectra with the radiometric correction

PCA was computed on radiometric corrected spectra (n=63). First and second components were not related to the time evolution (data not shown), probably because they were more related to unmodelled variability of spectra/images such as geometric effects or light variations. The third principal component (PC3) sensed the evolution of the leaves.

The global shape of the spectra can be observed in Figure I,3 (upper left). This is the typical shape of a plant leaf spectra (Siripatrawan et al., 2011; Xue & Yang, 2009) in which three main regions can be recognized: the “red edge” region between 680 and 740 nm approximately, a reflectance peak in the green region (500-600 nm), and a broad region at NIR with a high level of reflectance.

The Figure I.3 also shows that the global reflectance of the mean spectrum of each plastic on the last day was higher than the corresponding for the first day. That could be due to the changes in the structure and composition of the leaf along time (Jacquemoud & Baret, 1990). This phenomenon has already been described by several authors (Asner, 1998; Liy et al., 2010): when the plant leaves are aging, there is a decrease in the content of chlorophyll, and of other foliar pigments. Besides, water content decreases, which can produce an increase in the content of air between cells; consequently, the scattering increases. As a result, the global reflectance increases in all the visible range and mainly in the near infrared regions.

Figure I.3.- Upper left: mean radiometric corrected spectra of the leaves of each plastic (p1, p2, p3), on the calibration set, on first (d1) and last date (d7). Upper right: mean centered spectra of the same leaves. Lower left: PC3 loading from PCA applied on mean radiometric corrected spectra. Lower right: scores of PC3 on mean spectra of the leaves of calibration set along the time.

Figure I.3 (lower left), shows the loadings of PC3, related to the evolution of leaves during storage. The following parts can be observed: a first part in the visible range with negative values (between 400 and 500 nm) which is associated with the blue colour, and a second region with positive values (between 500 and 650 nm) which is associated with green and yellow colours. Therefore, it could be concluded that during the storage period, the blue coloration of the leaves decreases, whereas the green and yellow colorations increase.

Table I.2.- Spectral bands maximally sensitive to absorption by main pigments. Adapted by Gitelson et al. (2006).

Pigment	Wavelength range (nm)
Chlorophylls in anthocyanins free leaves (< 3 mg/m ²)	540-560 690-720
Chlorophylls in leaves containing anthocyanins	690-720
Carotenoids	510-520
Anthocyanins	540-560

Gitelson et al. (2006) described a conceptual model capable of estimating total chlorophylls, carotenoids and anthocyanins contents in leaves from many tree and crop species. They show different range of wavelengths maximally sensitive to absorption by the pigment of interest (Table I.2). Anthocyanin absorbs around 550 nm, consequently in leaves with anthocyanins only the region around 700 nm is used to estimate chlorophyll content, while in leaves without anthocyanins two regions of the spectra are sensitive to the content of chlorophyll (540-560 nm and 690-720 nm). These authors proposed an

estimation of chlorophyll content in leaves free of anthocyanin based on the ratio of the reflectance in NIR range and the reflectance at 540-560 nm.

According to Pavia et al. (2007), major pigments in spinach leaves are chlorophyll a and b and carotenoids, and then it could be feasible to consider the cited ratio. Figure I.3 (upper right) shows the radiometric corrected average spectra which have been mean centered, in order to improve the visualization of the changes that occurred in the cited ratio. It can be observed that this ratio increases during the storage period, because the NIR reflectance is higher in aged leaves and the reflectance at 540-560 nm is lower. Therefore, the chlorophyll content is likely to increase, but this fact is impossible in harvested leaves. As it has been explained previously, the water content of the leaves decreases during time. These two facts allow to extract the hypothesis that there is an increase in the relative concentration of chlorophyll because of the water decreasing is higher than the chlorophyll decreasing which could cause the described effect on the spectra (Figure I.3, upper left).

On the mean spectra, a shift towards the left at the red edge region for the 21 testing days can be observed. Figure I.4 (right) shows this more clearly. The loadings of the PC3 of the radiometric corrected spectra (Figure I.3, lower left) shows a big peak in the range between 680 and 760 nm, with its maximum at 707 nm. So PC3 could be related to the evolution of red-edge of the leaves. Vertical lines mark the most relevant wavelengths of the loadings at the red edge range: 680, 707 and 760 nm. The same wavelengths are marked on the mean spectra (Figure I.4, right). Those wavelengths delimit the characteristic sharp change in the spectra at red-edge region for leafy vegetables, 707 nm could be near the inflection point.

Figure I.4.- Left: lateral movements of spectra on red-edge region cause changes in the values of minus second derivative (-SD). Right: detailed range of the spectra on red-edge region.

Loadings of PC3 compute an approximation to the minus second derivative at 707 nm ($-SD_{707}$):

$$\text{Eq. I.5} \quad -SD_{707} = 2 \cdot R_{707} - R_{680} - R_{760}$$

Second derivative is related to the curvature at 707 nm: if this wavelength corresponds to the inflection point, the $-SD_{707}$ is equal to zero; on the contrary $-SD_{707}$ will be positive if the spectrum is shifting towards the left and negative if the spectrum is shifting towards the right (Figure I.4, left). Figure I.3 (lower right) shows an increase in the values of the scores for the samples along time, which is consistent with the displacement of the spectra to the left on the red edge region.

Several studies have reported that, when leaves are aging and chlorophyll content decreases, red edge suffers a displacement towards shorter wavelengths (Mutanga & Skidmore, 2007; Van der Meer & Jong, 2002); therefore, red edge could be used as a measurement for estimating the leaf chlorophyll content. Most of the reviewed and studied optical indexes include wavelengths from the red-edge region (Xue & Yang, 2009).

The analysis of the loadings of the PC3 on the NIR region (Figure I.3, lower left) allows identifying an approach to the calculation of the slope of the spectra between 760 and 1000 nm. Increasing slopes at the NIR range of the spectra along the evolution are reflected on increasing values of scores of PC3 (Figure I.3, lower right). This fact is in accordance with Asner (1998) who showed that, during aging, the slope of the spectra in the NIR region changes, becoming higher in died leaves than in healthy leaves.

3.2.2 Smoothed and Second derivative spectra

Figure I.5 (upper left) shows the mean spectra of the leaves subjected to RC and SG algorithm for each plastic on the first and the last days. The second derivative spectra of the reflectance spectra exhaust the absorbances as positive peaks. Thus, the main chlorophyll peak clearly appears around 680 nm in Figure I.5 (upper). In Figure I.5, the second highest peak appears around 510 nm and may be attributed to carotenoids (Gitelson et al., 2006; Table I.2). The third one, around 585 nm, should also be attributed to chlorophyll (Kleima et al., 2000). In these plots, it can be observed that chlorophyll and carotenoid peaks have slight increase in the values on the last day, whilst the peak at 585 nm does not change.

Figure I.5.- Upper left: mean radiometric corrected, second derivative spectra of the leaves of each plastic, on first and last date. Upper right: detailed range of the spectra in which the differences are relevant. Lower left: PC1 loading from PCA applied on RC plus second derivative corrected spectra. Lower right: scores of PC1 on mean spectra of the leaves of calibration set along testing time.

In the PCA performed on these spectra, PC1 was the most related one to the evolution of the leaves. The pre-processing of the spectra by means of SG algorithm seems to reduce the un-modelled information since the first score is directly related to the evolution of the leaves during time. The scores increased during the measurement period (Figure I.5, lower right).

The loadings reported in Figure I.5 (lower left), in association with the evolution of the corresponding scores, (Figure I.5, lower right), are in accordance with the preceding findings:

- Two clear positive peaks in correspondence with the chlorophyll and carotenoids peaks
- A quite flat shape in the 585 nm area

Moreover, the combination of the large negative peak at 710 nm and the positive one at 750 nm denotes a shift of the negative peak at 725 nm, noticeable in Figure I.5 (upper left). This peak corresponds to the end of the red edge of the reflectance spectra. Thus, the loadings sense in this area the red edge shift, as mentioned before.

Water has maximum absorptions centered near 1400 and 1900 nm, moreover water also has absorption bands centred on 970 nm and 1190 nm. However, the water peak at 970 nm does not appear in the second derivative spectra (Figure I.5, upper left), which could be due to the fact that this range corresponds to the less sensitive area of the hyperspectral detector.

3.2.3 Smoothed, Second derivative and SNV spectra

Figure I.6 (upper left) shows the same mean spectra with additional SNV pre-treatment. It can be observed that the differences in the spectra between first and last day nearly disappear. SNV seems to produce loss of information regarding the evolution of the leaves during time.

Figure I.6.- Upper left: mean radiometric corrected, second derivative and SNV corrected spectra of the leaves of each plastic, on first and last date. Upper right: detailed range of the spectra. Lower left: PC1 loading from PCA applied on RC plus second derivative and SNV corrected spectra. Lower right: scores of PC1 on mean spectra of the leaves of calibration set at first and last day. Vertical green lines mark the wavelengths 680, 707 and 760 nm, delimiting the sharp change in the spectra at red-edge region.

On the corresponding PCA, again the PC1 was the most related to the evolution of the leaves. Once more, wavelengths around 680, 707 and 760 nm showed the highest values in the loadings. It can be observed that the same shape appears in loadings of PC1 of RC plus SG (Figure I.5, lower left) and in loadings of PC1 of RC plus SG plus SNV (Figure I.6, lower left), but inverse that the corresponding loadings of PC3 of RC, because second derivative shows absorption zones as positive peaks.

3.3 DATA PROCESSING

3.3.1 ANOVAs for the comparison of the preprocessing procedures

Results of ANOVAs performed on the scores of the artificial images to test the efficiency of the pre-processing are shown in Table I.3.

In all the pre-processed images a significant evolution of the scores along the time could be observed (Table I.3). According to the lowest F of Fisher in ANOVA's, the pre-processing procedure including SNV could be the less sensitive to the evolution of the samples for the three sets of data. Multiplicative effect seems to be meaningful. Probably because multiplicative effect is related to scattering, which is due to changes of refractive indexes between cells and interstitial air.

Comparing the F values for RC and RC plus SG algorithm, it could not be concluded which procedure was the most segregating one. On validation set and on calibration plus validation set the RC showed the highest F value; on the contrary, the RC plus SG algorithm presented the highest F of Fisher on the calibration set.

As a result of the previous ANOVA's and in order to implement the simplest preprocessing procedures, only samples with RC were taken into account for further analysis.

Table I.3.- Analysis of variance comparing the scores of leaves at first date with the scores of leaves at last date, on each data set and on each data pre-processing. The scores considered for each case were PC3 scores for RC spectra; PC1 for RC plus SG and PC1 for RC plus SG plus SNV. Highest F-Fisher values are marked in bold characters.

		RC	RC + SG	RC + SG + SNV
Calibration Set n=31,000	<i>F - Fisher</i>	30,784	38,997	26,956
	<i>p-level</i>	0	0	0
Validation Set n=17,000	<i>F - Fisher</i>	31,878	19,225	14,199
	<i>p-level</i>	0	0	0
Calib. + Valid. Set n=48,000	<i>F - Fisher</i>	59,141	56,786	40,324
	<i>p-level</i>	0	0	0

Results of ANOVA's performed to evaluate the goodness of the plastics are shown in Table I.4. Considering the F of Fisher values, it could be concluded that leaves under plastic 1 presented the highest evolution; consequently, it could be noted that, on this experimental design, plastic 1 showed the worst behaviour on the preservation of leaves. On the other hand, the lowest F value of plastic 3 could indicate the best behaviour of this covering material. Plastic 2 showed an intermediate behaviour.

