UNIVERSITÀ DI FOGGIA

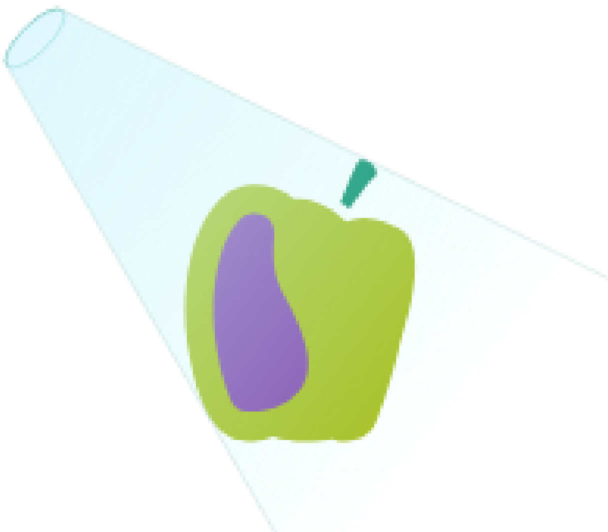
Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente

Doctoral Thesis in

Management of Innovation in the Agricultural and Food Systems of the Mediterranean Region

– XXXII cycle –

The potential use of non destructive optical-based techniques for early detection of chilling injury and freshness in horticultural commodities



Candidate:

Farahmand Babellahi

Tutor:

Prof. Maria Luisa Amodio

UNIVERSITÀ DI FOGGIA

Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente

Doctoral Thesis in

Management of Innovation in the Agricultural and Food Systems of the Mediterranean Region

– XXXII cycle –

The potential use of non destructive optical-based techniques for early detection of chilling injury and freshness in horticultural commodities

Candidate:

Farahmand Babellahi

Tutor:

Prof. Maria Luisa Amodio

UNIVERSITÀ DI FOGGIA

Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente

Doctoral Program in Management of Innovation in the Agricultural and Food Systems of the Mediterranean Region

– XXXII cycle –

Doctoral thesis on **"The potential use of non destructive optical-based techniques for early detection of chilling injury and freshness in horticultural commodities"** discussed at the Università di Foggia, 15/June/2020

Candidate:

Farahmand Babellahi

Tutor:

Prof. Maria Luisa Amodio (*Universita di Foggia, Italy*)

Committee members:

Prof. Giancarlo Colelli

Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente-Università di Foggia

Prof. Riccardo Guidetti

Dipartimento di Scienze Agrarie e Ambientali, Produzione, Territorio, Agroenergia - Università degli Studi di Milano

Prof. Vincenzo Fucilli

Dipartimento di Scienze agro-ambientali e territoriali-Università degli Studi di Bari Aldo Moro

به پایان آمد این دفتر

حکایت همچنان باقی است

سعدی شیرازی

This chapter of my life is finished

But its novel is continued

Saadi Shirazi

ACKNOWLEDGEMENT

I would like to express my special appreciation and thanks to my advisor **Prof. Maria Luisa Amdio** and my Ph.D. program coordinator **Professor Dr. Giancarlo Colelli** who have been tremendous mentors for me. I would like to thank you for encouraging my research and for allowing me to grow as a research scientist. Your advice on both research as well as on my career have been priceless. I would also like to thank **Professor Dr. Jitendra Paliwal** from the University of Manitoba and **Dr. Pavlos Tsouvaltzis** from the University of Thessaloniki for your precious positive impact that you made on my knowledge and personality.

I would especially like to thank the **Raytec Vision S.p.A** and in particular, **Dr. Raffaele Pezzoli** for supporting my Ph.D. program both financially and morally. I can say that without your supports this achievement was impossible.

A special thanks to my family. Words cannot express how grateful I am to my mother, father, and siblings for all of the sacrifices that you've made on my behalf. Your prayer for me was what sustained me thus far. At the end, I would also like to thank all of my friends who supported me during my doctorate period, and incented me to strive towards my goals.

Farahmand Babellahi,
PhD Course in Managing of innovation in Agricultural and Food Systems in the Mediterranean Area
Dipartimento di Scienze Agrarie, Degli Alimenti e Dell'Ambiente Universita di Foggia
Via Napoli 25, 71121 Foggia – Italy

Contents

PART I: INTRODUCTION

EXTENDED ABSTRACT

The increasing concern and awareness of the modern consumer regarding food including fruits and vegetables, has been oriented the research in the food industry to develop rapid, reliable and cost effective methods for the evaluation of food products including the traceability of the product history in terms of storage conditions. Since the conventional destructive analysis methods are time consuming, expensive, targeted and labor intensive, non-destructive methods are gaining significant popularity. These methods are being utilized by the food industry for the early detection of fruits defects, for the classification of fruits and vegetables on the basis of variety, maturity stage, storage history and origin and for the prediction of main internal constituents.

Since chilling injury (CI) occurrence is a major problem for chilling sensitive products, as tropical and sub-tropical fruit and vegetables, prompt detection of CI is still a challenge to be addressed. The incorrect management of the temperature during storage and distribution causes significant losses and wastes in the horticultural food chain, which can be prevented if the product is promptly reported to the correct temperature, before that damages become irreversible. For this reason, rapid and fast methods for early detection of CI are needed.

In the first work of this thesis, non-destructive optical techniques were applied for the early detection of chilling injury in eggplants. Eggplant fruit is a chilling sensitive vegetable that should be stored at temperatures above 12°C. For the estimation of CI, fruit were stored at 2°C (chilling temperature) and at 12°C (safe storage temperature) for a time span of 10 days. CIE L*a*b* measurements, reflectance data in the wavelength range 360–740 nm, Fourier Transformed (FT)-NIR spectra (800–2777 nm) and hyperspectral images in the visible (400–1000 nm) and near infrared (900–1700 nm) spectral range were acquired for each fruit. Partial least square discriminant analysis (PLSDA), supervised vector machine (SVM) and k-nearest neighbor (kNN) were applied to classify fruit according to the storage temperature. According to the results, although CI symptoms started being evident only after the

4th day of storage at 2°C, it was possible to discriminate fruit earlier using FT-NIR spectral data with the SVM classifier (100 and 92% non-error-rate (NER) in calibration and cross validation, respectively, in the whole data set. Color data and PLSDA classification possessed relatively lower accuracy as compared to SVM. These results depicted a good potential of for the non-destructive techniques for the early detection of CI in eggplants.

Similarly, in the second experimental part of the thesis, hyperspectral imaging in Vis-NIR and SWIR regions combined with chemometric techniques were used for the early estimation of chilling injury in bell peppers. PLSDA models accompanied by wavelength selection algorithms were used for this purpose, with accuracies ranging from 81% and 87% non-error-rate (NER) based on the wavelength ranges used and variables selected. PLSR models were developed for the prediction of days of cold storage resulting in R^2_{CV} = 0.92 for full range and R^2_{CV} = 0.79 using selected variables. Based on the results, it was concluded, that Vis-NIR hyperspectral imaging is a reliable option for on-line classification of fresh versus refrigerated fruit and for identifying early incidence of CI.

Inspired by the results obtained from previous studies a third study regarded the use of non-destructive techniques for the estimation of freshness of eggplants using color, spectral and hyperspectral measurements. To this aim, fruit were stored at 12°C for 10 days. Fruit were left at room temperature (20°C) for 1 day after sampling which was done with a 2-day interval, simulating one-day of shelf life in the market. PLSR models were developed using the spectral and hyperspectral data and the storage days, allowing safe assessment of the freshness of the fruits along with the utilization of SPA for variable reduction. The results depicted strong correlation between storage days, FT-NIR spectra and the hyperspectral data in the Vis-NIR range with accuracies as high as $R_C > 0.98$, $R_{CV} > 0.94$, RMSEC < 0.4 and RMSECV< 0.8, followed by lower accuracies using color data. The results of this study may set the basis to develop a protocol allowing a rapid screening and sorting of eggplants according to their postharvest freshness either upon handling in a distribution center or even upon the reception in the retail market.

In the last work, as a deeper investigation, the effect of temperature and storage time on the FT-NIR spectra was statistically investigated using ANOVA-simultaneous component analysis (ASCA) on eggplant fruit as a crop model. Also in this case, fruit were stored at 2 and 12 °C, for 10 days. Sensorial analysis, electrolyte leakage (EL), weight loss and firmness were used, as the reference measurements for CI. ASCA model proved that both temperature, duration of storage, and their interaction had a significant effect on the spectral changes over time of eggplant fruit. Followed by ASCA, PLSDA was conducted on the data to discriminate fruit based on the storage temperature. In this case, only the WL significant in the ASCA approach for temperature were considered, allowing to reach 87.4±2.7% as estimated by a repeated double-cross-validation procedure. The outcomes of all these studied manifested a promising, non-invasive, and fast tool for the control of CI and the prevention of food losses due to the incorrect management of the temperature in the horticultural food chain.

1. Chilling injury in horticultural commodities

Low-temperature storage is a postharvest technique commonly applied to prolong the shelf-life of horticultural produce, by slowing down the metabolism; however, tropical and subtropical commodities, that include more than half of the species on the Earth, are chilling sensitive (Lukatkin et al. 2012). These products, should be stored a temperature of 8-12 °C depending on the specie sensitivity. Storage at temperature lower than the critical chilling temperature will result in the occurrence of different chilling injuries which will decrease the marketability and the storage life of the product (Chien Yi Wang 1994).

Generally, low temperature induces membrane lipid phase transitions which causes a loss of membrane integrity and physiological dysfunction. It has been observed that the level of certain high melting phospholipids appears to be associated with the chill sensitivity of commodities Membranes and changes in their physical physiognomies are further associated as having a role in chilling injury (CI) by the detection that chilling stress educes an elaborate membrane retailoring response that leads to increased fluidity at reduced temperatures (PARKIN et al. 1989).

CI symptoms differ with commodities. Common CI symptoms in tropical horticultural commodities are pitting, discoloration, water soaked appearance, internal breakdown, and failure to ripen, loss of flavor and aroma, and decay (Hardenburg, Watada, and Wang 1986) (Fig.1). Symptom development depends not only on species and cultivars, but also on maturity, types of tissues, and other environmental factors, such as storage humidity. Pitting, circular or irregular-shaped pits on the fruit surface, is the most common and the first CI symptom in many tropical horticultural commodities (Chien Yi Wang 1994).