Table I.4.- Analysis of variance comparing the scores of the leaves at first date with the scores of the leaves at last date, on each cover plastic and on each data set. The considered scores were resulting of the projection of RC spectra onto PC3.

		Plastic 1	Plastic 2	Plastic 3
Calibration Set n=7,400	<i>F - Fisher</i>	35,014	22,530	3,230
	<i>p-level</i>	0	0	0
Validation Set n=3,900	<i>F - Fisher</i>	11,048	10,481	6,258
	<i>p-level</i>	0	0	0

Results of ANOVA's computed on the scores of individual leaves are shown in Table I.5. In general, regarding the average values of F of Fisher, the same conclusions as in previous analyses (Table I.4) about the behaviour of plastic films could be drawn. However, individual F values presented differences between leaves covered with the same plastic films. It could denote that the evolution rate was different for each leaf, and the initial freshness stage could be variable even in leaves belonging to the same commercial bag.

Table I.5.- Analysis of variance comparing the scores of each individual leaf at first and last date, and average values of the leaves with the same plastic on calibration and on validation sets.

	Number of leaf	Plastic film	F - Fisher	Average F - Fisher
Calibration Set n=800	1	1	13,536	7,453
	2		5,379	
	3		3,444	
	6	2	1,909	7,233
	7		12,899	
	8		6,890	
	11	3	722	524
	12		756	
	13		95	
Validation Set n=800	4	1	9,074	5,755
	5		2,437	
	9	2	3,494	2,033
	10		572	
	14	3	994	1,373
	15		1,752

Figure I.7.- Scores images obtained by projection of the hyperspectral radiometric corrected images on PC3. It is shown one leaf of each plastic from the validation set: left to right plastic 1, plastic 2 and plastic 3. Upper: first date. Lower: last date.

Figure I.7 shows scores images obtained by the projection of the RC hyperspectral images on PC3. One representative leaf of each plastic from the validation set on the first and last day is included. Differences among the three types of packing plastic appear clearly, in accordance with the results from ANOVA's (Tables I.4 and I.5); leaf under plastic 3 presented less evolution, and leaf under plastic 1 showed higher evolution rate. Remaining scores images (computed for all considered pre-processing procedures) were congruent regarding plastic behaviour identification: plastic 1 seems to be the worst type for protecting spinach leaves since their leaves evolved mainly in the score images (images not shown). Similar results are presented in Figures I.4, I.5 and I.6 (lower right), where the scores for plastic 3 during the time of the study experimented less evolution (see green lines).

Figure I.8.- Virtual image of loading 3 from the radiometric corrected data PCA. Two different leaves from plastic 3 on the seven tested days.

Figure I.8 illustrates the differences in the evolution rate and in the initial freshness stage of the leaves. Two leaves under plastic 3 are monitored through the storage. Evolution was apparently more intense in the periphery of the leaf. In the region near the peduncle, the evolution was lower. Additionally, veins of the leaves seemed to be more resistant to the evolution (lower scores values). It can be observed that the initial freshness stage of each leaf is very different.

4. CONCLUSIONS

The present research focused on the use of hyperspectral images (400 -1000 nm) in spinach, under three commercial packaging plastic films, to monitor their evolution during storage (21 days, 4°C). Different pre-processing procedures were applied to the images (radiometric correction, Savitzky Golay algorithm, and Standard Normal Variate combined with a Principal Component Analysis).

The effect of the variation of the transmittance of the plastic on the spectra of leaves was removed applying radiometric correction to the hyperspectral images. Therefore, this procedure may allow the monitoring of shelf-life in leafy vegetables through packaging transparent films.

The main changes observed on the spectra of spinach during storage period were: the shift of the red-edge to shorter wavelengths probably due to aging in leaves, as Van der Meer et al. (2002) described before; the increase in the global reflectance, mainly in the infrared region, probably due to changes in the structure of the leaves and loss of water (Jacquemoud & Baret, 1990); and the increase in the slope of the spectra at the infrared region due to the aging of leaves, as Asner (1998) stated.

A methodology for the evaluation of the different pre-processing procedures was proposed. ANOVA results show that all procedures were able to monitor the loss of freshness of the spinach leaves during the storage, although radiometric correction presented slight better performance in their ability to discriminate evolution stages.

Moreover, the behaviour of the plastic films along the storage period was evaluated for their ability to slow the loss of freshness. Remarkable variability on initial freshness stage and evolution rate was verified in leaves belonging to the same batch of packages, which indicates the advantages that could be gained if an image analysis system is applied to obtain an overall quality assessment of packaged products.

Further analysis has to be conducted to compare the effect of plastic films in the preservation of the leaves. To validate the obtained results, an extended analysis with an increased sample size will be necessary. The challenge for the next step of this research is the definition of multispectral indexes based on the loadings obtained from PCAs and on the pattern of the spectra, capable of following the evolution during storage and removing the effect of the plastic films.

SECTION II

USING SPINACH RESULTS IN THE CASE OF A DIFFERENT LEAFY VEGETABLE: WATERCRESS

USING SPINACH RESULTS IN THE CASE OF A DIFFERENT LEAFY VEGETABLE: WATERCRESS

ABSTRACT

The aim of the present work is to monitor the shelf-life of watercress by means of hyperspectral images. Specific models for watercress along with other models previously developed for spinach have been applied and compared in the present section. The artificial images of scores obtained, applying each model, have been compared by means of Analysis of Variance and Wilks- λ . All models applied were able to monitor the aging of the leaves. However, the models developed using watercress spectra were more suitable for monitoring the shelf-life of watercress leaves along the storage. There were some differences between the models applied for spinach and for watercress leaves. It seems to be necessary to study the spectra of each leafy vegetable independently for developing prediction models abler to monitor the aging of the leaves.

Keywords: Image analysis, non-destructive assessment, radiometric correction, ready to use leafy vegetables, shelf-life, watercress, hyperspectral.

1. INTRODUCTION

The success of ready-to-eat fresh vegetables has caused in the food industry the need to search more varieties in the vegetables offered to the consumers, in order to satisfy them and keep awake their interest on these products. The development of new types of ready-to-eat salads, using new vegetables, is increasing the sales of these products. Vegetables such as salad rocket (*Erucula sativa*), alfalfa sprouts (*Medicago sativa*), baby leaves or watercress (*Nasturtium officinale*) are being processed and commercialized in recent years although they were not commonly used some years ago.

Watercress is an aquatic perennial vegetable, from the *Brassicaceae* family, with wide culinary applications. The raw watercress leaves can be used as salad or steamed. They have a great amount of vitamin C and pro-vitamin A, folic acid, iodine, iron, proteins and, especially, calcium and sulfur compounds, causing its characteristic smell and its nutritional benefits (Gonçalves et al., 2009).

Understanding the evolution and deterioration processes of watercress leaves during their shelf-life is a key factor for commercializing this vegetable as minimal processed fresh product. To establish the optimal shelf-life of watercress leaves allows the industry to offer high quality products to the consumer, with optimal sensory conditions.

Hyperspectral imaging, as shown in section I, could be used for monitoring the freshness of spinach leaves during their shelf-life. This technology provides wide and complex spectral information, that implies to create prediction models, easy to apply to the images.

The objective of this section is to study the feasibility of those “spinach” models on watercress leaves, with the purpose to monitor the evolution of watercress. Also, new prediction models for watercress are proposed. Finally, similarities and differences between the models of both leafy vegetables are discussed. Obtaining a common model able to be applied in different leafy vegetables could allow the monitoring of freshness in mixed salads directly, which is a great advantage in industrial applications.

2. MATERIALS AND METHODS

An experimental design has been carried out, similar to the one conducted in spinach leaves, described by Lara et al. (2013). For that, 15 leaves from a commercial bag of ready-to-eat watercress leaves have been selected and put individually into Petri dishes, with a reference material (the same grey plastic than in the case of spinach), and covered with a plastic film (polypropylene 35 microns width) for simulating similar storage conditions than in the original plastic bag (figure II.1). The Petri dishes were stored at 4°C during 16 days.

Figure II.1.- Leaves selection from a watercress commercial bag and placement of the leaves inside Petri dishes for their conservation and image acquisition.

Along this period, hyperspectral images of the leaves surface were acquired, through the plastic film, two times per week, each 3-4 days, with a total of 5 acquisitions. The test was not prolonged because of the evolution of the watercress leaves, that became dried and started to wrinkle. One of the 15 leaves was removed during the test due to its fast evolution. For that, the spectral analysis was carried out finally over 14 leaves.

The same hyperspectral imaging system used in the experiment with spinach leaves was used in this case (see section I). The image acquisition conditions are shown in table II.1. The samples were scanned acquiring all the leaf surface, obtaining a data hypercube for each image. The hyperspectral relative reflectance images were computed.

Table II.1.- Configuration of the image acquisition conditions.

Spectral binning	4 (189 wavelengths)
Frames to average	5
Scan length	80 mm
Slit	25 μm
Exposition time	50 ms

The spectra corresponding to the pixels of the leaf surface were extracted from each hyperspectral image, for each leaf on each tested day. Depending on the leaf size, the number of spectra extracted ranged from $n=2624$ to $n=50213$. The mean spectrum of the leaf on each image was computed and a matrix of spectral data was created, containing 70 mean spectra (14 leaves $\times$ 5 days). The mean spectrum of the reference material for each image was also calculated, with the aim of applying the radiometric correction.

2.1 PREPROCESSING PROCEDURES

The preprocessing procedures described in this point were successively applied on the matrix containing the mean spectra of the watercress leaves.

2.1.1 Radiometric correction

Radiometric correction is applied to avoid the effect of the plastic film in the signal (see Material and Methods of Section I).

2.1.2 Multiplicative and additive effect correction

As in the case of spinach spectra, Savitzky-Golay smoothing and differentiation algorithm (SG) was applied to the spectra: a polynomial of order three was fitted to 21 wavelengths width and the second derivative function was applied to the smoothed spectra.

After SG algorithm, the multiplicative effect was corrected by Standard Normal Variate scaling method (SNV). See explanation at Material and Methods of Section I.

2.2 DATA PROCESSING

After the preprocessing procedures of spectra, three independent data matrixes were obtained to analyze: a) the matrix corresponding to the mean spectra of watercress leaves with radiometric correction (RC); b) the matrix corresponding to those spectra corrected with RC and SG algorithm; and c) the matrix corresponding to those spectra corrected with RC, SG algorithm and SNV normalization. A Principal Component Analysis (PCA) was done on each of these matrixes. The loadings obtained on each PCA were studied, and those abler to sense the evolution of the leaves during time were selected.

The retained loadings, calculated from the spectra of watercress leaves, were added to the three loadings retained in the case of spinach leaves (Section 1 of this research). All these loadings were used to project the adequately processed hyperspectral images of watercress leaves in order to obtain virtual

images of scores potentially capable of detect and monitor the evolution of the leaves during their shelf-life.

Virtual images of scores were analysed and compared. Because of the evolution of the leaves, some of them became smaller and the available surface to acquire spectra were reduced. For that, 7 of the 14 leaves were selected and compared by means of Analysis of Variance (ANOVA) and by means of Wilks- λ , with the aim of establishing which model was able to detect better the differences between the images corresponding to the first and the last day. The same number of pixels from the surface of each leaf was considered ($n= 38000$ pixels) and the parameters F-Fisher and Wilks- λ were calculated. The model which shows greatest values of both parameters was selected as the most suitable to monitor the evolution of the leaves. The hyperspectral images of the 14 watercress leaves were then projected onto the best loading retained to check differences in the evolution of each leaf independently, and to monitor the evolution of the leaves day by day.