Particularly, flesh browning and darkening of seeds and pulp tissue, color changes are internal symptoms of CI in eggplant, whereas pitting in the skin and calyx discoloration are that can be seen externally (Concellón, Añón, and Chaves 2007a). For bell peppers, the most common indications for CI are calyx browning, surface pitting, seed discoloration. For pomegranates, the external symptoms are the brown discoloration of the skin, surface pitting, and susceptibility to decay organisms, and in the same manner, pale aril color and brown discoloration of the white segments separating the arils could happen for the internal matters (Mirdehghan et al. 2007)

In some tropical and subtropical produce, CI may even cause failure in the ripening process. For instance, this disorder is a regular phenomenon in avocados, bananas, mangos, melons, papayas, sapodilla, and tomatoes. Moreover, there are more symptoms that are related to specific crops. In bananas, for example, sub epidermal brown streaking of vascular tissues can occur; membranous staining and mahogany browning are particular symptom in lemon and potatoes, respectively (C.Y. Wang 2010).

Fig.1. Effect of discoloration (left), water soak appearance (center), and surface pitting (right) in banana, peach, and nectarine (photo credit: Adel Kader and Don Edwards, UC Davis)

During the last decade, various researches have been conducted for diminishing chilling-induced injuries. Temperature conditioning, intermittent warming, controlled atmosphere storage, and chemical treatments, are the most frequent techniques that have been used regarding this purpose.

Temperature conditioning

Temperature conditioning includes two techniques: Low Temperature Conditioning (LTC) and High Temperature Conditioning (HTC). In LTC, chilling-sensitive fruit and vegetables are subjected to a temperature a little above the chilling range. This phenomenon helps the crops to enhance the tolerance to chilling during successive low storage temperature. As a sequence, this technique makes an adaptive response in fruit and vegetables to chilling stress resulting in several physiological and biochemical variations (C.Y. Wang 2010). On the other hand, HTC is a short term treatment with temperature above 35°C. In this case, the commodities get exposed to one type of stress leading to stimulation of some elements which can protect against another type of stress. HTC can be done by hot water dip, hot forced air, or vapor heat (Klein and Lurie 1991).

Intermittent warming

Intermittent warming is another alternative method interposing to the low temperature storage with one or more short periods of warm temperature that allow to increase the storage time of some chilling-sensitive produce. In order to use this method, the CI should be detected at the early stage in order to expose fruit to worming before that symptoms become irreversible (P. Li et al. 2016a).

Intermittent warming ordinarily induces higher metabolic activities and permits the tissue to metabolize excess intermediates or toxic materials accumulated during chilling

Controlled atmosphere storage

Controlled atmosphere storage in some particular fruit inhibits the solubilization and depolymerization of chelator-soluble wall polyuronides and considerably reduced the surge in pectinesterase and β-galactosidase activities which is associated with CI (Alba-Jiménez et al. 2018; Ali et al. 2004; L. Li et al. 2019). In this regard, Lurie (1993) observed that the exposition to 10% CO_2 and 10% O_2 for 6 weeks could prevent CI in the nectarine cultivars 'Fantasia', 'Flavortop', and 'Flamekist'. On the other hand, Ketsa and Klaewkasetkorn (1995) reported that for Rambutan fruit, 0.3–0.7 % CO_2 and 16.1–19.5 % O_2 delayed the CI development compared to fruit stored in air. Moreover, Gracia (1997) compared the effect of different O_2 and CO_2 mixtures (5% CO_2 + 5% O_2, 15% CO_2 + 2% O_2 and air) for 'Hass' avocado stored at 2°C for 30 days, founding that fruit stored in 15% CO_2 with 2% O_2 showed less CI symptoms.

Chemical treatment

Chemical treatment such as methyl jamonate (MeJA) and salicylic acid have been used also for reduction of CI which leads to six different effects:

1) Higher membrane integrity due to higher unsaturated/saturated fatty acids (unSFA/SFA) ratio which causes reduction in enzymatic activities during storage at chilling temperature and led to maintenance of membrane integrity.
2) higher HSPs gene expression and accumulation that produce a stress-responsive family of proteins whose molecular weights range between 15 and 115 kDa
3) higher antioxidant system activity;
4) Higher arginine pathway activity leading to higher polyamines, nitric oxide (NO), and proline accumulation. Arginine is a metabolically multifunctional amino acid that play an important role for constructing block of proteins and as a precursor for the biosynthesis of signaling molecules such as polyamines (putrescine, spermidine, spermine), proline NO) which can be fairly vital in enhancing the tolerance to CI.
5) Higher phenylalanine ammonia lyase (PAL)/polyphenol oxidase (PPO) enzymatic activity ratio along with higher DPPH scavenging capacity leading to lower browning. ParticularlyPAL activity stimulated by the CI induces an increase of total phenols (TP) that accumulates in vacuoles; a membrane selective permeability loss occurs; PPO activity increases in cytoplasm that is responsible for flesh or internal browning; phenolics compounds accumulated in vacuoles leak to cytoplasm due to loss of vacuole membrane (tonoplast) selective permeability and contribute to IB incidence and
6) Higher -aminobutyric acid (GABA) shunt pathway activity. GABA is a four carbon, non-protein amino acid which plays a crucial role as a signaling molecule in response to postharvest stress such as CI. In addition to anti-chilling function of GABA in fruits and vegetables, GABA plays an important role in

human health due to its antihypertensive effects (Aghdam and Bodbodak 2013; Cai et al. 2011; Mae et al. 2012; Sayyari et al. 2016).

2. Detection of chilling injury

2.1. Conventional techniques

Many methods have been conventionally applied for the detection and evaluation of CI.

In general, for all chilling sensitive commodities, visual symptoms can be notices. Alterations in the appearance of fruit often involve changes to the chemical content and structure of the cell wall and plasma membrane (Liu et al. 2015), as observed for bell peppers. Changes in the cell walls can be detected trough firmness measurements; however, changes in the composition of the cell wall are caused by the action of hydrolytic enzymes, such as polygalacturonase (PG) and pectin methyl esterase (PME), which are therefore indicators of CI. Moreover, since CI causes cell membrane damage and, as a consequence, affects the effectiveness of membranes as barriers to solute diffusion, electrolyte leakage is considered to provide an indirect measure of this damage (Murata 1990). On the other hand, malondialdehyde content, antioxidant enzymes including catalase, peroxidase, ascorbate peroxidase and glutathione reductase, ascorbate-glutathione cycle, organic acid content, and activities and relative gene expressions of Ascorbate Peroxidase (APX) were used for the CI assessment in bell peppers(Endo et al. 2019; L. Liu et al. 2015; Q. Wang et al. 2012; Y. Wang et al. 2019)

As, reactive oxygen species (ROS) scavengers, also the antioxidant enzymes activity may decline by exposing fruit to low temperature, as observed for the eggplant. Therefore, antioxidant enzymes activity assessment could be reference for CI evaluation (Zheng et al. 2008).

Phenolic content is another parameter for CI evaluation which can be decreased by induction of CI in the fruit. On the other hand, ,pH, the activity and relative gene expression of peroxidase (POD) and catalase (CAT) are the most dominant factors that are being measured for the evaluation of CU in eggplants (Concellón et al. 2012; Concellón, Añón, and Chaves 2005, 2007b; Fan et al. 2016; Shi et al. 2018).

2.2. Non-destructive and innovative techniques

Non-destructive techniques are significantly advantageous over the chemical analysis in terms of cost, speed, time and reliability of the expected results. Most importantly, non-destructive techniques are not a direct replacement of the conventional methods, but can be a useful tool to assist these techniques. In case of the non-destructive methods no sample preparation is needed and upon the development of prediction/classification model, the prediction process becomes quite quicker. In order to evaluate the changes in chlorophyll content caused by CI, Lurie, ronen, and Meier (1994) studied Pulse Amplitude Modulated (PAM) fluorimeter to measure changes in photosynthetic competency (quantum yield (Fm/Fe), photochemical quenching (Qp), and non-photochemical quenching (Qnp)) associated with CI in whole green bell peppers. Similarly, Kosson's (2003) investigated the effect of storage at various chilling temperatures for 18 days on chlorophyll fluorescence parameters and CI occurrence of green pepper (Capsicum annuum) . Due to this purpose, the chlorophyll fluorescence parameters including minimum fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv), plus Fv/Fm were measured. The results of this study showed a gradual decrease of Fv/Fm fluorescence (as an effective factor) from about 0.85 for fresh-harvested pepper to 0.55 after 3 days of storage at 1 °C and to 0.45 after 18 days of storage. For assessment of chlorophyll change, Hashim et al. (2013) used a different technique. They employed laser diodes emitting at 660 and 785 nm to acquire images of backscattered light from intact banana fruits to monitor chlorophyll and texture changes resulted by CI.

Firmness also as an index for CI evaluation has been used in several studies. For instance, Verlinden, De Smedt, and Nicolaı (2004) evaluated texture changes related to CI by acoustic firmness measurements and ultrasonic wave propagation. Hale et al. (2013) used two non-destructive instruments, an acoustic firmness sensor (AWETA), and a vis/NIR DA-meter to classify ‘August Fire’ nectarines into maturity stages based on both fruit firmness and ethylene production as result of CI.

Nowadays, smartphones and their applications are getting popular in case of applying in the agricultural and horticultural researches. In this regard, Novas et al. (2019) developed an application for a smartphone that automates the process of CI and applied it to zucchini, one of the most sensitive vegetables.. The smartphone used in that work was a commercial Samsung Galaxy S5 G900F working in visible spectrum range. The built-in camera was a 16 MP (f/2.2, 31 mm, 1/ 2.600, 1, 12 mmm). When used to extract frames from the video capture, the resolution used was 720p at 30 fps. To demonstrate the validity, the authors compared the results to standard method. They also compared different varieties allowing thresholds of hue and saturation settings to be adapted to these different varieties.

Among non-destructive optical-based techniques, NIR spectroscopy and hyperspectral imaging have shown promising results as reported in various papers. In the following sections, these techniques will be described.

3. Optical-based techniques

3.1. Spectroscopy

3.1.1. Basic concepts

Optical radiation covers the wavelength range of 100 nm to 1000 µm of the electromagnetic spectrum. This range splits into the ultraviolet (UV) region from 100 to 380 nm, the visible (VIS) light ranging from 380 to 780 nm, and the infrared (IR) radiation of wavelengths above 780 nm (Fig.2). IR region itself, is subdivided to the near infrared (NIR) region covers wavelengths from 780 nm up to 2500 nm, mid infrared (MIR) covers the region from 2500 nm to 25 µm, and far infrared (FIR) the contiguous region up to 1000 µm (Porep, Kammerer, and Carle 2015).

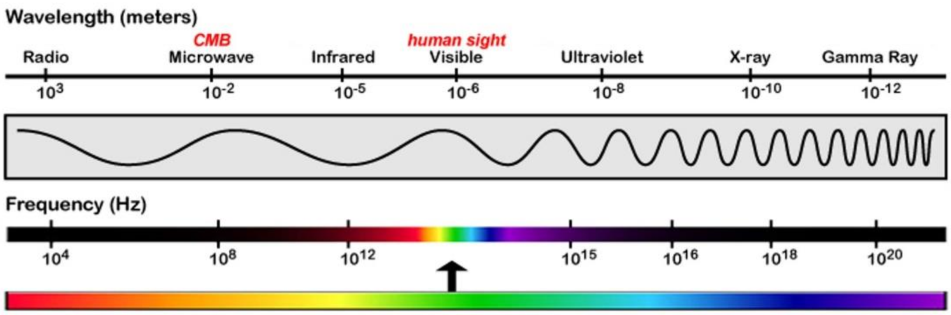


Fig.2. Electromagnetic spectrum

Fundamentally, when the infrared (IR) light radiates a molecule, ta discrete amounts of energy at specific resonant frequencies, is absorbed causing the vibration of specific molecule bonds (Larkin 2017; Smith 2011). Different kind of vibrations are then generated, namely stretching, bending, scissoring, wagging, rocking, twisting or deformation modes as shown in Fig. 3 (Bureau, Cozzolino, and Clark 2019).

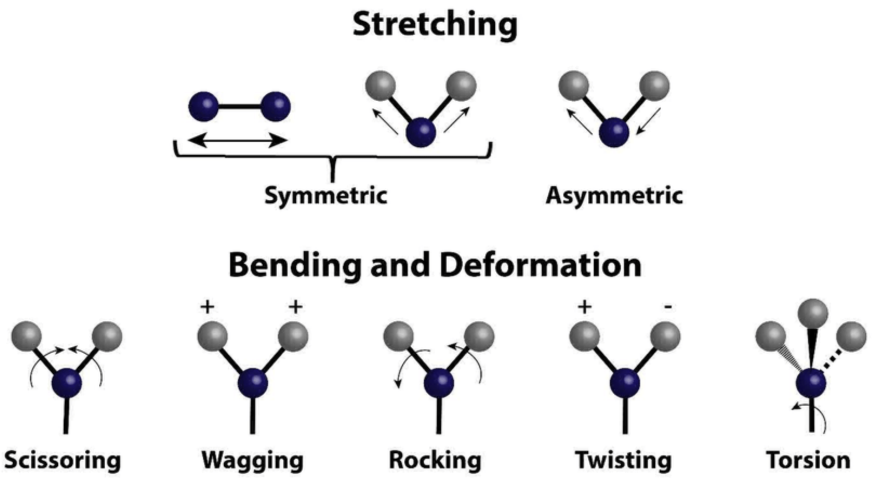


Fig.3. Various vibration kinds in molecules (Photo credit: (Sitarski 2017))

In the NIR spectroscopy, radiated NIR can be measured either in reflected or transmitted mode. In fact, the radiating energy penetrates the sample, and scattering and absorption processes affect the reflected or transmitted spectra. This transformation is due to the chemical composition of the product, and also on its light scattering properties which are associated with the microstructure (Nicolaï et al. 2007a).

Spectra obtained by NIR spectroscopy includes overlapping absorptions corresponding mainly to overtones and combinations of vibrational types involving C–H, O–H, N–H and S–H chemical bonds (Osborne 2006), and particularly C–H bonds and water O–H bonds controlling the hydrated material (Nicolaï et al. 2014).

Historically, Norris (1964) was the first researcher who had applied NIR spectroscopy in agricultural applications by evaluating the moisture in grain. Later on, by soaring the importance of non-destructive techniques, NIR spectroscopy started to play a crucial role in postharvest technology, from the fact that numerous producers demanded on/in- line sorting systems using NIR technology to assess different quality aspects.

3.1.2. Instrumentation

Instrumentation for near-IR (NIR) spectroscopy is comparable to instruments for the UV-visible and mid-IR ranges. NIR spectrophotometer consists of a light source which is typically a tungsten halogen light bulb, sample presentation accessory, monochromator, detector, and optical components, such as lenses, collimators, beam splitters, integrating spheres and optical fibers. Spectrophotometers could be categorized based on the monochromator. In a filter instrument, the monochromator is a wheel holding a number of absorption or interference filters, although, it’s spectral resolution is limited. On the other hand, scanning monochromator instrument consists of a grating or a prism that separate the specific frequencies of the radiation either entering or leaving the sample. In this case, the wavelength separator rotates so that the radiation of the individual wavelengths subsequently reaches the detector. Fourier transform NIR (FT-NIR) instruments use an interferometer, especially for wavelengths above ~1000 nm. Depending on the sample, the spectrum can be quantified in either reflection or transmission (Nicolaï et al. 2007a; Stratis et al. 2001).

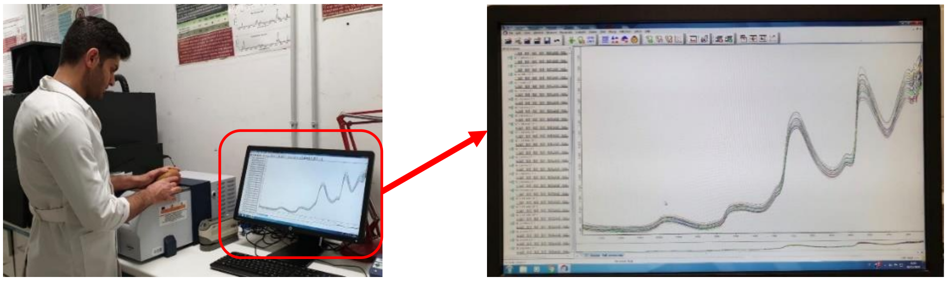


Fig.4. Spectra acquiring by Multi-Purpose Analyzer (MPA) (Bruker Inc.) spectrometer and OPUS software

3.1.3. Application

Fruit and vegetables are a particular group of daily food. Due to having a vast range of size, color, shape, and chemical composition, as well as, vary even when harvested at the same place and same time, grading them on the basis of their quality and physio-chemical aspects is very essential. Indeed, performing a conventional analytical technique is very time-consuming, labor intensive, and comparatively expensive. Nowadays, NIR spectroscopy as a non-destructive technology is suitable to the measurement of the aforementioned aspects.

Regarding online application, Zude et al. (2010) investigated banana and papaya spectra (VIS-NIR) by utilizing optical geometry for non-invasive remittance analysis in-situ as well as diffuse reflectance readings with a laboratory spectrophotometer. Considering different maturity stages and after CI level, spectral variations were examined. Spectral variations were analyzed in fruits at different maturity stages as well as after CI treatment. Fruit maturity was estimated by means of chlorophyll changes analyzed in the red and near infrared wavelength ranges of the fruit spectra.

In another aspect of online practice, Hara et al. (2018) used Raman spectroscopy by applying different excitation wavelengths (532 nm, 785 nm, and 1064 nm) to identify an appropriate wavelength for the quantitative analysis of carotenoids in tomatoes. It was figured out that a regression model designed using the 785 nm-excited Raman spectra is showing a better result than the 532 nm- and 1064 nm-excited Raman spectra.

In the offline and lab scale applications, Farneti, el al. (2012) conducted a research to realize the possibility of spectroscopy for detection of CI in tomatoes by assessing Lycopene as a factor of CI in tomatoes using remittance VIS spectroscopy as a test panel and HPLC as a reference. It was found to relate closely to the lycopene level as measured by HPLC measurements of pericarp tissue. Additionally, Huang, Lu, and Chen (2018) assessed the quality of tomato fruit by using a newly developed spatially-resolved spectroscopy (SRS) system with 30 detection optic fibers covering the wavelength range of 550–1650 nm and comparing its performance with two conventional single-point (SP) spectroscopic instruments covering the visible and shortwave near-infrared (Vis/SWIR) (400–1100 nm) and near-infrared (NIR) (900–1300 nm) regions, respectively. The result of this work manifested combinations of two or more SR spectra resulted in better, more consistent SSC and pH predictions. SR predictions of pH ($r_p = 0.819$) were better than for SP Vis/SWNIR ($r_p = 0.743$) and NIR ($r_p = 0.741$) predictions, whereas SR predictions of SSC ($r_p = 0.800$) were comparable to SP NIR predictions ($r_p = 0.810$) but better than SP Vis/SWNIR predictions ($r_p = 0.729$). For estimating the prior storage period of lamb's lettuce based on visible/near infrared reflectance spectroscopy, Jacobs et al. (2016) conducted a research. Different variable selection techniques were combined in this research to improve the accuracy and robustness of the prediction model, while decreasing the number of used wavelengths. The final model used only 10% of the original wavelengths, while the root mean squared error of cross validation decreased from 6.0 to 3.6 days. Moreover, NIR spectroscopy is an advantageous tool also for authenticity and adulteration detection in agricultural produce as Wilde et al. (2019) used. In their research, Near and Fourier-Transform Infrared Spectroscopy has been combined with chemometrics to screen for the substitution of black pepper with papaya seeds, chili and with non-functional black pepper material such as black pepper husk, pinheads and defatted spent materials. Reasonable separation performance between black pepper and adulterated samples could be shown and after running a binary classification model with an external test set an area under the receiver operator characteristic curve of 0.98 for both, the NIR and FT-IR model was obtained.

3.2. Hyperspectral imaging

3.2.1. Basic concepts

Hyperspectral imaging technique integrates imaging technique and spectroscopy simultaneously, obtaining spatial and spectral information at the same time. Hyperspectral images are comprised of a stack of images of the same sample over a large number of contiguous wavebands for each spatial position for a targeted study formulating a 3D data cube (Ariana and Lu 2008). The spatial dimensions of the hyperspectral images are contained in the x rows and y columns while the pixels in the z direction possess the spectral information.