3. RESULTS AND DISCUSSION

3.1 INTERPRETATIONS OF THE FEATURES OF WATERCRESS LEAVES SPECTRA AND PRINCIPAL COMPONENTS RETAINED

3.1.1 Reflectance spectra with the radiometric correction

Figure II.2 shows the mean spectra of the watercress leaves analyzed ($n=14$) on each of the five tested days. Similar patterns than in the case of spinach leaves can be observed with three main features:

- A reflectance peak in the visible range, between 500 and 660 nm.
- The “red edge”, located between 680 and 740 nm.
- High relative reflectance in the infrared region.

In figure II.2, it can be observed an increment of global reflectance in the spectra along the experiment. In the case of spinach, this fact was explained due to structural changes in the leaves, which cause a decrease in the water content of the cells and an increase of air in the interstitial spaces. This fact induces an increase of scattering in the reflected light and, for that, an increase in the global reflectance of the spectra (Jacquemoud and Baret, 1990; Asner, 1998).

Figure II.2.- Left: mean spectra, corrected with RC, of the watercress leaves on each tested day. Right: detailed range of spectra on red-edge region.

Moreover, a displacement to the left of the red edge position can be clearly observed when the leaves aged. Several authors noticed that, due to the aging of the leaves and chlorophyll degradation, the red edge suffers a displacement to shorter wavelengths (Mutanga and Skidmore, 2007; Van der Meer and Jong, 2002).

In the PCA computed on the reflectance spectra with RC, none PC showed a clear relationship with the evolution of the leaves along storage period (data not shown). Therefore, in this data set, none of the loadings calculated was selected for generating a prediction model.

3.1.2 Smoothed and second derivative spectra

Figure II.3 (upper) shows the mean spectra of the watercress leaves, subjected to RC and SG algorithm, on each day. These pretreated spectra are very similar to those of spinach. When second derivative is computed on the reflectance spectra, the absorbances appear as positive peaks.

Figure II.3.- Upper: mean radiometric corrected, second derivative spectra of the watercress leaves on the tested days. Middle left: PC1 loadings from PCA applied on RC + SG corrected spectra. Middle right: scores of PC1 on mean spectra of the leaves along testing time. Lower left: PC3 loadings from PCA applied on RC + SG corrected spectra. Lower right: scores of PC3 on mean spectra of the leaves along testing time.

The patterns of the RC + SG corrected spectra could be characterized by three main features: 1) a high positive peak around 680 nm followed by a high negative peak around 740 nm, approximately. Both peaks are placed close to the red edge region of the spectra and a shift to the left can be observed on the spectra along the days of storage. The great positive peak could be due to the main absorbance peak of chlorophyll. 2) A succession of positive and negative peaks between 500 and 585 nm, approximately. These peaks are placed in the green and yellow regions of the visible spectrum and could be due to carotenoids (Gitelson et al., 2006; Table I.2) and chlorophyll (Kleima et al., 2000). A shift to the left can be also observed in the spectra of the last days in this region that could be due to a decrease in chlorophyll content that causes this “green edge” displacement (Gitelson et al., 2003). 3) A slight negative peak around 940 nm that decreases along the storage. This peak would be attributed to the water content of the leaves.

There were two principal components that seem to be related to the evolution of the leaves during time: PC1 and PC3. In the scores of both principal components, there is a clear and progressive rising trend along the period of evolution.

3.1.3 Smoothed, second derivative and SNV spectra

Figure II.4 (upper) shows the mean spectra of the watercress leaves subjected to RC, SG algorithm and SNV pretreatment. The observations that can be done seeing the shape of the spectra are similar to the previous pretreatments (RC+SG). The resulting spectra are very similar when the multiplicative effect has been corrected, although the main differences previously seen in the spectra seem to be more clear in this case. The main differences during storage can be observed in the wavelengths corresponding to the absorption bands of the different pigments (chlorophyll and carotenoids), the red edge region and the small negative peak at 940 nm.

Again, two different principal components were retained as the most related to the evolution of the leaves during time: PC1 and PC2. In both cases, the shape of the corresponding loadings is very similar to the shape of the PC1 and PC3 retained in the case before, respectively.

3.2 PRINCIPAL COMPONENTS RETAINED

The following principal components were retained as the most related with the evolution of the watercress during time:

- PC1 and PC3 from PCA applied on RC + SG corrected spectra.
- PC1 and PC2 from PCA applied on RC + SG + SNV corrected spectra.

For comparison, in order to study how specific are the loadings observed in a leafy vegetable applying them directly on other, the loadings retained in the case of spinach leaves (Section I) were recovered. These loadings were (see figure II.5):

- PC3 from PCA applied on RC corrected spectra.
- PC1 from PCA applied on RC + SG corrected spectra.
- PC1 from PCA applied on RC + SG + SNV corrected spectra.

Figure II.4.- Upper: mean RC+SG+SNV spectra of the watercress leaves on the tested days. Middle left: PC1 loading from PCA applied on RC+SG+SNV corrected spectra. Middle right: scores of PC1 on mean spectra of the leaves along testing time. Lower left: PC2 loading from PCA applied on RC+SG+SNV corrected spectra. Lower right: scores of PC2 on mean spectra of the leaves along testing time.

Figure II.5 shows the similarities between the loadings of the principal components retained for spinach spectra (RC+SG and RC+SG+SNV spectra) and some of those retained for watercress spectra (PC1 RC+SG and PC1 RC+SG+SNV). The loadings shows the same shape in both cases, with similar positive and negative peaks, in the same spectral bands.

Figure II.5.- Left: PC1 loadings from PCA applied on RC+SG corrected spectra of watercress (blue) and spinach leaves (red). Right: PC1 loadings from PCA applied on RC+SG+SNV corrected spectra of watercress (blue) and spinach leaves (red).

As was previously stated, for watercress leaves spectra, two different principal components seem to be related with the aging of the leaves in each data set (RC+SG spectra and RC+SG+SNV spectra). The shape of the loadings of each PC is quite different. Figure II.6 shows each PC loadings, from RC+SG+SNV data set, overlaid on the mean spectra of the watercress leaves of each day.

PC1 loadings are shown in Figure II.6 (up). The most relevant values are centred in the range between 675 and 750 nm, approximately, with a minor peak around 500 nm and a slight peak in the infrared region, around 940 nm. The peak around 500 nm could be attributed to one of the absorbance peaks of carotenoids (see Table I.2, Section I) (Gitelson et al., 2006). The range between 675 and 750 nm is related to one of the absorbance peaks of chlorophyll, around 680 nm, and to the red edge. It could be reflecting the displacement of the red edge position and the loss of chlorophyll typically associated with the aging of leaves (Mutanga and Skidmore, 2007; Van der Meer and Jong, 2002), that can be observed in the watercress leaves mean spectra. The peak at 940 nm is very small but coincident with the peak that appears in the NIR region of the spectra.

PC2 loadings are shown in Figure II.6 (down). The shape of these loadings is more complex than in the previous case, however, most of the interpretation carried out in that case is identical in this principal component. In the visible range of the spectra, it can be observed two different positive peaks, centred at 490 nm and 550 nm respectively, with a negative peak between them. The first peak (around 500 nm) could be due to carotenoids absorption. The second peak (550 nm) is coincident with the peak observed in the spectra in this region. It could reflect the changes in reflectance that the spectra show during time, due to changes in chlorophyll and anthocyanins contents. The spectral bands maximally sensitive to absorption of anthocyanins, according to Gitelson et al. (2006), are in the range between 540 – 560 nm, and that range can be used also for chlorophyll quantification in anthocyanins free leaves (see Table I.2, Section I). Moreover, in the visible range, there are two consecutive positive peaks more, centred at 672 and 723 nm respectively. These peaks could be reflecting the changes in chlorophyll absorption and the red edge displacement. In the NIR region, there is a peak at 940 nm, bigger than the peak of the PC1 in that wavelength, followed by a negative peak beyond 970 nm. The peak at 940 nm is also coincident with the peak of the spectra in the NIR region. The negative peak around 970 nm could be related to the absorption of water and could reflect changes in water content of the leaves along the storage.

Figure II.6.- Mean spectra, corrected with RC, of the watercress leaves on each tested day. The most relevant loadings from PCA applied on RC+SG+SNV corrected spectra of watercress leaves are overlaid. Up: PC1 loadings. Down: PC2 loadings.

PC2 loadings are more complex than PC1 loadings but both are retaining mainly the same information in the visible range of the spectra. However, in the NIR range, PC2 shows bigger peaks between 940 and 970 nm. Those peaks, mainly the negative peak at 970 nm, are not so pronounced in PC1 loadings of watercress, and less in PC1 loadings of spinach. Those peaks could reflect the more pronounced dehydration in watercress leaves than in spinach leaves along time.

3.3 DATA PROCESSING

The hyperspectral images of the watercress leaves were projected onto those seven loadings mentioned before, with the aim of obtaining virtual images of scores able to monitor the evolution of the leaves. The virtual images obtained are compared, leaf by leaf, by means of analysis of variance (ANOVA) and Wilks- λ parameter.

Scores images corresponding to each loading were considered. ANOVAs were carried out comparing the pixels of each leaf, in the first and in the last tested day. The F-Fisher values obtained are shown in table II.2, using 7 of the 14 leaves used in the test (those with more surface available). The Wilks- λ values were computed similarly, and the results are shown in table II.3.

Table II.2.- F-Fisher values obtained in each ANOVA computed on the scores images corresponding to each loading used. Pixels (n=38000) from 7 of the 14 watercress leaves are compared, each leaf independently, between the first and the last tested day. The greatest F-Fisher value obtained for each leaf is marked in bold.

Number of leaf		Ld 3 Spinach RC	Ld 1 Spinach RC+SG	Ld 1 Spinach RC+SG+SNV	Ld 1 Watercress RC+SG	Ld 3 Watercress RC+SG	Ld 1 Watercress RC+SG+SNV	Ld 2 Watercress RC+SG+SNV
1	F	136	12861	6554	9845	232397	7492	314562
	p	$2 \cdot 10^{-31}$	0	0	0	0	0	0
2	F	66	729	77	398	253351	11	276726
	p	$4 \cdot 10^{-16}$	0	$2 \cdot 10^{-18}$	0	0	$4 \cdot 10^{-26}$	0
4	F	214	5357	3384	4735	133695	3842	172918
	p	0	0	0	0	0	0	0
5	F	35650	67358	65182	65886	50081	69294	66801
	p	0	0	0	0	0	0	0
6	F	1323	13671	10175	12222	223604	11366	291248
	p	0	0	0	0	0	0	0
9	F	8048	31170	22675	28089	128528	24514	91948
	p	0	0	0	0	0	0	0
12	F	27638	60146	55996	61952	73731	60729	91005
	p	0	0	0	0	0	0	0

Table II.3.- Wilks- λ values obtained for each scores image corresponding to each loading used. Pixels (n=38000) from 7 of the 14 watercress leaves are compared, each leaf independently, between the first and the last tested day. The greatest Wilks- λ value obtained for each leaf is marked in bold.

Number of leaf	Ld 3 Spinach RC	Ld 1 Spinach RC+SG	Ld 1 Spinach RC+SG+SNV	Ld 1 Watercress RC+SG	Ld 3 Watercress RC+SG	Ld 1 Watercress RC+SG+SNV	Ld 2 Watercress RC+SG+SNV
1	0.002	0.169	0.086	0.129	3.058	0.099	4.139
2	0.001	0.010	0.001	0.001	3.337	0.001	3.641
4	0.003	0.070	0.045	0.062	1.759	0.051	2.275
5	0.469	0.886	0.858	0.867	0.659	0.891	0.879
6	0.017	0.180	0.134	0.161	2.941	0.150	3.832
9	0.106	0.410	0.298	0.370	1.691	0.323	1.210
12	0.364	0.791	0.737	0.815	0.971	0.799	1.197

According to the F-Fisher values obtained for each leaf applying each model, it can be seen that there are significant differences between the leaves in the first and the last tested day in all cases. All models seem to detect the evolution of the leaves during the time. However, the model based on loading 2 from the spectral data set of watercress leaves, corrected with RC + SG + SNV, is the most sensitive detecting the evolution of the leaves. There are only two leaves in which other models show greater F-Fisher values and, in those cases, the difference is quiet small. Results for PC1 RC+SG of spinach and

watercress are quite similar. The same occurs with results for PC1 RC+SG+SNV in both leafy vegetables. As shown before, figure II.5, the loadings of these PCs were almost identical so, these results verify such observation.

Wilks- λ values are in most of the cases lower than one so, this indicates that the internal variance of the scores in the leaves is very high in comparison to the variance due to evolution. However, loading 2 RC+SG+SNV of watercress leaves shows values higher than one, so that PC could be able to detect the evolution of the leaves.

When the spinach loadings are used directly on the watercress leaves, the results obtained are modest. There are significant differences between first and last tested days in those cases, but not as strong as using some of the own loadings obtained from the spectra of watercress leaves. The differences between spinach and watercress loadings mentioned before, mainly the differences in NIR region, could justify why spinach loadings does not work as good as PC2 loadings of watercress. Watercress leaves suffered a more pronounced dehydration process than spinach leaves along the storage and that effect is not so reflected in spinach loadings. PCs of watercress have been also tested in spinach spectra with poor results (not shown). As shown, there are slight spectral differences on the behaviour of each leafy vegetable. Lee et al. (2014) tried to develop a common model for detecting bovine faeces on both Romaine lettuce and baby spinach leaves. Correlation analysis was used to select the most significant waveband pairs for two-band ratio and difference methods in distinguishing contaminated and uncontaminated leaf areas. For that investigation, two-band ratios using bands at 665.6 nm and 680.0 nm (F665.6/F680.0) for lettuce and at 660.8 nm and 680.0 nm (F660.8/F680.0) for spinach effectively differentiated all contamination spots applied to the lettuce and spinach leaves, respectively. Both ratios used almost the same spectral bands, with slight but relevant differences.

Figure II.7 shows the virtual images of scores obtained by projection of the hyperspectral images of the watercress leaves (correspondingly corrected) onto the best principal component able to detect the evolution of leaves along the storage: PC2 from the spectra of watercress leaves corrected with RC+SG+SNV. The fourteen leaves are shown in the first and the last days of the test. All the leaves evolve during time but with different rates. Some of the leaves suffered changes in shape during storage, becoming smaller and with curved edges, reducing the available surface for acquiring the spectra.

Figure II.7.- Scores images obtained by projection of the hyperspectral images of the leaves, correspondingly corrected, on PC2 from the spectra of watercress leaves corrected with RC+SG+SNV. It is shown the 14 watercress leaves studied, the number of each leaf is marked in red. Upper row: first date. Lower row: last date.

As shown in tables II.2 and II.3, the F-Fisher and Wilks- λ values obtained for each leaf are very variable. This demonstrates, as in the case of spinach, that each leaf ages at different speed. There are leaves evolving faster than others, depending also in the initial stage of freshness (figure II.8).

Figure II.8.- Scores images obtained by projection of the hyperspectral images of the leaves, correspondingly corrected, on PC2 from the spectra of watercress leaves corrected with RC+SG+SNV. It is shown two different watercress leaves along the five testing days.

4. CONCLUSIONS

The procedure developed for monitoring the evolution of spinach leaves through transparent plastic films presented at Section I has been validated in a different leafy vegetable: watercress leaves.

Such as in the work carried out in spinach leaves, the effect of the variation of the plastic film transmittance has been removed applying the radiometric correction.

Four new principal components, computed from a set of spectra of watercress leaves, have been added to the PCs retained in the case of spinach as the most related to the evolution of the leaves along the storage. After projection of the hyperspectral images of watercress leaves on each PC, the virtual images obtained in each case have been compared by using ANOVA and Wilks- λ statistics, in order to determine which PC showed the greatest differences between the pixels of the leaves at the first and the last tested day.

The PCs calculated in the case of spinach leaves can be used in watercress leaves for detecting their evolution, showing significant differences between the leaves at the first and the last day, however, the results are modest. Applying the PCs calculated using the spectra of watercress leaves, the results obtained are better, showing greater differences between the pixels of the leaves in the different stages of evolution.

The loadings retained as the most related to the evolution of the watercress leaves, and those retained in the case of spinach, show the main information in the same spectral bands, with sharpened peaks in the range of 500-550 nm, due to carotenoids, anthocyanins and chlorophylls; in the band of 680 nm, due to chlorophyll; and in the range between 700 and 760 nm, due to the red edge of the leaves.

However, in the case of watercress, the most interesting loading shows also information in the NIR region, between 940 and 970 nm. This fact can be the reason why, in this case, other PCs different to those retained in the case of spinach offer better results.

Thus, it seems to be necessary to analyse the spectra of each leafy vegetable species independently, in order to obtain the most satisfying results for monitoring its shelf-life using hyperspectral imaging. Although each leafy vegetable, spinach and watercress, seems to have different behaviour during its shelf-life, there are some common changes that allow to obtain prediction models very similar, able to monitor the evolution of the leaves in both cases. This fact can be relevant for monitoring the shelf-life of mixed salads and ready-to-eat leafy vegetables prepared with different leaves together.

SECTION III

HYPERSPECTRAL IMAGING TO EVALUATE THE EFFECT OF IRRIGATION WATER SALINITY ON LETTUCE

HYPERSPECTRAL IMAGING TO EVALUATE THE EFFECT OF IRRIGATION WATER SALINITY ON LETTUCE

ABSTRACT

Salinity is one of the most important stress factors in crop production, particularly in arid regions. This research focuses on the effect of salinity on the growth of lettuce plants; three solutions with different levels of salinity were considered and compared (S1=50, S2=100 and S3=150 mM of NaCl) with a control solution (Ct=0 mM NaCl). The osmotic potential and water content of the leaves were measured, and hyperspectral images of the surfaces of 40 leaves (10 leaves per treatment) were taken after two weeks of growth. The mean spectra of the leaves were pre-processed by means of a Savitzky-Golay algorithm and standard normal variate normalization. Principal component analysis was then performed on the calibration set (n=28 leaves), yielding an initial model for salinity effect detection. A second model was subsequently proposed based on an index computing an approximation to the second derivative at the red edge region. Both models were applied to the hyperspectral images of the calibration (n=28) and validation sets (n=12) to obtain the corresponding artificial images. Those virtual images were studied using analysis of variance in order to compare their ability for detecting salinity effects on the leaves. Both models showed significant differences between each salinity level, and the hyperspectral images allowed observations of the distribution of the salinity effects on the leaf surfaces, which were more intense in the areas distant from the veins. However, the index-based model was simpler and easier to apply because it was based solely on the reflectance at three different wavelengths, thus allowing for the implementation of less expensive multispectral devices.

Keywords: Image analysis, hyperspectral, salinity, lettuce, non-destructive assessment.

1. INTRODUCTION

Soil and irrigation salinity are key factors in the growth of most vegetables and can cause many problems in agriculture, particularly in arid and semi-arid regions (Lamsal et al., 1999; Shannon et al., 1994). Industrial development and population growth have increased the contamination and salinization of surface and underground water and thus of agricultural soils. Saline soils cover a significant area in the southeast of Spain—a major region of horticultural crops. Lettuce (*Lactuca sativa* L.) is one of the most important crops in this region, the major producer area in the European Union. This production is primarily employed for the fresh consumption of a ready-to-eat product. Acosta et al. (2011) reported some peaks value of 6.4 dS/m electrical conductivity, which corresponded with 50 mM. This high EC observed in the soils in the SE area of Spain is mainly the result of poor quality irrigation water used in agriculture.

Lettuce is moderately sensitive to salinity; when the electrical conductivity (EC) is greater than 1.3 dS/m, growth is affected, decreasing its yield by 13% for each unit of EC above that level (Ünlükara et al., 2008).

Salinity primarily affects crop growth in two ways: a) by increasing the osmotic potential of the soil, making water less available to plants, and b) by creating excessive concentrations of specific elements (Yamaguchi and Blumwald, 2005). Herbaceous crops affected by salinity at moderate levels do not show visible damage in their leaves and appear to be normal, although their growth and yield decrease. They may have deep green and more succulent leaves with more density and thickness (Maas and Hoffman, 1977). However, when excess salinity induces imbalances in concentrations of certain mineral elements, such as Cl, Na, B or Ca, necrosis, chlorosis and tip burn can appear in the leaves (Bie et al., 2004; Eraslan et al., 2007; Carassay et al., 2012).

Hyperspectral imaging has been widely employed to detect salinity levels in soils and canopies through remote sensing and the development of numerous indexes for estimating saline concentrations in relation to reflectance at different wavelengths (Poss et al., 2006; Hamzeh et al., 2013). This remote sensing technique has been applied to many different crops and plants, including sugarcane (Hamzeh et al., 2013), cotton, corn, cogon grass, reeds, saltcedar, suaeda, and aeluropus (Zhang et al., 2011). In addition, there are numerous remote sensing studies at the laboratory level that have observed the effects of water stress (Tucker, 1980; Harris et al., 2006; Clevers et al., 2010) and nutrient deficiencies (Al-Abbas et al., 1974; Masoni et al., 1996; Pacumbaba and Beyl, 2011). However, there are not many studies at the laboratory level that have observed changes in the optic behaviour of leaf tissues due to increased saline concentrations. This work applies hyperspectral imaging techniques as a non-destructive procedure to identify the influence of saline stress on newly harvested “baby” lettuce in order to detect any distribution of the effects on the surface of the leaves.

The first part of this paper explains the materials and methods employed to obtain the hyperspectral images and other analytical measurements; it also outlines the data and processed hyperspectral images used to obtain and evaluate the models and identify the effects of different saline concentrations on the leaves. The second part of the paper describes the obtained results and the applied models while validating the different leaf samples with an extensive discussion.

2. MATERIALS AND METHODS

2.1 MATERIALS AND ANALYTICAL MEASUREMENTS

The lettuce plants (*Lactuca sativa* L. var *capitata* variety ‘Tempo neutro’) were grown from seeds. The seeds were placed into polystyrene cylinders that were positioned through holes in the plastic lid of containers with Hoagland’s nutrient solution inside, thus enabling the roots to be in contact with the nutrient solution. The composition of the nutrient solution was 7 mM K⁺, 4 mM Ca⁺², 14 mM NO₃⁻, 1 mM Mg⁺², 1 mM PO₄⁻², 1 mM SO₄⁻², 20 µM Fe⁺², 2.5 µM B⁺³, 2 µM Mn⁺², 2 µM Zn⁺², 0.5 µM Cu⁺² and 0.5 µM Mo⁺⁶; it was maintained between pH levels of 5.5 and 6.5 through routine replacement of the hydroponic solution. After 14 days, plants with similar development were selected and placed in containers (five plants per container) using 15 plants per treatment. Four salt treatments were then applied—a control treatment without NaCl (Ct) and three levels of salt through the addition of different

concentrations of NaCl to the nutrient solution: S1 (50 mM NaCl), S2 (100 mM NaCl) and S3 (150 mM NaCl). The application of salt treatments was conducted for a total of 10 days. The average electrical conductivities (EC) of the nutrient solution during the experiments were 1.23 dS/m, 6.09 dS/m, 10.77 dS/m and 14.95 dS/m, for Ct, S1, S2 and S3, respectively. The experiments were performed in a controlled growth chamber with a temperature (T) of 23 °C/18 °C (day/night), a photoperiod of 16 h/8h (light/darkness) and a photosynthetically active radiation of 400 $\mu\text{mol}\cdot\text{m}^{-2}/\text{s}$. The leaves were harvested at the end of the experiments and a sample of 10 leaves per treatment, with similar size, was selected for the acquisition of hyperspectral images. Figure III.1 shows, as an example, RGB images of one leaf per treatment.