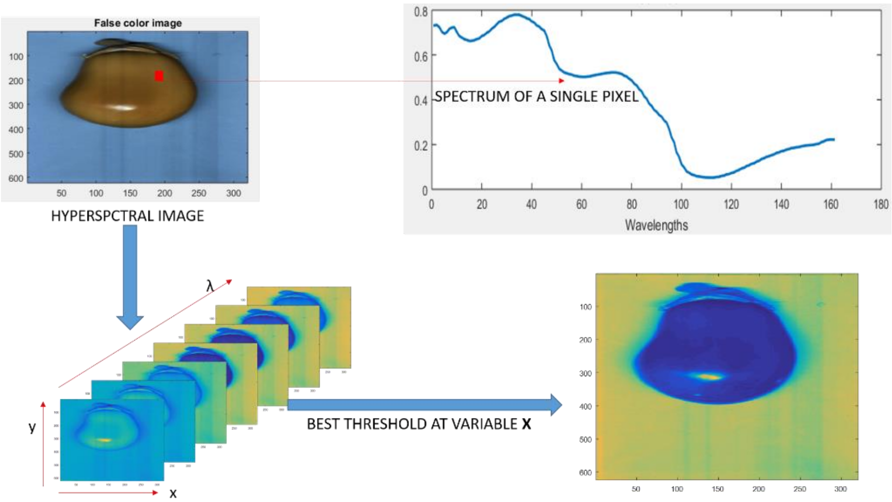


Fig.5. A hyperspectral image

Table 1 shows the significance of the hyperspectral imaging over conventional imaging and spectroscopic techniques. It can be seen that hyperspectral imaging possesses a lot of potentiality in terms of providing nutritional information non-destructively and mapping of nutritional profiles along with defect and damage detection.

Table 1. Overview of differences between imaging, spectroscopy and hyperspectral imaging

Aim of analysis	Spatial information	Spectral information	Mapping of chemical composition
Imaging	**YES**	**NO**	**NO**
Spectroscopy	**NO**	**YES**	**NO**
Hyperspectral imaging	**YES**	**YES**	**YES**

For every pixel in the hyperspectral image the whole spectrum is registered allowing the characterization of the internal composition of the sample in that specific position. The composition of the sample under consideration can be accessed by the spectral dimension whereas the spatial dimension represents the visual location of the compound in question, hence hyperspectral image as a whole providing information to "what and where" in the food sample the desired compound exists (D. Wu and Sun 2013).

3.2.2. Components of hyperspectral imaging

Excitation sources (light), devices for wavelength dispersion and area detectors are the integral components of hyperspectral imaging systems. Light sources are responsible for the illumination of the sample under study hence constituting an essential component of the optical system. Halogen lamps, light emitting diodes (LEDs), lasers and tunable light sources are most significantly used. Halogen lamps are categorized as broadband illumination sources and are commonly used in the visible and near-infrared spectral ranges. An incandescent emission is generated by a filament giving rise to a light source as an output. In this case, a smooth continuous spectrum is generated by the light in the visible and the near infrared regions (Q. Wu, Xu, and Xu 2019). A few disadvantages of the halogen lamps are, a shorter life span, rapid heating, voltage fluctuations affecting output stability and sensitivity to vibrations. A newly used technology named LEDs possess the capability to produce a narrow band light in different wavelengths of the ultraviolet, visible and near infrared regions along with producing high intensity broadband white light. Voltage fluctuations, temperature and certain intensity issues have been listed as the disadvantages of the LEDs as compared to halogens, since LEDs have comparatively lower intensity and increasing the number of LEDs in the bulbs results in grainy light. Presently, in the food research mostly halogen lamps are being used (Amodio et al. 2017; Ariana and Lu 2008; H. Yang, Wu, and Cheng 2011).

Raman and fluorescence applications mostly use monochromatic sources of light namely lasers (Jiménez-Carvelo et al. 2017; C.-C. Yang et al. 2012). Monochromaticity, directionality and coherence are considered as the major characteristics of laser light. In case of tunable light sources, a dispersion device is used between the sample and the light source which works on the basis of area scan mode but are considered as weak light sources for in-line or on-line food analysis applications.

Wavelength dispersion devices are one of the integral part of the hyperspectral imaging systems and serve the purpose of dispersing the broadband light into different wavelengths. The most commonly used wavelength dispersion devices include filter wheels, imaging spectrographs, liquid crystal tunable filters, and Fourier transform imaging spectrographs. Two categories of image spectrographs are transmission gratings and reflectance gratings. The grating is superimposed on a transparent surface in case of transmission gratings whereas, the surface is reflective with a grating superimposed on it in case of the reflectance grating as indicated by the name. The flowchart diagram of the components of a line scan hyperspectral imaging system is shown in Fig. 6 (Qin et al. 2017).

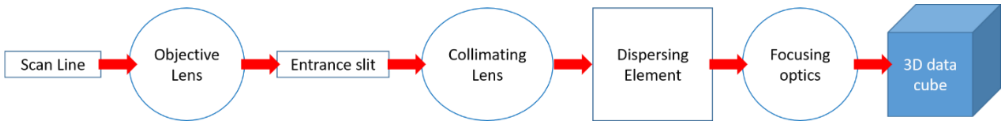


Fig.6. Flow chart diagram of components of line scan hyperspectral imaging system

Reflectance gratings are most commonly used gratings since they have greater advantages over the transmission gratings such as high-quality images with low distortion and larger field size (Bannon and Thomas, 2005). Reflection grating consists of an entrance slit, two spherical mirrors that are concentric, a convex reflection grating that is aberration-corrected, and a detector. When the light ray enters the slit, it is reflected to the reflection grating by one of the two mirrors resulting in the dispersion of the incident beam in a way that the direction of the light propagation is a function of its wavelength (Sun,2010). The second mirror reflects this dispersed light towards the detector forming a continuous spectrum at different pixels. The reflection spectrographs provide a high signal to noise ratio (S/N) and perform well in low light conditions.

Detectors are responsible for converting the incident light photons into electrons and hence quantifying the intensity of the acquired light. The most commonly used area detectors in hyperspectral imaging systems are charge coupled devices (CCD) cameras which contain photovoltaic semiconductor devices. The semiconductor line or area arrays used in most spectral imaging devices are silicon (Si), indium gallium arsenide (InGaAs) and indium antimonide (InSb) most commonly. Since it is economical in terms of cost, temperature range and simple processing; silicon is widely used in semiconductor devices. As shown in Fig.7, silicon (Si) arrays are sensitive towards a wavelength range from 400-1000 nm while in case of longer wavelengths i.e. from 1000-5000 nm, the indium antimonide (InSb) and indium gallium arsenide (InGaAs) are more sensitive. Overlapping detector elements with cooling arrangements are used for sensitivity optimization in different wavelength regions especially for the near infrared regions enhancing the efficiency of the hyperspectral imaging devices.

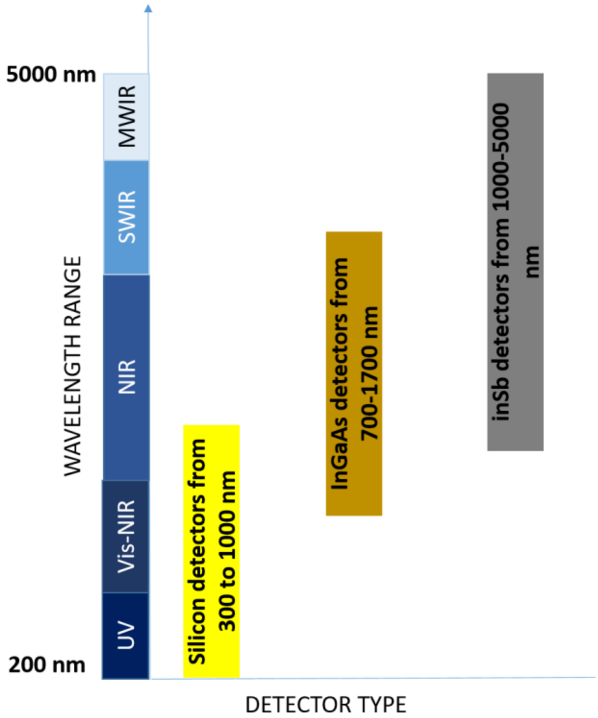


Fig.7. Sensitivity ranges of detector materials over the electromagnetic spectrum

3.2.3. Hyperspectral image acquisition mode

There are three different modes conventionally used for hyperspectral image acquisition; point scan, line scan and area scan. Fig. 8 depicts the three acquisition methods.

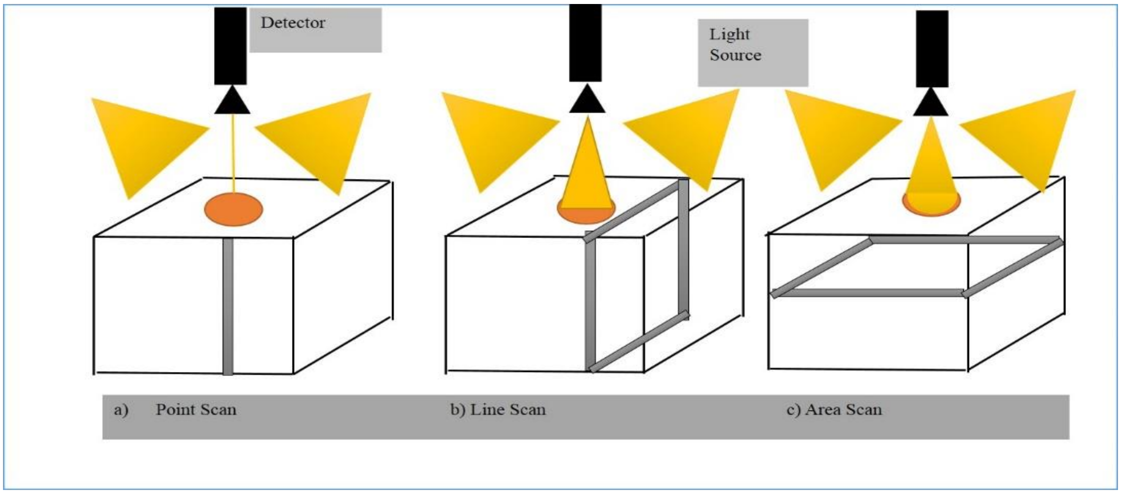


Fig.8. Acquisition modes of hyperspectral images (*adopted from Amodio et al., 2017)*

Area scanning or staring imaging mode, also known as focal plane scanning imaging in which the image field of view is fixed and images are acquired wavelength by wavelength making it a wavelength scanning method. In case of point scan as known as whiskbroom method, the spectrum of a single point is measured and the next spectrum is acquired by moving the sample. The push broom or line scan method which is most commonly used in food analysis acquires a spectrum from a sample line which is further instantaneously recorded with the help of an array detector. The line scan method is highly compatible with the conveyor belt system and is the most used in the food industry for online applications.

A 2D grating and detector array are used in line scan devices for scanning an entire line instead of a single pixel at a time.

In this way the acquired image will be a 2D image, where scanned line will take place in pixel row and simultaneously on the second dimension the spectrum of each point is acquired.

4. Chemometric tools

The spectral information acquired from the food products is analyzed towards the formulation of prediction or classification models using multivariate and chemometric tools. Chemometrics is the science of extracting information from the chemical systems using mathematical, statistical and other methods. NIR prediction models are developed with the aim of correlating spectral information of the training datasets to the desired quality parameters acquired using reference methods. Once the calibration model is developed and internally cross validated then the model performance is evaluated by introducing external unknown samples for prediction purposes of relevant parameters. In order to extract the relevant information from the calibration spectral dataset, the first step aims at separating the chemical information from physical changes, using mathematical pre-treatments of spectral signals. Unwanted effects are always a part of the spectroscopic signals referred to as ‘noise’ which might be associated to the instrumentation for spectral acquisition, changes in various environmental factors and signal variations due to sample nature. In case of food analysis, the most commonly used pretreatment to get rid of the scattering and noise in the spectra, are multiplicative scatter correction (MSC), Standard Normal Variate (SNV), and Detrend (DT) (Martens, Naes, and Naes 1992; Nicolaï et al. 2007b; Roy et al. 1993) and derivatives (Savitzky and Golay 1964).

After the spectral data has been preprocessed then the next step is the development of a calibration equation to correlate spectral information/signals with the chemical features for prediction of quality parameters of unknown samples (Guthrie, Liebenberg, and Walsh 2006). In order to develop this relationship between the spectra and the chemical features, regression techniques are used (Cozzolino et al. 2006, 2009; Martens, Naes, and Naes 1992; N1s et al. 1996; Nicolaï et al. 2007b; Reeves, McCarty, and Meisinger 1999). The techniques used for this purpose include, linear regression methods for calibration development (De Jong 1990; Lodder 2002)such as Multiple Linear Regression (MLR), the Principal Component Regression (PCR) and the Partial Least Squares (PLS) (Perez-Marin, Garrido-Varo, and Guerrero 2007).

4.1. Principal component analysis (PCA)

Large multivariate data is decomposed into a limited number of independent factors which are linear combinations of the original variables using the PCA models (Lodder 2002). PCA is a data exploration tool which provides information about the spectra population in terms of variables, sample distribution and trends in the data and abnormal sample detection. This technique is aimed to describe the maximum variability of the samples by reducing the information contained in all the variables to a limited number of principal components (PCs) which can be also used to detect sample outliers (samples with different spectral behavior). Each PC provides the direction of maximum variability in the data and all PCs are orthogonal to each other. Mathematical representation of a PCA model can be given as:

$$X = TP^T + E \quad \text{(Equation 1)}$$

Where, X is the data matrix, T represents the loadings, P depicts the scores and E stands for residuals/error. Indeed, achieved scores refer to the distance of the projected samples to the center of the new axis. On the other hand, since the orthogonal PCs are projected in a new space the relationship between this new axis and the old axis is represented by the loadings (Bro and Smilde 2014). In another word, the scores in a PCA model represent the samples while the loadings are affiliated with the variables/wavelengths under study.

4.2. Regression models

Regression model is used to describe a group of methods that summarize the degree of association between one variable (or set of variables) and another variable (or set of variables). Multivariate linear regression (MLR) is very similar to the simple regression. The difference is that in case of simple regression one independent variable is correlated with one dependent variable while in case of MLR many independent variables attempt to correlate with one dependent variable at the same time utilizing the least squares method hence finding the smallest sum of squares of residuals. In case of MLR, the data is converted into information when the variables or factors are in a fewer number, possessing no significant collinearity or redundancy along with having good relationship to responses. Therefore, in case of MLR a linear relationship (first order) is established between the measured characteristics from m number of independent variables (x_j where j=1 - m) and a dependent variable (y) hence mathematically depicting the relationship as:

$$y = b_1x_1 + b_2x_2 + \cdots + b_mx_m + e \qquad \text{(Equation 2)}$$

Where, y is the dependent variable, x_1 to x_m are the independent variables, b_j are the coefficients and e is the residual or error term. Equation 2 can therefore also be represented as:

$$y = \sum_{j=1}^{m} b_jx_j + e \qquad \text{(Equation 3)}$$

When the number of observations are less than the number of factors, MLR might provide models that would fits the training data very well but will fail to provide good and reliable results for the prediction of the new data (unknown samples), a phenomenon known as overfitting. Out of a large number of measured/recorded factors, only a few underlying factors exist that are responsible to contribute towards the variation of the response to a large extent. This is the point where the partial least squares regression (PLSR) come into play aiming towards the extraction of these underlying factors, followed by the prediction of Y-scores from these extracted factors (X-scores). The regression model is then simplified as the relationship is concentrated on the smallest possible number of underlying variables. When the number of variables/factors is large possessing high collinearity among them, and when there is a need to take into account the reference value of the parameter for each sample along with the spectral information then PLSR is the best choice to use (Jansen et al. 2005). PLSR emphasizes to predict the responses rather than grasping the sense of the underlying relationships between these responses, which can further be easily extended for the prediction of several quality attributes simultaneously, in which case the algorithm is known as PLS2 (Lavine 2003). The mathematical comparison of the outer relationship can be done with the PCA in this case which in case of X block is:

$$\boldsymbol{X} = \boldsymbol{TP'} + \boldsymbol{E} = \sum t_r p_r' + E \qquad \text{(Equation 4)}$$

Similarly, for the Y block the outer relation can be represented as:

$$\boldsymbol{Y} = \boldsymbol{U}\boldsymbol{Q}' + \boldsymbol{F} = \sum u_r q_r' + F \qquad \text{(Equation 5)}$$

The goal is to provide a description of Y in the best possible way and minimizing ||F|| along with simultaneous achievement of a meaningful relationship between X and Y. Plotting the scores of X block (t) and scores of Y block (u) for each component results in the provision of the inner relationship (linking X and Y). In case of a simple linear model the inner relationship can be mathematically depicted as:

$$\hat{u} = b_r t_r \qquad \text{(Equation 6)}$$

Where, $b_r = u_r' t_r / t_r' t_r$ and is the regression coefficient in case of the PLSR model.

As an additional equation Y=TBQ^'+F provides the mixed relationship where ||F|| is minimized. In this case the blocks receive each other's scores using the iterative method resulting in the provision of a better understanding of the inner relationship. Weights are introduced to achieve orthogonality among the X scores (Geladi and Kowalski 1986). The predictive ability of a PLSR calibration model is tested by the application of internal/cross-validation (Shenk and Westerhaus 1995). In this case the calibration dataset is divided into several sub-groups (depending on the number of samples); upon the development of a calibration equation every validation group is taken one by one and is predicted by using the model built on the remaining groups hence preventing the possibility of overfitting (Williams and Norris 1987; Shenk and Westerhaus 1995). The steps to formulate a calibration model are shown in the flow chart depicted in Fig. 9.

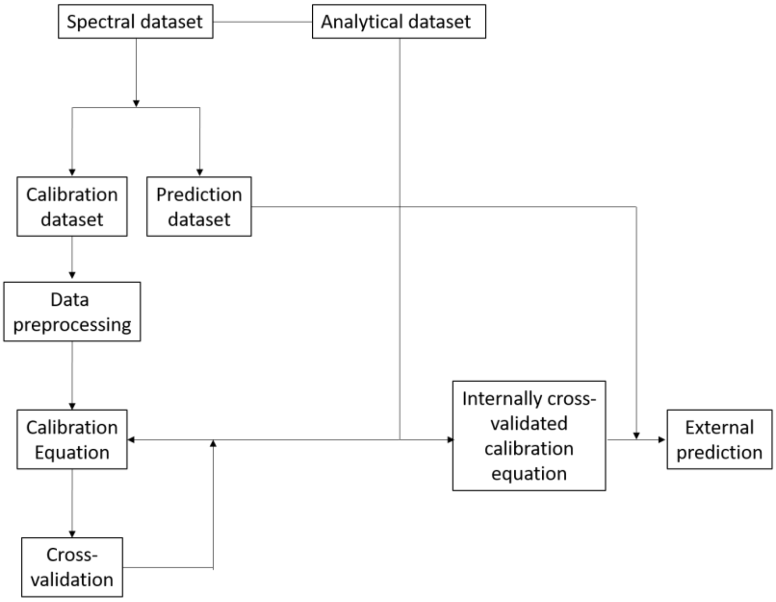


Fig. 9. Steps involved in the development of a calibration model leading to prediction

For the performance evaluation of the internally cross-validated calibration model, the statistical parameters including the standard error of calibration (SEC), the coefficient of determination between predicted and measured parameters (R2), the standard error of cross validation (SECV) and coefficient of determination for cross validation (R2) are compared (Williams and Norris 1987). Similar parameters are brought under consideration while evaluating the performance of external prediction. All the aforementioned errors are taken under consideration for the model performance analysis since errors are always associated to the chemometric methods due to sampling, sample preparation, and instrumental noise and can only be partially reduced. Sampling errors are not a function of the underlying process but they are the real errors, which are significant cause of deviation in the measurements. The sample preparation errors can occur during the various stages of the chemical process; errors in all these stages are often called 'uncertainty'. Instrumental noise is another type of error that occurs due to the measurement process or instrument because of the various factors influencing instruments such as the effect of fluctuating voltages on filters and lamps.

4.3. Classification models

For the classification purposes, in case of food analysis, partial least squares discriminant analysis (PLSDA) is widely used which is a supervised algorithm based on the relationship between the sample characteristics and their recorded spectral intensities. In this case for calibration purposes training of the PLSDA model is done to compute the membership values assigning a different membership value to every class. The aim of the PLSDA model is to develop a precise prediction threshold, therefore, the sample values above this prediction threshold are assigned to a specific class. The PLSDA model accuracy can be evaluated using statistical parameters including sensitivity, true positive rate, specificity, true negative rate and non-error rate. Sensitivity refers to the ability of the PLSDA model to correctly classify the samples whereas the specificity refers to the correct identification of the samples belonging to another class. Once the sensitivity and the specificity of the samples are measured, these can lead to the development of valuable indices such as the non-error rate (NER) which basically represents the percentage of the correctly classified samples and is the average of true positive rates calculated over the number of classes. Mathematically, the non-error rate (NER) can be depicted as:

$$Sensitivity\ (SEN) = \frac{true\ positives}{(true\ positives + false\ negatives)} \quad \text{(Equation 7)}$$

$$Specificity\ (SPEC) = \frac{true\ negatives}{(true\ negarives + false\ positives)} \quad \text{(Equation 8)}$$

$$NER = \frac{\sum_{i=1}^{n} Sens}{n} \quad \text{(Equation 9)}$$

Where, n represents the number of classes.

5. Hyperspectral image processing

In order to make the hyperspectral image applicable and beneficial, there are some common methods that should be done, as described in the process operation flow in Fig. 10.