Figure III.1.- RGB image of one leaf per saline treatment. Apparently, no visible symptoms of stress can be observed.

After image acquisition, the water content and osmotic potential were determined for the harvested leaves. The leaf water contents were analysed following the method described by Agüero et al. (2011) with modifications. Twenty grams of leaf pieces per treatment were taken, homogenized with a commercial grinder, and divided into three samples of five grams per treatment. The samples were dried in an oven (Thermocenter T C40/100, Salvis Lab, Rotkreuz, Switzerland), at 65 °C until achieving a constant weight. The water content (WC) was calculated as:

$$\text{Eq. III.1} \quad \% \text{ WC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

For the calculation of the osmotic potential (ψ_s), three 5-g samples of leaf pieces per treatment were collected and frozen at -20 °C. The samples were centrifuged at 2800 g (9.8 m/s^2) for 15 minutes (Centronic centrifuge, J.P. Selecta, Barcelona, Spain). The supernatant was analysed with a micro-

osmometer (Roebeling 13DR, Löser Messtechnik, Berlin, Germany) to determine osmolarity. The osmotic potential (ψ_s) was calculated with the Van't Hoff equation (1887):

$$\text{Eq. III.2} \quad \psi_s = -R \times T \times c_s$$

Where R is the ideal gas constant ($\text{m}^3 \cdot \text{Pa} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$), T is the temperature (K) and c_s is the osmolarity ($\text{Osm} \cdot \text{m}^{-3}$).

2.2 HYPERSPECTRAL IMAGES

Hyperspectral images from the adaxial surfaces of 40 selected leaves were taken with a hyperspectral vision system, consisting of an EMCCD Luca-R camera (AndorTM Technology, Northern Ireland) coupled to a VIS-NIR spectrometer (Headwall Photonics Hyperspec-VNIRTM) working in the range of 400–1000 nm. A total of 189 wavelengths were considered along this range, thus obtaining a spectral resolution of 3.2 nm. The spectrometer of the camera was equipped with a progressive line-by-line scan spectrograph with a slit of 25 μm . The spatial resolutions in the line direction and in the scan direction were 0.26 mm/pixel and 0.1 mm/pixel, respectively. A halogen lamp was used for illumination. Headwall HyperspecTM software was used to control the equipment. Samples were scanned by acquiring the entire surface of the leaf (scan length = 100 mm). A hypercube dataset was obtained from each image. Relative reflectance hyperspectral images were simultaneously computed by the software of the camera. White reference (barium sulphate) and dark current signal (acquired with the objective of the camera covered by a black tap) were acquired before each batch of images. Each line of the image was then corrected pixel by pixel by subtracting the dark current and dividing the result by the white reference minus the dark current.

2.2.1 Data processing and computation of models

The hyperspectral images of lettuce leaves were randomly divided in two different groups: a calibration set with seven leaves per treatment (28 leaves in total) for generating the models and a validation set with the three remaining leaves of each treatment (12 leaves in total) for testing the models.

For each hyperspectral image, 32.000 spectra from the green surface of the leaves, avoiding manually the mid-rib, were selected and the mean spectrum of each leaf was calculated. For the calibration set with 28 mean spectra, two different spectra pre-treatments were applied: a second derivative with regard to wavelength by means of a Savitzky-Golay algorithm (SG) (Savitzky and Golay, 1964) using a width of 21 wavelengths, a polynomial order of 3 and a second derivative; and a normalization by means of a Standard Normal Variate algorithm (Barnes et al., 1989) (SNV).

For the set of pre-treated spectra, a principal component analysis (PCA) was performed in order to obtain an initial model. The hyperspectral images projected onto the principal components produced the corresponding artificial images of scores.

The first derivative of the leaves mean spectra were also computed by means of the Savitzky-Golay algorithm (width of 21 wavelengths, polynomial order 3, first derivative) in order to analyse the lateral movements in the red edge area (Richardson et al., 2002, Li et al., 2010). As a result of these analyses and the loadings obtained in the PCA, an index that computes an approximation to the second derivative at the red edge region was proposed. This index was applied to the hyperspectral images of the leaves from the calibration set, thus obtaining the corresponding virtual images.

In order to evaluate the ability of the proposed index in comparisons to others previously applied, a collection of indexes described in the literature were computed on a representative group of spectra of the calibration set (7000 spectra per treatment). Some of them are specifically related to salinity and others related to water, pigments and chlorophyll content under stress conditions. One-way ANOVA's and Tukey's least significant difference procedure were carried out for each index comparing the salinity treatments. Also scores resulting from PCA of pre-treated spectra was included in such comparison.

Both models (SG + SNV model and red edge index model) were applied to the leaves of the calibration and validation sets to obtain virtual images. A segmentation procedure was applied in the images in order to discard the pixels of nerves. Firstly, artificial images were computed based on the combination of planes 730, 680 and 550 nm, where the main differences between nerve and green area was observed; then the Otsu method was applied to these images to obtain the mask that separated the green areas and the nerves. And finally these masks were applied onto the scores and index images.

2.2.2 Virtual images analysis

The relative histograms (256 intensity levels) of the virtual images of the red edge index in the calibration set, were considered. Further, a non-supervised classification method according to Ward (Ward, 1963), was applied to the relative histograms. Ward's classification method was applied by computing the matrix of Euclidean distances between each pair of individuals (histograms), grouping the closest individuals, and hierarchically merging groups (or individuals) whose combination gave the least Ward Linkage Distance, that is, the minimum increase of the within sum of squares of the new-formed group (Herrero-Langreo et al, 2011). The within-group sum of squares of a cluster is computed as the sum of the squares of the distance between all objects in the cluster and the centroid of the cluster.

Ward Linkage Distance (D_{ward}) between two groups a and b is mathematically described as:

$$\text{Eq. III.3} \quad D_{ward}(a,b) = SS_{ab} - [SS_a + SS_b]$$

Where SS is the sum of squares within group a (SS_a), group b (SS_b), or both groups merged (SS_{ab}).

Ward's non supervised classification of the histograms was tested using the relative histograms of the validation set leaves. These histograms were assigned to the different clusters generated by Ward's classification calculating the Euclidean distance between the relative histogram of each leaf and the representative histogram of each established cluster. The minimum Euclidean distance obtained for each histogram determined the cluster to which each leaf was assigned.

3. RESULTS AND DISCUSSION

3.1 EFFECT OF SALINITY ON WATER CONTENT AND OSMOTIC POTENTIAL

The water content and osmotic potential data are shown in Table III.1. The water content of the leaves decreases when saline concentration increases from 100 mM NaCl to 150 mM NaCl, while similar values are obtained in the control and in the samples subjected to 50 mM NaCl. The same trend can be observed with the osmotic potential of the leaves. This fact suggests that lettuce plants growing in saline conditions have more difficulty in acquiring water from the soil and maintain less water in their tissues, which concurs with previous research (Eraslan et al., 2007).

Table III.1.- Water content and osmotic potential of the lettuce leaves treated without NaCl (Ct) and treated with 50, 100 and 150 mM NaCl (S1, S2 and S3, respectively).

Saline Treatment	Water content (%). Mean $\pm$ STD	Osmotic potential (MPa). Mean $\pm$ STD
Ct	94.53 $\pm$ 0.09	-0.65 $\pm$ 0.04
S1	94.54 $\pm$ 0.12	-1.08 $\pm$ 0.09
S2	93.15 $\pm$ 0.18	-1.49 $\pm$ 0.11
S3	92.70 $\pm$ 0.07	-2.06 $\pm$ 0.09

3.2 EFFECT OF SALINITY ON SPECTRAL FEATURES

The mean spectrum of each saline treatment was computed (calibration set, $n = 7$ leaves per treatment). Figure III.2A shows the mean spectra of each saline treatment. It can be seen that the Ct global reflectance is lower than the others, primarily in the infrared range. This fact can be related to certain physical properties inducing lower scattering in the leaves of the Ct leaf samples.

Figure III.2.- A) Mean relative reflectance spectra of the leaves of each saline treatment. Calibration set, $n = 28$ leaves (4 saline treatments $\times$ 7 leaves). B) same spectra corrected with SNV normalization to eliminate multiplicative effect. C) zoom in the visible range of SNV corrected spectra. See Table 1 for abbreviations.

Crops often have a higher NIR reflectance level under water stress conditions (Tucker, 1980; Clevers et al., 2010; Harris et al., 2006) due to air replacing water between the cells and producing optical discontinuities and scattering. Blum (1988) noted that crops under salt stress conditions typically show the same symptoms as under water stress conditions. Salinity affects the osmotic potential of the plants and the water availability of the soil; as such, the water absorption capacity is affected (Yamaguchi and Blumwald, 2005). As shown in Table 1, the osmotic potential and water content in the lettuce leaves decreases when the salinity increases, mainly in S2 and S3 treatments. This fact could induce water stress in the plants that could justify the higher NIR reflectance observed in the leaves of those treatments.

Other effects observed in the crops under saline stress include the loss of growth and turgor, as well as the destruction of cellular membranes (Leidi and Pardo, 2002). Moreover, an accumulation of salt in the leaves can alter the refractive index of the water. According to Gitelson et al. (2003), the increase in NIR reflectance could be caused by an increase in the thickness and density of the leaves. This fact has been observed in leaves under moderate salinity levels (Maas and Hoffman, 1977). All of these described effects can affect the scattering process, and this scattering can induce a multiplicative effect on the spectra in the NIR region. This multiplicative effect has been removed applying SNV.

Figure III.2B shows the mean spectra of the leaves in each saline treatment corrected with SNV normalization in order to remove the multiplicative effect. Differences among each spectrum can be observed in two areas: 1) the peak near 550 nm and 2) the valleys near 480 and 680 nm.

The visible region of the spectra results from the absorbency of the different pigments of the leaves, primarily chlorophyll but also carotenoids and flavonoids. The reflectance peak near 550 nm is due to the significant absorption of chlorophyll in the blue and red regions and the reflection in the green region. The height of the peak of each spectrum can be compared by computing the difference between the reflectances at 550 and 510 nm. A progressive decrease in the height of the peak was observed from the Ct to the S3 treatments as the salinity increased. Many authors have related the changes in the visible region of the spectra to the decrease of pigment content in the leaves, primarily chlorophyll (Knippling, 1970; Al-Abbas et al., 1974; Gitelson et al., 2003). When the chlorophyll content decreases, an increase of reflectance in the visible region of the spectra generally occurs due to the decrease in absorbance in the blue and red regions. This causes the spectra to flatten, resulting in a decrease in the height of the peak near 550 nm. Some research indicates that the total chlorophyll and carotenoid content decreases in plant leaves under salt stress (Parida and Das, 2005). Gitelson et al. (2002) introduced a carotenoid reflectance index (CRI_{550}), based on the reflectances at 510 and 550 nm, to calculate the carotenoid content in the leaves. Applying this index to these spectra, a decrease in the carotenoid content was observed as the salinity increased (Ct=0.78; S1=0.74; S2=0.65; S3=0.55).

$$\text{Eq. III.4} \quad CRI_{550} = (1/R_{510}) - (1/R_{550})$$

Chlorophyll absorption peaks were observed in the 480 and 680 nm bands, along with a progressive increase of reflectance with increasing salt concentrations. This could indicate a slight loss of chlorophyll in the leaves when the salinity increased.