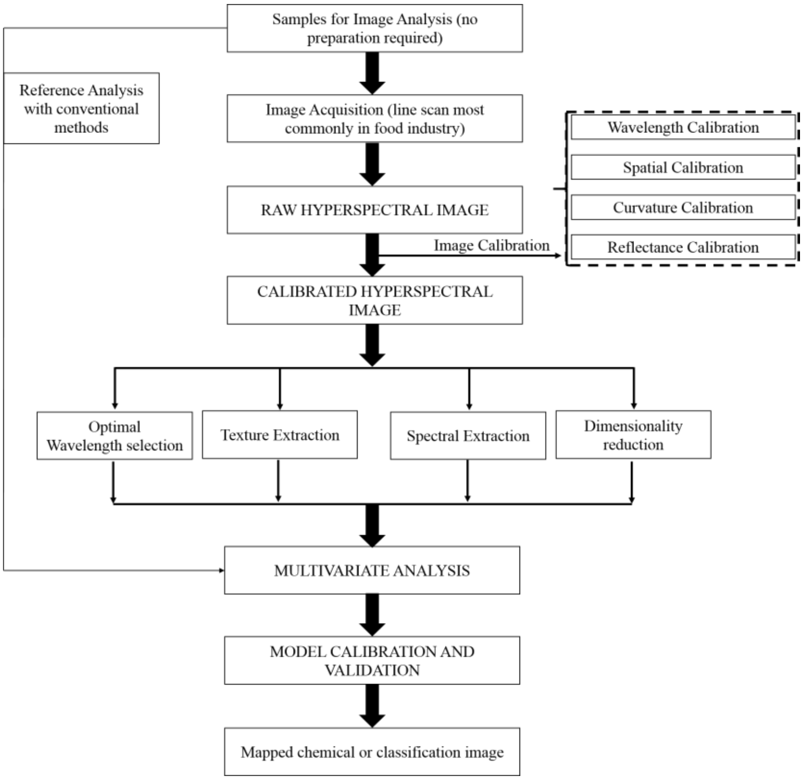


Fig.10. Hyperspectral image processing flow chart (*adopted from Chaudhry et al., 2018)*

Firstly, the hyperspectral image should be calibrated. For this purpose, mainly, wavelength, spatial and curvature calibration is done followed by reflectance calibration (Wu and Sun 2013). The major aims for the calibration of the hyperspectral imaging systems include the standardization of the spectral axis, determination of correct operation of the system, acquisition and validation of the spectral data and diagnosing of instrumental errors. The succeeding process is image segmentation which is a process of dividing the image into different parts or regions, in other words into sets of pixels simplifying the image. An image is, in fact, comprised of many pixels and each pixel can be similar to the corresponding neighboring pixels with respect to a particular characteristic or property as intensity, color values, or textural characteristics. Following the thresholded image, data of hyperspectral must be extracted due to the specific objective (e.g. extraction of averaged pixel data, pixel-based data extraction, and extraction of texture information). In a hyperspectral image each pixel possesses its own spectra, which can be used for the characterization of the composition of that specific pa and the spatial images provide the surface feature information. The composition of the sample under consideration can be accessed by the spectral dimension whereas the spatial dimension helps in visualizing the location of a particular chemical compound or in other words, mapping the constituent concentrations. In the mapping procedure, hyperspectral images at the feature wavebands will be first extracted and constructed into a multispectral data-cube (x, y, $\lambda_{selected}$); subsequently, the data-cube is unfolded into a two-dimensional matrix (x×y, $\lambda_{selected}$). At this point a calibration models can be built, as described in par. 1.5, obtaining the regression or classification coefficients. The vector (x×y) are then multiplied by the coefficients and then re-folded into a color map where the predicted parameters are represented by different color for each pixel, based on a concentration color scales. Therefore, physiochemical distribution can be visualized clearly on the color map (Pu and Sun 2015). As example, in Fig. 11 the concentration map of soluble solids for a fennel slice is shown, as reported from Amodio et al. (2018).

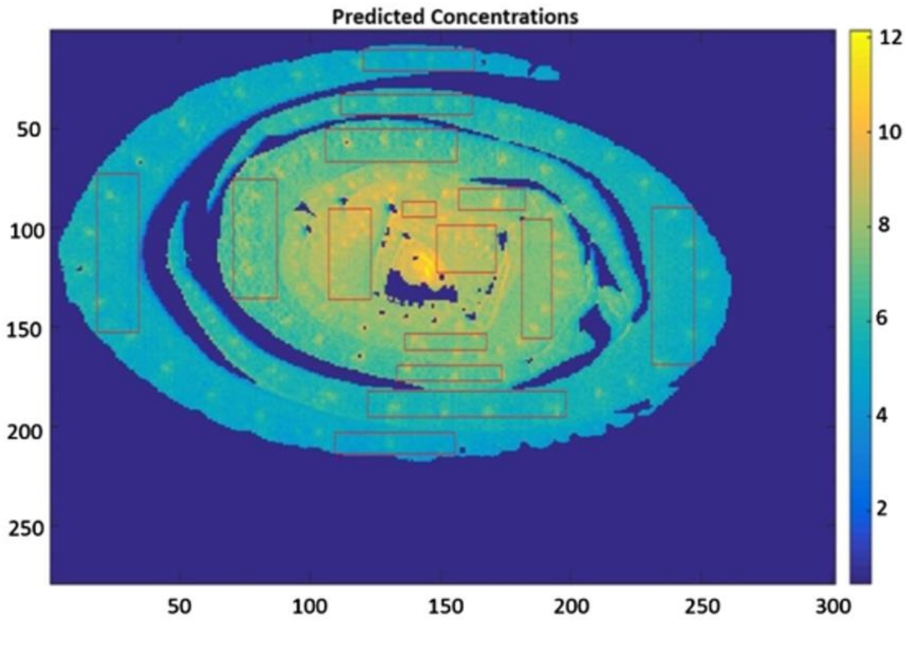


Fig. 11. Distribution map of SSC concentration over a fennel section (*from Amodio et al., 2018)*

Reference

“About the Editor A2 - Sun, Da-Wen BT - Hyperspectral Imaging for Food Quality Analysis and Control.” 2010. : vii–viii. https://www.sciencedirect.com/science/article/pii/B9780123747532100188.

Aghdam, Morteza Soleimani, and Samad Bodbodak. 2013. “Physiological and Biochemical Mechanisms Regulating Chilling Tolerance in Fruits and Vegetables under Postharvest Salicylates and Jasmonates Treatments.” *Scientia Horticulturae* 156: 73–85. http://dx.doi.org/10.1016/j.scienta.2013.03.028.

Alba-Jiménez, J.E. et al. 2018. “Chilling Injury Is Associated with Changes in Microsomal Membrane Lipids in Guava Fruit (Psidium Guajava L.) and the Use of Controlled Atmospheres Reduce These Effects.” *Scientia Horticulturae* 240: 94–101. https://www.sciencedirect.com/science/article/pii/S0304423818303522 (November 6, 2019).

Ali, Zainon Mohd, Lieng-Hong Chin, Muthusamy Marimuthu, and Hamid Lazan. 2004. “Low Temperature Storage and Modified Atmosphere Packaging of Carambola Fruit and Their Effects on Ripening Related Texture Changes, Wall Modification and Chilling Injury Symptoms.” *Postharvest Biology and Technology* 33(2): 181–92. https://www.sciencedirect.com/science/article/pii/S0925521404000511 (November 6, 2019).

Amodio, Maria Luisa, Imperatrice Capotorto, Muhammad Mudassir Arif Chaudhry, and Giancarlo Colelli. 2017. “The Use of Hyperspectral Imaging to Predict the Distribution of Internal Constituents and to Classify Edible Fennel Heads Based on the Harvest Time.” *Computers and Electronics in Agriculture* 134: 1–10. https://www.sciencedirect.com/science/article/pii/S0168169916307220 (May 13, 2019).

Ariana, Diwan P, and Renfu Lu. 2008. “Quality Evaluation of Pickling Cucumbers Using Hyperspectral Reflectance and Transmittance Imaging: Part I. Development of a Prototype.” *Sensing and Instrumentation for Food Quality and Safety* 2(3): 144–51. https://doi.org/10.1007/s11694-008-9057-x.

Babalar, Mesbah et al. 2018. “Arginine Treatment Attenuates Chilling Injury of Pomegranate Fruit during Cold Storage by Enhancing Antioxidant System Activity.” *Postharvest Biology and Technology* 137: 31–37. https://www.sciencedirect.com/science/article/pii/S0925521417303538 (November 6, 2019).

Bro, Rasmus, and Age K Smilde. 2014. “Principal Component Analysis.” *Analytical Methods* 6(9): 2812–31. http://dx.doi.org/10.1039/C3AY41907J.

Bureau, Sylvie, Daniel Cozzolino, and Christopher J. Clark. 2019. “Contributions of Fourier-Transform Mid Infrared (FT-MIR) Spectroscopy to the Study of Fruit and Vegetables: A Review.” *Postharvest Biology and Technology* 148: 1–14. https://www.sciencedirect.com/science/article/pii/S0925521418305660 (November 7, 2019).

Cai, Yuting, Shifeng Cao, Zhenfeng Yang, and Yonghua Zheng. 2011. “MeJA Regulates Enzymes Involved in Ascorbic Acid and Glutathione Metabolism and Improves Chilling Tolerance in Loquat Fruit.” *Postharvest Biology and Technology* 59(3): 324–26. http://dx.doi.org/10.1016/j.postharvbio.2010.08.020.

Clark, C. J., and S. K. Forbes. 1994. “Nuclear Magnetic Resonance Imaging of the Development of Chilling Injury in ‘Fuyu’ Persimmon (Diospyros Kaki).” *New Zealand Journal of Crop and Horticultural Science* 22(2): 209–15.

Concellón, Analía, María C. Añón, and Alicia R. Chaves. 2005. “Effect of Chilling on Ethylene Production in Eggplant Fruit.” *Food Chemistry* 92(1): 63–69.

———. 2007a. “Effect of Low Temperature Storage on Physical and Physiological Characteristics of Eggplant Fruit (Solanum Melongena L.).” *LWT - Food Science and Technology* 40(3): 389–96.

———. 2007b. “Effect of Low Temperature Storage on Physical and Physiological Characteristics of Eggplant Fruit (Solanum Melongena L.).” *LWT - Food Science and Technology* 40(3): 389–96. https://www.sciencedirect.com/science/article/pii/S0023643806000211 (November 6, 2019).

Concellón, Analía, María J. Zaro, Alicia R. Chaves, and Ariel R. Vicente. 2012. “Changes in Quality and Phenolic Antioxidants in Dark Purple American Eggplant (Solanum Melongena L. Cv. Lucía) as Affected by Storage at 0 °C and 10 °C.” *Postharvest Biology and Technology* 66: 35–41. https://www.sciencedirect.com/science/article/pii/S0925521411002900 (November 6, 2019).

Cozzolino, D. et al. 2009. “A Brief Introduction to Multivariate Methods in Grape and Wine Analysis.” *International Journal of Wine Research* 1(1): 123–30.

Cozzolino, D et al. 2006. “Analysis of Grapes and Wine by near Infrared Spectroscopy.” *Journal of Near Infrared Spectroscopy* 14(5): 279–89. https://doi.org/10.1255/jnirs.679.

Fan, Linlin et al. 2016. “Methyl Jasmonate Delays Postharvest Ripening and Senescence in the Non-Climacteric Eggplant (Solanum Melongena L.) Fruit.” *Postharvest Biology and Technology* 120: 76–83. https://www.sciencedirect.com/science/article/pii/S0925521416301041

(October 16, 2019).

Farneti, Brian, Rob E. Schouten, and Ernst J. Woltering. 2012. “Low Temperature-Induced Lycopene Degradation in Red Ripe Tomato Evaluated by Remittance Spectroscopy.” *Postharvest Biology and Technology* 73: 22–27. https://www.sciencedirect.com/science/article/abs/pii/S092552141200110X (March 7, 2020).

Geladi, Paul, and Bruce R. Kowalski. 1986. “Partial Least-Squares Regression: A Tutorial.” *Analytica Chimica Acta* 185: 1–17. https://www.sciencedirect.com/science/article/pii/0003267086800289 (February 2, 2020).

Guthrie, J A, C J Liebenberg, and K B Walsh. 2006. “NIR Model Development and Robustness in Prediction of Melon Fruit Total Soluble Solids.” *Australian Journal of Agricultural Research* 57(4): 411–18. https://doi.org/10.1071/AR05123.

Hale, G. et al. 2013. “Using Non-Destructive Methods to Correlate Chilling Injury in Nectarines with Fruit Maturity.” *Acta Horticulturae* 1012: 83–90.

Hara, Risa et al. 2018. “Excitation Wavelength Selection for Quantitative Analysis of Carotenoids in Tomatoes Using Raman Spectroscopy.” *Food Chemistry* 258: 308–13. https://www.sciencedirect.com/science/article/pii/S0308814618305223 (November 11, 2019).

Hardenburg, R E, A E Watada, and C Y Wang. 1986. Agriculture Handbook, USDA *The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks.*

Hashim, Norhashila et al. 2013. “An Approach for Monitoring the Chilling Injury Appearance in Bananas by Means of Backscattering Imaging.” *Journal of Food Engineering* 116(1): 28–36.

Huang, Yuping, Renfu Lu, and Kunjie Chen. 2018. “Assessment of Tomato Soluble Solids Content and PH by Spatially-Resolved and Conventional Vis/NIR Spectroscopy.” *Journal of Food Engineering* 236(May): 19–28. https://doi.org/10.1016/j.jfoodeng.2018.05.008.

Jacobs, Bert A.J.G. et al. 2016. “Estimation of the Prior Storage Period of Lamb’s Lettuce Based on Visible/near Infrared Reflectance Spectroscopy.” *Postharvest Biology and Technology* 113: 95–105. https://www.sciencedirect.com/science/article/pii/S092552141530168X (November 11, 2019).

Jansen, Jeroen J. et al. 2005. “ASCA: Analysis of Multivariate Data Obtained from an Experimental Design.” *Journal of Chemometrics* 19(9): 469–81.

Jiménez-Carvelo, Ana M. et al. 2017. “Chemometric Classification and Quantification of Olive Oil in Blends with Any Edible Vegetable Oils Using FTIR-ATR and Raman Spectroscopy.” *LWT* 86: 174–84. https://www.sciencedirect.com/science/article/pii/S0023643817305297 (February 2, 2020).

De Jong, Sijmen. 1990. “Multivariate Calibration, H. Martens and T. Naes, Wiley, New York, 1989. ISBN 0 471 90979 3. Price: £75.00, US$138.00. No. of Pages: 504.” *Journal of Chemometrics* 4(6): 441. https://doi.org/10.1002/cem.1180040607.

Klein, Joshua D, and Susan Lurie. 1991. “Postharvest Heat Treatment and Fruit Quality.” *Postharvest News and Information* 2(1): 15–19.

Kosson, R. 2003. “Chlorophyll Fluorescence and Chilling Injury of Green Pepper as Affected by Storage Conditions.” (1999): 379–85.

Larkin, Peter. 2017. *Infrared and Raman Spectroscopy: Principles and Spectral Interpretation*. Elsevier.

Lavine, Barry. 2003. “A User-Friendly Guide to Multivariate Calibration and Classification, Tomas Naes, Tomas Isakson, Tom Fearn and Tony Davies, NIR Publications, Chichester, 2002, ISBN 0-9528666-2-5, £45.00.” *Journal of Chemometrics* 17(10): 571–72. https://doi.org/10.1002/cem.815.

Li, Ling et al. 2019. “New Insights into the Chilling Injury of Postharvest White Mushroom (Agaricus Bisporus) Related to Mitochondria and Electron Transport Pathway under High O2/CO2 Controlled Atmospheres.” *Postharvest Biology and Technology* 152: 45–53. https://www.sciencedirect.com/science/article/pii/S0925521418307075 (November 6, 2019).

Li, Peiyan, Fei Yin, Lijun Song, and Xiaolin Zheng. 2016a. “Alleviation of Chilling Injury in Tomato Fruit by Exogenous Application of Oxalic Acid.” *Food Chemistry* 202: 125–32. https://www.sciencedirect.com/science/article/pii/S0308814616301388 (November 6, 2019).

———. 2016b. “Alleviation of Chilling Injury in Tomato Fruit by Exogenous Application of Oxalic Acid.” *Food Chemistry* 202: 125–32.

Liu, Hui, Weibo Jiang, Jiankang Cao, and Lin Ma. 2018. “A Combination of 1-Methylcyclopropene Treatment and Intermittent Warming Alleviates Chilling Injury and Affects Phenolics and Antioxidant Activity of Peach Fruit during Storage.” *Scientia Horticulturae* 229: 175–81. https://www.sciencedirect.com/science/article/pii/S0304423817306829 (November 6, 2019).

Liu, Ling et al. 2015. “Intermittent Warming Improves Postharvest Quality of Bell Peppers and Reduces Chilling Injury.” *Postharvest Biology and Technology* 101: 18–25.

Lodder, Robert A. 2002. “Handbook of Near-Infrared Analysis, 2nd Ed., Revised and Expanded. Practical Spectroscopy Series Volume 27 Edited by D. A. Burns (NIR Resources) and E. W. Ciurczak (Purdue Pharma LP). Dekker: New York. 2001. Xv + 814 Pp. $225.00. ISBN: 0-8247-0534-3.” *Journal of the American Chemical Society* 124(19): 5603–4. https://doi.org/10.1021/ja015320c.

Lukatkin, Alexander, Ausra Brazaityte, Ceslovas Bobinas, and Pavelas Duchovskis. 2012. “Chilling Injury in Chilling-Sensitive Plants: A Review.” *Agriculture* 99(2): 111–24.

Lurie, S. et al. 2011. “Chilling Injury in Stored Nectarines and Its Detection by Time-Resolved Reflectance Spectroscopy.” *Postharvest Biology and Technology* 59(3): 211–18. https://www.sciencedirect.com/science/article/pii/S0925521410002413 (November 6, 2019).

Lurie, S., R. ronen, and S. Meier. 1994. “Determining Chilling Injury Induction in Green Peppers Using Nondestructive Pulsed Amplitude Modulated (PAM) Fluorometry.” *Journal of the American Society for Horticultural Science* 119(1): 59–62.

Martens, Harald, Tormod Naes, and Tormod Naes. 1992. *Multivariate Calibration*. John Wiley & Sons.

Mirdehghan, S.H. et al. 2007. “Reduction of Pomegranate Chilling Injury during Storage after Heat Treatment: Role of Polyamines.” *Postharvest Biology and Technology* 44(1): 19–25. https://www.sciencedirect.com/science/article/abs/pii/S0925521406003085 (March 6, 2020).

Murata, Takao. 1990. “Relation of Chilling Stress to Membrane Permeability.” *Chilling injury of horticultural crops*: 201–9.

Nıs, T., P. Baardseth, H. Helgesen, and T. Isaksson. 1996. “Multivariate Techniques in the Analysis of Meat Quality.” *Meat Science* 43: 135–49. https://www.sciencedirect.com/science/article/abs/pii/0309174096000617 (February 2, 2020).

Nicolaï, Bart M. et al. 2007a. “Nondestructive Measurement of Fruit and Vegetable Quality by Means of NIR Spectroscopy: A Review.” *Postharvest Biology and Technology* 46(2): 99–118. https://www.sciencedirect.com/science/article/pii/S0925521407002293 (November 7, 2019).

———. 2007b. "Nondestructive Measurement of Fruit and Vegetable Quality by Means of NIR Spectroscopy: A Review." *Postharvest Biology and Technology* 46(2): 99–118. https://www.sciencedirect.com/science/article/abs/pii/S0925521407002293 (February 2, 2020).

Nicolaï, Bart M et al. 2014. "Non-Destructive Evaluation: Detection of External and Internal Attributes Frequently Associated with Quality and Damage." In *Postharvest Handling*, Elsevier, 363–85.

Norris, Karl H. 1964. "Design and Development of a New Moisture Meter." *Agric. Eng* 45(7): 370–72.

Novas, N. et al. 2019. "Development of a Smartphone Application for Assessment of Chilling Injuries in Zucchini." *Biosystems Engineering* 181: 114–27.

Osborne, Brian G. 2006. "Near-infrared Spectroscopy in Food Analysis." *Encyclopedia of analytical chemistry: applications, theory and instrumentation*.

PARKIN, K L et al. 1989. "CHILLING INJURY. A REVIEW OF POSSIBLE MECHANISMS." *Journal of Food Biochemistry* 13(2): 127–53. https://doi.org/10.1111/j.1745-4514.1989.tb00389.x.

Pentzer, W T, and P H Heinze. 1954. "Postharvest Physiology of Fruits and Vegetables." *Annual Review of Plant Physiology* 5(1): 205–24. https://doi.org/10.1146/annurev.pp.05.060154.001225.

Perez-Marin, D, A Garrido-Varo, and J E Guerrero. 2007. "Non-Linear Regression Methods in NIRS Quantitative Analysis." *Talanta* 72(1): 28–42.