Differing views have been reported regarding the observed changes in visible range with increased salinity. Some studies related to vegetative growth under salinity conditions showed a decrease in the peak at 550 nm and in the visible region with increased salinity in the plants or soil (Hamzeh et al., 2013; Zhang et al., 2011), which is consistent with our findings. However, other studies showed either an increase in reflectance in the visible region, primarily near 550 nm (Li et al., 2010; Peñuelas et al., 1997a), or no differences in the visible range under salt stress conditions (Wang et al., 2002b).

3.3 SPECTRAL PREPROCESSING

3.3.1 First derivative of the spectra

Figure III.3A shows the first derivative mean spectra of each saline treatment, where the maximum value corresponds with the red edge. A magnification of the red edge region can be seen in Figure III.3B.

A displacement to the right in the peak, though slight and progressive, can be observed in the S3 treatment (Figure III.3B). However, numerous authors found that a displacement to the left of the red edge typically occurs when plants suffer environmental conditions of stress (water, nutritive, or salt stress) (Gitelson et al., 2003; Mariotti et al., 1996; Masoni et al., 1996). This displacement to the left is typically related to the loss of activity and the degradation of chlorophyll (Ustin et al., 2009). Lara et al. (2013) confirmed this fact in spinach leaves during shelf life and attributed it to aging processes and the loss of cell structure and chlorophyll. However, the effect of aging in harvested leaves over time is not similar to the effect of saline stress in freshly harvested leaves.

Figure III.3.- A) first derivative mean spectra of the leaves of each saline treatment. Calibration set, n= 28 leaves (4 saline treatments x 7 leaves). B) zoom on the red edge. See Table 1 for abbreviations.

Li et al. (2010) performed a test with castor bean seedlings under salinity conditions of 0, 100, 200, and 300 mM of NaCl. They observed a displacement to the right of the red edge at 100 mM NaCl and displacements towards the left edge for higher concentrations. This salt concentration (100 mM) is in the range of the highest concentrations of the present research. The salinity may initially cause a displacement of the red edge to the right until reaching a certain level of salt concentration, subsequently shifting to the left when the salinity stress increases; this concurs with Li et al. (2010).

3.3.2 Second derivative and SNV normalization. Principal Component Analysis

Figure III.4A shows the 28 mean pre-treated spectra (SG+SNV) corresponding to the seven leaves from the calibration set for each salt treatment. Because the pre-treated spectra compute the second derivative, they derive a type of 'curvature' of the raw spectra. Peaks at 550 nm and 510 nm of the pre-treated spectra decrease with the salinity level, so the curvature of the spectra decrease with increased salinity. This concurs with the previously observed flattening process of the spectra as the salinity level increases.

The first principal component (PC1) from the PCA, explaining a 94.46% of variance, was retained as the most related to the changes caused by the salt concentration in the leaves. The PC1 loadings are shown in Figure III.4B. The projections of the mean spectra for the PC1 (scores) for each of the four salinity groups are presented in Figure III.4C.

Two regions of high loading values can be observed (Figure III.4B), corresponding to the major regions with differences in the pre-treated spectra (Figure III.4A): one is located at approximately 500-600 nm and the other occurs at the red edge region. The remaining wavelengths have loading values close to zero.

PC1 computes the slope between 550 and 510 nm; as the salinity level increases, the slope (that is negative in this range) decreases. This concurs with the evolution of the scores. In addition, the pre-treated spectra show a displacement to the right at the red edge region. PC1 also computes the curvature of the pre-treated spectra at 710 nm. When the pre-treated spectra move towards the right, the curvature increases and the scores increase.

Scores progressively evolve towards higher values with increasing levels of salinity. Therefore, both previously mentioned regions could be related to the salt concentration of the leaves. These observations concur with the changes observed in the spectra.

Figure III.4.- A) mean SG+SNV pre-treated spectra of the leaves of calibration set for each saline treatment. B) PC1 loadings from PCA applied on second derivative and SNV processed spectra. C) PC1 scores of mean spectra of the leaves of calibration set for each saline treatment (Ct, S1, S2 and S3 treatments, x axis), mean values of each group are shown in red.

Figure III.5 shows the images of scores obtained from the projection of the hyperspectral images of the leaves from the calibration set, corrected with SG and SNV, on the PC1. The differences between Ct and S3 leaves are clear, evolving from deep blue pixels (low score value) to orange and red pixels (high score value) when the salinity concentration increases.

Figure III.5.- Comparison of scores obtained by projection of the hyperspectral images from calibration set, corrected with Savitzky-Golay algorithm and SNV, on PC1.

3.3.3 Index on the red edge region

As previously explained, a slight displacement to the right of the red edge region was observed on the first derivative of the spectra, which could be related to the moderate increase in the salt concentration of the leaves. The PC1 loading shows the red edge region as the most sensitive area to changes in the spectra induced by salinity. Consequently, an index (LSI) based on the most relevant wavelengths of PC1 loading (Figure III.4B) is proposed.

This LSI index is an approximation to the second derivative at 710 nm and it is a value related to the red edge displacements (Lara et al. 2013):

$$\text{Eq. III.5} \quad \text{LSI} = [(R_{675} + R_{745}) / 2] - R_{710}$$

The LSI was applied to each pixel of the hyperspectral images of the calibration set leaves in order to generate corresponding virtual images of this second model and predict the salinity effects on the leaves (Figure III.6).

Figure III.6.- Comparison of scores obtained computing LSI index on the hyperspectral images of the calibration set leaves.

Clear differences between the LSI values from the pixels of the leaves corresponding to the control treatment (Ct) and the pixels of the leaves corresponding to the highest salinity treatment (S3) were observed (Figure III.6). The increase in the LSI values noted in the images responds to a lateral displacement of the red edge to the right (Lara et al., 2013; Diezma et al. 2013), which concurs with the findings obtained from the first derivative spectra analysis.

3.3.4 LSI and other spectral indexes

Table III.2 presents eleven narrow band indexes that are related to pigment, water content and salinity effects, all of them extracted from the bibliography.

Table III.3 presents the results of the one-way ANOVA's and Tukey-Kramer tests applied on these indexes, LSI and PCA scores, considering as grouping variable the salinity treatment. LSI ($F=3440.2$), GIR ($F=1340.2$), $NDVI_{green}$ ($F=1160.7$), $Chl_{red\ edge}$ ($F=1024.1$), $NDVI_{705}$ ($F=874.2$), Chl_{green} ($F=737.2$), $NDVI_{far\ red}$ ($F=702.8$) and RPI ($F=686.4$) showed consistent trends and all of them were able to distinguish the four groups of salinity treatments. Among these indexes the three with the highest segregation ability had been specifically defined for sensing salinity effects (LSI, GIR and $NDVI_{green}$). However, SRVI ($F=119.7$), also for salinity detection, together with NDVI ($F=137.3$), PSRI ($F=119.8$) and WI ($F=53.4$) did not give consistent trend, neither clear distinction between salinity classes. The scores of PC1 showed similar segregating ability than LSI ($F=2845.88$).

Although seven of the eleven indexes reviewed from literature, showed good performance for salinity effect detection, the LSI index, specifically defined for images of lettuces acquired under laboratory conditions, exhibited the best behaviour, with the highest F-value (Table III.3).

Table III.2.- Indexes used for comparison with LSI index.

Index	Formula
NDVI, related to chlorophyll content (Tucker, 1979)	$NDVI = (R_{800} - R_{670}) / (R_{800} + R_{670})$
$NDVI_{705}$, related to chlorophyll content (Gitelson & Merzlyak, 1994)	$NDVI_{705} = (R_{750} - R_{705}) / (R_{750} + R_{705})$
Red Edge Position Index, related to chlorophyll content (Gitelson et al., 1996)	$RPI = (R_{750} / R_{700})$
Related to chlorophyll content in anthocyanin free leaves (Gitelson et al. 2006)	$Chl_{green} = (R_{760} / R_{550}) - 1$ $Chl_{red\ edge} = (R_{760} / R_{705}) - 1$
Plant Senescence Reflectance Index (Merzlyak et al., 1999)	$PSRI = (R_{680} - R_{500}) / (R_{750})$
Related to water content (Peñuelas et al., 1997b)	$WI = (R_{900} / R_{970})$
Green region NDVI, related to salinity (Poss et al., 2006)	$NDVI_{green} = (R_{550} - R_{670}) / (R_{550} + R_{670})$
Far red region NDVI, related to salinity (Poss et al., 2006)	$NDVI_{far\ red} = (R_{710} - R_{670}) / (R_{710} + R_{670})$
Simple Ratio Vegetation Index, related to salinity (Wang et al., 2002b)	$SRVI = (R_{830} / R_{660})$
Green and Indigo Ratio, related to salinity (Rud et al., 2011)	$GIR = (R_{436} / R_{554})$

3.4 VALIDATION OF THE MODELS TO EVALUATE SALINITY EFFECTS

3.4.1 *Application of the models on the validation set*

In order to test the performance of the prediction models generated on the calibration set, both models (SG+SNV and LSI index) were applied to the hyperspectral images of the leaves of the validation set, thus obtaining corresponding virtual images for each case. Differences between the leaves corresponding to the Ct treatment (Figure III.7A) and the S3 treatment (Figure III.7B) were observed in both models. It can be seen that the virtual images of both models for the same leaf showed a similar distribution of pixel values. Both types of virtual images were able to sense the effect of salinity in a similar way. However, the LSI-based model was simpler and faster to apply because it is based solely on the reflectance at three different wavelengths.

As seen in the images, the pixels from the external parts of the leaves are the most affected by the salinity. The salinity effect is more intense in the areas furthest away from the veins of the leaves. In an intact plant, water is transported to the leaves through the xylem of vascular bundles that branch into a fine network of nerves through the leaf (Holbrook, 2006). Salinity restricts water uptake and transpiration and thus reduces water and nutrient uptake and transport to young leaves (Lazof and Bernstein, 1999). This limitation in the xylem transport due to the effect of salinity could explain why the most affected areas of the leaves correspond to areas with thin venation (leaf margin).

Marginal necrosis is a typical foliar symptom in plants suffering from salinity stress (Winsor and Adams, 1987; Poss et al., 1999). In lettuce, tip burn is a physiological disorder displayed as necrosis in the margins of young developing leaves and is commonly observed under saline conditions (Saure, 1998; Carassay et al. 2012). In lettuce, these symptoms have recently been associated with local oxidative stress in leaf margins that appear before symptom expression (Carassay et al., 2012). This pattern is coincident with the distribution of the most affected pixels by salinity in our images. Because of this, hyperspectral images could be used to detect symptoms of salt stress in leaves before visible damage expression and thus avoid significant economic losses in crop production.

Table III.3.- Analysis of variance (F-Fisher and p-value) comparing the values of indexes for the four salinity classes (n=7000 spectra per class belonging to the calibration set of leaves). Mean values and standard error of the different indexes per salinity class. Means followed by the same letter are not significantly different by Tukey-Kramer test; different letters implies there is significant difference between means.

Index	F-Fisher	p-value		Mean	Standard error		Index	F-Fisher	p-value		Mean	Standard error	
LSI	3440.2	0	Ct	0.01765	0.00045	a	WI	53.4	$2.03 \cdot 10^{-34}$	Ct	1.28100	0.00036	a
			S1	0.03130	0.00045	b				S1	1.27616	0.00036	b
			S2	0.05175	0.00045	c				S2	1.27872	0.00036	c
			S3	0.07829	0.00045	d				S3	1.28220	0.00036	d
NDVI	137.3	$2.45 \cdot 10^{-88}$	Ct	0.82469	0.00080	a	NDVI _{green}	1160.7	0	Ct	0.53842	0.00108	a
			S1	0.80969	0.00080	b				S1	0.50228	0.00108	b
			S2	0.80345	0.00080	c				S2	0.47366	0.00108	c
			S3	0.80714	0.00080	d				S3	0.45415	0.00108	d
NDVI ₇₀₅	874.2	0	Ct	0.45693	0.00076	a	NDVI _{far red}	702.8	0	Ct	0.68830	0.00102	a
			S1	0.46466	0.00076	b				S1	0.65912	0.00102	b
			S2	0.47856	0.00076	c				S2	0.63788	0.00102	c
			S3	0.50774	0.00076	d				S3	0.62661	0.00102	d
RPI	686.4	0	Ct	3.48135	0.00864	a	SRVI	119.7	$5.35 \cdot 10^{-77}$	Ct	10.53830	0.04345	a
			S1	3.54853	0.00864	b				S1	9.64646	0.04345	b
			S2	3.64758	0.00864	c				S2	9.46616	0.04345	c
			S3	3.99075	0.00864	d				S3	10.04068	0.04345	d
ChI _{green}	737.2	0	Ct	2.26632	0.00731	a	GIR	1340.2	0	Ct	0.35211	0.00100	a
			S1	2.28408	0.00731	a				S1	0.38783	0.00100	b
			S2	2.39855	0.00731	b				S2	0.41381	0.00100	c
			S3	2.69576	0.00731	c				S3	0.43755	0.00100	d
ChI _{red edge}	1024.1	0	Ct	1.78446	0.00583	a	Scores PC1	2845.88	0	Ct	-1.0902	0.000227	a
			S1	1.84552	0.00583	b				S1	-0.6474	0.000228	b
			S2	1.94359	0.00583	c				S2	0.0851	0.000199	c
			S3	2.20687	0.00583	d				S3	1.1235	0.000212	d
PSRI	119.8	$4.38 \cdot 10^{-77}$	Ct	-0.00536	0.00013	a							
			S1	-0.00706	0.00013	b							
			S2	-0.00552	0.00013	a							
			S3	-0.00366	0.00013	c

Figure III.7.- Virtual images obtained with both models calculated on the leaves of the validation set. Left: PC1 model. Right: LSI model. From top to bottom: Ct, S1, S2 and S3 treatments, respectively.

3.4.2 Average histograms of the leaves

The average relative histogram of the LSI images for each salinity treatment is showed in Figure III.8, on the left for calibration set and on the right for validation sets.

Figure III.8.- Average histograms from the leaves of each saline treatment, applying model based on index LSI. Left: calibration set. Right: validation set.

The histograms show a progressive displacement to the right, related to the increase in salt concentration of each treatment. Ct and S1 treatments seem to be very similar. The main differences appear in the treatments S2 and S3, in accordance to the analysis of variance computed on the LSI index of the mean spectra.

3.4.3 Non supervised classification according to Ward's method

Applying Ward's classification on the histograms of the leaves from calibration set, the corresponding dendrogram was obtained. For a linkage distance of 0.8, two clusters of leaves were grouped (Figure III.9, left). Figure III.9 (right) shows the average histograms of each cluster.

Figure III.9.- Left: dendrogram of Ward classification. Horizontal red line indicates the considered linkage distance. Right: average histograms of two clusters considered in the Ward classification. Leaves from calibration set.

Further, the leaves included in each cluster were examined. Cluster 1 grouped all the leaves of Ct treatment, six of the seven leaves of S1 treatment and two leaves of S2 treatment. Cluster 2 grouped all the leaves of S3 treatment, five of the seven leaves of S2 treatment and one leaf of S1 treatment (Table III.4). So, Cluster 1 or "low salinity group" is related, in general, with the leaves from Ct and S1 treatments; and Cluster 2 or "high salinity group" is related with the leaves from S2 and S3 treatments.

Table III.4.- Number of leaves, of each saline treatment, grouped on each cluster considered. Calibration set (n=28).

	Ct treatment	S1 treatment	S2 treatment	S3 treatment
Cluster 1	7	6	2	0
Cluster 2	0	1	5	7

Table III.5 shows the Euclidean distances of each leaf of validation set to the average histogram of each cluster. Minimum distance is marked in bold and, it indicates the cluster in which the leaf is assigned.

Leaves from Ct and S1 treatments are grouped correctly in Cluster 1 (minimum Euclidean distance), and leaves from the higher salinity treatments (S2 and S3) are grouped in the Cluster 2. Leaf number 28, corresponding to S2 treatment, is in the middle of both clusters, showing a very similar Euclidean distance to both groups.

Table III.5.- Euclidean distance of each leaf (validation set) to each cluster considered on Ward classification. Minimum value is in bold characters.

Treatment	Leaf number	Cluster 1	Cluster 2
Ct	8	0,092	0,361
	9	0,233	0,530
	10	0,049	0,387
S1	18	0,047	0,409
	19	0,085	0,448
	20	0,159	0,303
S2	28	0,208	0,243
	29	0,317	0,175
	30	0,367	0,116
S3	38	0,576	0,156
	39	0,298	0,155
	40	0,536	0,105

4. CONCLUSIONS

Hyperspectral images were applied in a non-destructive way to evaluate the effect of salinity on lettuce leaves at harvest. These images were studied in order to determine the best combination of wavelengths for diagnosing the problem of salinity in lettuce leaves.

Two models were proposed able to sense salinity effect, based on the features of selected spectra of leaves with different rate of affection. Average spectra of the leaves were considering for models computation. Previously, average spectra and hyperspectral images were submitted to identical pre-processing procedures: second derivative and normalization by Standard Normal Variate procedure. The first model was based on a principal component analysis. PC1 was the most related to the changes in the leaves induced by salt concentration. By projecting the preprocessed hyperspectral images onto the PC1, the corresponding virtual images of scores showed clear differences among the leaves for each saline treatment, with concordant evolution trend with increasing salinity levels.

The second model was based on an optical index (named Level Salinity Index LSI) combination of three wavelengths at the red edge region. Several optical indexes from literature computed on randomly selected spectra of the hyperspectral images exhibited also the ability to sense the salinity effect on the leaves. However, LSI was the most discriminative index, compared to them. This fact can indicate that the detailed interpretation of the spectra behaviour can enhance the performance of indexes in each particular cultivar and condition. Once the index was proposed, a less expensive multispectral system (in comparison to hyperspectral one) can be developed to compute the respective virtual images (only three wavelengths were actually required).

Analysis of variance applied on those indexes and on LSI and PC1 scores, using only about 1% of pixels extracted at random from the total population of pixels of each leaf, showed significant differences between each salinity level, and congruent trends with increasing salinity; in an industrial application, this allows for the implementation of a hyperspectral or multispectral device with smaller spatial resolution. This can simplify the technology, managed data volume and hardware requirements of the system.

Hyperspectral images allowed detecting the distribution of the salinity effects on the surface of the leaves, which are more intense in the areas furthest from the veins. Marginal necrosis in leaves under salinity stress can be detected by the artificial images before the damage was visible.

A non-supervised classification, according Ward's method, was applied on the histograms of the LSI images of the leaves of calibration set. The histograms of the leaves were correctly separated into two different clusters, corresponding to two levels of salinity. Further, the Euclidean distance of the histograms of the validation set to the average histograms of each group, allowed the classification of the leaves according to the salinity with a 91'66 percentage of well classified.

Further research will be necessary to validate these models with larger data sets and salinity ranges, and also different varieties and growing conditions, than those examined in this study.

DISCUSSION OF RESULTS

Hyperspectral imaging was successfully applied to monitor aging of RTU leafy vegetables during shelf-life. Two different species of green leafy vegetables were considered: spinach and watercress. Results confirmed that several differences exist in the behaviour between both vegetables during aging, but most relevant spectral bands related to the evolution were common, allowing developing common models able to be applied simultaneously in different products.

Main changes observed during shelf-life in the reflectance spectra of both, spinach and watercress leaves, were centred in the same spectral ranges:

- Reflectance peak in the visible range around 550 nm.
- Chlorophyll absorption band around 680 nm.
- Red edge area, in the range between 700 and 760 nm.
- Global NIR reflectance.

According to the literature, those spectral areas are the most related to aging, senescence and stress symptoms in leaves. When leaves become senescent, due to aging or stress factors, the content of pigments in the tissues varies. This fact has a spectral response centred in the visible region of the spectra, where the pigments absorb radiation. In spinach and watercress leaves samples, such response was observed around 550 nm, which are the most sensitive absorption bands of carotenoids and anthocyanins (Gitelson et al., 2006); and around 680 nm, which is one of the absorption bands of chlorophyll. Moreover, a displacement of the red edge position to the left is typically observed in senescent leaves (Mutanga and Skidmore, 2007; Van der Meer and Jong, 2002), and it occurred also in spinach and watercress leaves during shelf-life. Finally, the increase at NIR reflectance range was also observed in spinach and watercress leaves. It is usually attributed to changes in the structure of the leaf, due to loss of water and increase in the content of air between cells, which induce an increase of scattering (Jacquemoud and Baret, 1990; Asner, 1998).

Models developed independently for both leafy vegetables were based on the same spectral bands, reflecting the previously commented spectral changes associated to senescence. They showed the main information in the visible range, between 500 and 550 nm, due to pigments content; the chlorophyll absorption band at 680 nm; and the displacement of red edge position, between 700 and 760 nm, approximately. It allows developing a common prediction model able to be applied in both vegetables, and probably in many other green leafy vegetables. In fact, models developed for spinach were able to monitor aging in watercress leaves, and vice versa.

Better results were obtained applying the own models developed on each vegetable, because they reflected the slight differences that could exist in the behaviour of each leafy vegetable during aging. Watercress leaves suffered a higher dehydration during shelf-life than spinach leaves. This fact was reflected in the own models showing also relevant areas in the NIR region, between 940 and 970 nm, related to the structural changes that dehydration can induce. However, a common model based in the common spectral changes observed can be segregative enough to monitor aging, or as a tool for a preliminary inspection of freshness. Moreover, it allows to inspect directly mixes of leaves or green salads, not only each vegetable independently. It would be a great advantage for industrial applications.

Lateral displacement of red edge seemed to be one of the most important facts to predict senescence of leaves, and it can be sensed by using only three different wavelengths. There are many existing indexes to predict pigments content based on the visible spectral bands detected as relevant to monitor aging of spinach and watercress leaves (Gitelson et al., 2001, 2002, 2006). It can be possible to simplify the models developed into multispectral indexes retaining the same relevant information related to aging of leaves. A common multispectral index for monitoring aging and freshness of leafy vegetables would allow developing cheap and fast multispectral devices able to be easily used in the industrial production lines.

As it was also shown, the initial state of freshness of the different leaves in a commercial bag of RTU spinach or watercress is very variable. The overall shelf-life of those RTU products will be affected by the initial state of freshness of each individual leaf. The models developed allowed inspecting freshness of the leaves and detecting aging symptoms before they can be seen by human eye, which is another great advantage.

On the other hand, the effect of water salinity on lettuce leaves for RTU purposes was evaluated by using hyperspectral imaging. Salinity, as a stress factor, affected the spectral response of the leaves in the same spectral bands than aging or senescence. Main changes observed when salinity increased were centred in the absorption range of pigments (500-550 nm); and the lateral displacement of red edge. Prediction models developed to evaluate the effect of salinity were focused on those spectral features. However, displacement of red edge showed to be the most relevant fact associated with increasing levels of salinity, and LSI index was proposed. This index was based on three different wavelengths characterizing the position of red edge, and it can be seen that is quite similar to the index proposed previously to sense the lateral displacement of red edge position in spinach leaves during aging. Salinity seems to affect the health of the leaves and it may induce a spectral response similar to aging or loss of freshness.

LSI index was compared with up to eleven different indexes found in the literature, developed to evaluate salinity effect in crops and plants. LSI index showed the highest segregation ability among them for sensing salinity effects. The spectral bands included in the index and prediction model proposed in this research, and also in the indexes found in the literature, were mainly related to the spectral bands associated to the pigments content, displacement of red edge and the water content in the NIR range. Those are the typical spectral ranges where changes associated to aging and senescence occur, as it was discussed before. Probably, indexes developed to evaluate salinity effects on crops and plants are sensing, in fact, state of health or senescence due to this stress factor.

Salinity levels considered in this research produced a lateral displacement to the right of the red edge position of the spectra of lettuce leaves samples. This may be interpreted, according to the literature, as an increase of the health or freshness of the lettuce leaves. As it was explained before, herbaceous crops affected by salinity at moderate levels do not show visible damages and may have deep green and more succulent leaves (Maas and Hoffman, 1977). Scuderi et al. (2011), employing the same salinity conditions than this research, noticed an improvement of the properties of fresh-cut lettuce with increasing levels of salinity, delaying the decay process and increasing the shelf-life. Chisari et al. (2010) observed a reduction of oxidative enzymes on fresh-cut lettuce leaves grown under salinity conditions. All these facts are in accordance with the displacement of the red edge position to the right observed in this research. Moderate salinity levels may enhance the quality and shelf-life of fresh-cut lettuce leaves, improving their capabilities for RTU purposes.

LSI is a three wavelengths based index able to sense the displacement of red edge position due to non-biotic factors, such as salinity, affecting leafy vegetables. Lateral displacement of red edge position also occurred due to aging and loss of freshness of leaves. Maybe LSI index, or a slight modification of it, can be applied to evaluate the overall capability, as a combination of freshness and quality, of leafy vegetables to be used as RTU products, since all the spectral changes affecting the quality of leafy vegetables are mainly centred in the red edge range.

GENERAL CONCLUSIONS

This research demonstrates the potential of hyperspectral imaging as a tool for a fast and non-destructive evaluation of quality parameters in leafy vegetables, such as spinach, watercress and lettuce.

Aging was successfully monitored during shelf-life of two different species of leafy vegetables: spinach and watercress. Models were developed in each case, based on the same spectral bands, related to the variation in the pigments content and the lateral displacement to shorter wavelengths of the red edge position when leaves become senescent. Effects of aging and loss of freshness can be sensed by this technology before they can be detected by human eye, which can prevent economic losses to the industry and improve the quality of the RTU products offered to the consumer.

Shifting of red edge position was the most important indicator associated to aging of leaves, and it was sensed by using only three wavelengths. Models developed can be simplified into multispectral indexes easier and faster to apply at industrial level for inspecting the freshness of leafy vegetables.

Although the best performance was obtained with own models developed for each leafy vegetable, models developed for spinach leaves were able to monitor the evolution of watercress leaves, and vice versa. This fact suggests the possibility to obtain a common model appropriate for a variety of leafy vegetables, allowing the inspection of salads and mixes of leaves directly.

Radiometric correction proposed in this research allowed to obtain useful spectral information by acquiring the hyperspectral images of the leaves directly through commercial packaging films. Transmittance of the films can vary during time and it can affect the spectral response of the leaves. Radiometric correction removes this effect from the spectra allowing the inspection of packed leafy vegetables directly on the industrial line or along the product distribution.

Hyperspectral imaging was also successfully applied to evaluate salinity effect on leafy vegetables, such as lettuce. A prediction model and a three wavelengths based spectral index (LSI) were proposed and compared with other indexes from literature. Both models showed the best ability to sense the salinity effect on lettuce leaves.

Only 1% of the pixels acquired were needed to sense the effect of salinity on the leaves. This fact suggests the implementation of this index into a cheaper multispectral device, with less spatial resolution, simplifying the technology, the data volume and hardware requirements of the system.

Spectral response of the leaves to salinity was centred mainly in the red edge range, producing a lateral displacement to the right of the red edge position. Lateral movements of the red edge position are associated to aging, state of freshness or stress affecting the leaves. Shifting to the left indicates loss of freshness, senescence or stress. Shifting to the right indicates, therefore, freshness and vigour of the leaves. A moderate level of salinity can enhance the shelf-life of RTU leafy vegetables, as literature state and this finding suggests.

Aging of leaves during shelf-life and salinity effect produced spectral changes in similar spectral ranges, and there were several similarities in the models proposed on each case, since all of them reflected changes in the red edge position. The findings reached in this research facilitate the further development of a multispectral index (probably based on a slight modification of LSI) for the evaluation of leafy vegetables to be used as RTU products. This index would be easy to implement in cheaper multispectral devices for the inspection of the freshness leaf by leaf in the industrial line, allowing the quality segregation of the leaves and improving the shelf-life of the final RTU product.

FUTURE WORK

Further research should be directed to develop that common multispectral index proposed in the general conclusions of this research. Findings showed along this work suggest that possibility, due to the similarities observed in the prediction models developed, not only to monitor aging in spinach and leaves, but also in those developed for evaluating the effect of salinity stress. Factors affecting the freshness or vigour of leaves can be sensed in a quite similar way so it would be possibly to obtain that common index. Further steps for future research would be:

- To validate the results obtained in spinach and watercress leaves considering larger data sets, different storage conditions, and also different varieties of those vegetables, in order to consider the most internal variability as possible. To obtain refined prediction models for each case.
- To explore the feasibility of this technology based on spectral response of other green leafy vegetables during shelf-life, for example: lettuce, cabbage, wild rocket, mache or tatsoi. To create prediction models for each case able to inspect the freshness of the leaves during time.
- To analyse the differences and similarities among all the models developed on each case, trying to find a common model suitable for monitoring the freshness in all the cases.
- To understand deeply the prediction models (i.e. physiological based explanation, pigments content evolution...), studying the main spectral bands involved in the changes occurred when leaves age. To select the most important wavelengths for each case and to define a multispectral index able to monitor properly the evolution of most of the leafy vegetables studied.
- To validate the multispectral index proposed on large data sets of different leafy vegetables for evaluating the real capability of the index to detect different states of freshness in different species.

If a successful general multispectral index for inspecting freshness and aging of leafy vegetables can be developed, the next steps should be to test such index to inspect overall quality of leaves for RTU products, not only from the freshness point of view, but also according to the effect of different stress factors that can affect the quality of leaves, such as salinity, drought, nutritive imbalances... In the case of salinity, higher levels of NaCl may be tested to establish the salinity level that starts to negatively affect the quality of lettuce leaves, shifting the red edge to shorter wavelengths. It would allow improving the lettuce growing conditions to obtain the best quality of the leaves for RTU purposes.

Another challenge would be to explore the applicability of this multispectral index on non-green leafy vegetables. To analyse the spectral response along shelf-life of other leafy vegetables, such as lollo rosso, radicchio, red leaf lettuce, and purple kale; compared with the green ones.

Finally, the big challenge would be to check the behaviour of the multispectral index on real industrial conditions. For that, two steps should be considered:

- To acquire spectral data directly on commercial bags of RTU leafy vegetables, through packaging films, analysing the difficulties that can appear when the position of the leaves is not controlled and both sides of the leaves can be exposed. To identify and minimize those difficulties and limitations for industrial applications, especially in the case of a mix of leaves.
- To test this technology and the multispectral index at industrial level. To study the feasibility of spectral acquisition of samples under real industrial environments, for example, directly on the conveyor.

These proposed steps are ambitious but they can constitute the guidelines to implement multispectral imaging devices to inspect the quality of RTU leafy vegetables on the industrial production lines. The final objective would be to develop an only and common multispectral index for most of the applications, but it would be also a great advantage to obtain independent indexes for each leafy vegetable variety.

RESULTS DISSEMINATION

1. PUBLICATIONS

Table 12 gathers the conference presentations carried out along this thesis. The experimental work done in Section I and Section II for monitoring the shelf-life of spinach and watercress leaves was presented, in a preliminary stage, in the *International Conference of Agricultural Engineering CIGR-AgEng* 2012.

Table 13 gathers the SCI publications and other articles published along this thesis. The results obtained in Section I were published in 2013 in *Journal of Food Engineering*. First results obtained in Section III, detecting the effect of salinity on lettuce leaves, were published in 2013 in the magazine *Horticultura*, nº310; and they were also exposed in the *SEAgIng-SECH* congress, in 2013. Final results from this Section III have been recently submitted to the journal *Applied Sciences* and they have been accepted to be published (after corrections).

Table 12.- Conference presentations published along the development of this thesis.

MONITORING SHELF-LIFE IN LEAFY VEGETABLES
Diezma, B., Lara, M.A., Molina, M., Lleó, L., Ruiz-Altisent, M., Artés-Hernández, F., Roger, J.M. "Monitoring leafy vegetables through packaging films with hyperspectral images". Poster P-1321 presented at International Conference of Agricultural Engineering CIGR-AgEng 2012. Valencia (Spain). 8-12 July 2012.

Table 13.- SCI publications and other articles published along the development of this thesis.

MONITORING SHELF-LIFE IN LEAFY VEGETABLES
Lara, M.A., Lleó, L., Diezma-Iglesias, B., Roger, J.M., Ruiz-Altisent, M. 2013. Monitoring spinach shelf-life with hyperspectral image through packaging films. <i>Journal of Food Engineering</i> 119, 353–361.
SALINITY EFFECT IN LEAFY VEGETABLES
Lara, M.A., Diezma, B., Lleó, L., Roger, J.M., Garrido, Y., Gil, M.I., Ruiz-Altisent, M. "Aplicación de imagen hiperespectral para observar el efecto de la salinidad en hojas de lechuga". 2013. <i>Horticultura</i> , 310.
Lara, M.A., Diezma, B., Lleó, L., Roger, J.M., Garrido, Y., Gil, M.I., Ruiz-Altisent, M., 2014. Hyperspectral images for the detection of salinity effect on lettuce leaves. <i>VII Congreso Ibérico de Agroingeniería y Ciencias Hortícolas: Innovar y Producir para el Futuro. Innovating and Producing for the Future</i> , pp. 1644-1649.
Lara, M.A., Diezma, B., Lleó, L., Roger, J.M., Garrido, Y., Gil, M.I., Ruiz-Altisent, M. "Hyperspectral imaging to evaluate the effect of irrigation water salinity on lettuce". <i>Applied Sciences</i> (accepted).

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