Porep, Jan U., Dietmar R. Kammerer, and Reinhold Carle. 2015. "On-Line Application of near Infrared (NIR) Spectroscopy in Food Production." *Trends in Food Science & Technology* 46(2): 211–30. https://www.sciencedirect.com/science/article/pii/S0924224415002174 (November 7, 2019).

Priya Sethu, K.M., T.N. Prabha, and R.N. Tharanathan. 1996. "Post-Harvest Biochemical Changes Associated with the Softening Phenomenon in Capsicum Annuum Fruits." *Phytochemistry* 42(4): 961–66. https://www.sciencedirect.com/science/article/abs/pii/003194229600057X (March 7, 2020).

Pu, Yuan-Yuan, and Da-Wen Sun. 2015. "Vis–NIR Hyperspectral Imaging in Visualizing Moisture Distribution of Mango Slices during Microwave-Vacuum Drying." *Food Chemistry* 188: 271–78. https://www.sciencedirect.com/science/article/pii/S0308814615006779

(February 2, 2020).

Qin, Jianwei et al. 2017. “Line-Scan Hyperspectral Imaging Techniques for Food Safety and Quality Applications.” *Applied Sciences (Switzerland)* 7(2).

Reeves, J B, G W McCarty, and J J Meisinger. 1999. “Near Infrared Reflectance Spectroscopy for the Analysis of Agricultural Soils.” *Journal of Near Infrared Spectroscopy* 7(3): 179–93. https://doi.org/10.1255/jnirs.248.

Roy, Soumya et al. 1993. “Determination of Moisture Content of Mushrooms by Vis—NIR Spectroscopy.” *Journal of the Science of Food and Agriculture* 63(3): 355–60. https://doi.org/10.1002/jsfa.2740630314.

Rui, Huaijing et al. 2010. “Effects of Heat Treatment on Internal Browning Andmembrane Fatty Acid in Loquat Fruit in Response to Chilling Stress.” *Journal of the Science of Food and Agriculture* 90(9): 1557–61.

Sayyari, Mohammad et al. 2011. “Acetyl Salicylic Acid Alleviates Chilling Injury and Maintains Nutritive and Bioactive Compounds and Antioxidant Activity during Postharvest Storage of Pomegranates.” *Postharvest Biology and Technology* 60(2): 136–42. http://dx.doi.org/10.1016/j.postharvbio.2010.12.012.

Sayyari, Mohammad, Morteza Soleimani Aghdam, Fakhreddin Salehi, and Fardin Ghanbari. 2016. “Salicyloyl Chitosan Alleviates Chilling Injury and Maintains Antioxidant Capacity of Pomegranate Fruits during Cold Storage.” *Scientia Horticulturae* 211: 110–17. http://dx.doi.org/10.1016/j.scienta.2016.08.015.

Shi, Junyan et al. 2018. “Low-Temperature Conditioning Enhances Chilling Tolerance and Reduces Damage in Cold-Stored Eggplant (Solanum Melongena L.) Fruit.” *Postharvest Biology and Technology* 141(March): 33–38. https://doi.org/10.1016/j.postharvbio.2018.03.007.

Sitarski, Anna M. 2017. “Development of Spectroscopic Methods for Dynamic Cellular Level Study of Biochemical Kinetics and Disease Progression.” : 242. http://digitalcommons.library.umaine.edu/etd%0Ahttp://digitalcommons.library.umaine.edu/etd/2652.

Smith, Brian C. 2011. *Fundamentals of Fourier Transform Infrared Spectroscopy*. CRC press.

Song, Huwei et al. 2017. “A Cold-Induced Phytosulfokine Peptide Is Related to the Improvement of Loquat Fruit Chilling Tolerance.” *Food Chemistry* 232: 434–42. https://www.sciencedirect.com/science/article/pii/S0308814617306167 (November 6, 2019).

Stratis, Dimitra N et al. 2001. “Comparison of Acousto-Optic and Liquid Crystal Tunable Filters for Laser-Induced Breakdown Spectroscopy.” *Applied* *spectroscopy* 55(8): 999–1004.

Verlinden, Bert E., Veerle De Smedt, and Bart M. Nicolaı̈ 2004. “Evaluation of Ultrasonic Wave Propagation to Measure Chilling Injury in Tomatoes.” *Postharvest Biology and Technology* 32(1): 109–13. https://www.sciencedirect.com/science/article/pii/S0925521403002369 (November 6, 2019).

Wang, C.Y. 2010. “Alleviation of Chiling Injury in Tropical and Subtropical Fruits.” *Acta Horticulturae* 864: 267–74. http://ucce.ucdavis.edu/files/datastore/234-2405.pdf.

Wang, Chien Yi. 1994. “Chilling Injury of Tropical Horticultural Commodities POSTHARVEST TECHNIQUES FOR REDUCING CI.” *Hortscience* 29(9): 986–88.

Wang, L. et al. 2019. “Key Tomato Volatile Compounds during Postharvest Ripening in Response to Chilling and Pre-Chilling Heat Treatments.” *Postharvest Biology and Technology* 154: 11–20. https://www.sciencedirect.com/science/article/pii/S0925521418312146#aep-article-footnote-id9 (November 6, 2019).

Wilde, Amelie S., Simon A. Haughey, Pamela Galvin-King, and Christopher T. Elliott. 2019. “The Feasibility of Applying NIR and FT-IR Fingerprinting to Detect Adulteration in Black Pepper.” *Food Control* 100: 1–7. https://www.sciencedirect.com/science/article/pii/S095671351830639X (November 11, 2019).

Wu, Di, and Da-Wen Sun. 2013. “Advanced Applications of Hyperspectral Imaging Technology for Food Quality and Safety Analysis and Assessment: A Review — Part I: Fundamentals.” *Innovative Food Science & Emerging Technologies* 19: 1–14. https://www.sciencedirect.com/science/article/pii/S1466856413000775 (February 2, 2020).

Wu, Qifang, Jun Xu, and Huirong Xu. 2019. “Discrimination of Aflatoxin B 1 Contaminated Pistachio Kernels Using Laser Induced Fluorescence Spectroscopy.” *Biosystems Engineering* 179: 22–34. https://doi.org/10.1016/j.biosystemseng.2018.12.009.

Yang, Chun-Chieh et al. 2012. “Red to Far-Red Multispectral Fluorescence Image Fusion for Detection of Fecal Contamination on Apples.” *Journal of Food Engineering* 108(2): 312–19. https://www.sciencedirect.com/science/article/abs/pii/S0260877411004341 (February 2, 2020).

Yang, Huqing, Fenghua Wu, and Jiyu Cheng. 2011. “Reduced Chilling Injury in

Cucumber by Nitric Oxide and the Antioxidant Response.” *Food Chemistry* 127(3): 1237–42. http://www.sciencedirect.com/science/article/pii/S0308814611002548.

Zheng, Yonghua, Raymond W.M. Fung, Shiow Y. Wang, and Chien Y. Wang. 2008. “Transcript Levels of Antioxidative Genes and Oxygen Radical Scavenging Enzyme Activities in Chilled Zucchini Squash in Response to Superatmospheric Oxygen.” *Postharvest Biology and Technology* 47(2): 151–58. https://www.sciencedirect.com/science/article/abs/pii/S0925521407002190 (March 7, 2020).

Zude, M, J Sasse, and H Schallnus. 2010. “NON-INVASIVE SENSING OF FRUIT DEVELOPMENT IN BANANA AND PAPAYA BY MEANS OF A SPECTROSCOPIC APPROACH.” In *Acta Horticulturae*, International Society for Horticultural Science (ISHS), Leuven, Belgium, 277–81. https://doi.org/10.17660/ActaHortic.2010.880.32.

PART II: EXPERIMENTAL

General objectives

The general objective of this thesis was to assess the potentiality of various non destructive optical-based techniques for early detection of chilling injury (CI) and freshness of fresh produce. In this regard, three optical instruments i.e. spectroscopy, hyperspectral imaging, and colorimeter in different spectral range were employed in combination with several chemometric methods to develop simplified classification and regression models for aforementioned objectives.

Generally, by using eggplant and bell pepper fruit as models and testing the methods, this thesis was divided to following goals:

- Evaluation of the potentiality of FT-NIR spectroscopy, hyperspectral imaging and colorimeter techniques coupled with supervised classification algorithm, for the early detection of eggplants fruit stored at chilling temperature;
- Evaluation of the potentiality of hyperspectral imaging in VIS-NIR and SWIR range and chemometrics for early detection of chilling injury in green bell peppers
- Evaluation of the potentiality of FT-NIR spectroscopy, hyperspectral imaging and color measurements for the assessment of eggplant fruit freshness
- Characterization of the effects of storage time and temperature on spectral changes induced by chilling injury in eggplant fruit

EARLY DETECTION OF EGGPLANT FRUIT STORED AT CHILLING TEMPERATURE USING DIFFERENT NON DESTRUCTIVE OPTICAL TECHNIQUES AND SUPERVISED CLASSIFICATION ALGORITHMS

Pavlos Tsouvaltzis[1,], Farahmand Babellahi[2], Maria Luisa Amodio[2] and Giancarlo Colelli[2]

[1] *Department of Horticulture, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece*

[2] *Department of Science of Agriculture, Food, and Environment, University of Foggia, 71100, Foggia, Italy*

Published in the *Postharvest Biology and Technology* Journal (2020)

DOI: 10.1016/j.postharvbio.2019.111001

ABSTRACT

Eggplant fruit is a chilling injury sensitive vegetable and should not be stored at lower than 12^{o}C postharvest, although fruit are often placed in temperatures as low as 0- 5^{o}C. For this reason, a rapid early detection of eggplants previously stored at chilling temperatures would allow early removal of those fruit from the market. Eggplant fruit (cv. Fantasy) were stored either at 2^{o}C (chilling injurious temperature) or at 12^{o}C (safe storage temperature) for 10 days. Every 2 days, fruit from each group were sampled and left at room temperature, for one additional day. Color measurements in the CIE L*a*b* mode and reflectance data in the wavelength range 360-740 nm, Fourier Transform (FT)-NIR spectra (800- 2777 nm) and hyperspectral images at the visible (400-1000 nm) and near infrared (900-1700 nm) part of the electromagnetic spectrum were also acquired on each fruit. Three supervised algorithms; partial least square (PLS), supervised vector machine (SVM) and k-nearest neighbor (kNN) were applied to classify fruit according to the storage temperature. Chilling injury (CI) was subjectively evaluated, according to the presence of black seeds or of brown discolored flesh area. According to the results, although chilling injury symptoms started being evident only after the 4th day of storage at 2^{o}C, it was possible to discriminate fruit earlier, since day 2, by processing the FT-NIR spectral data with the SVM classifier (100 and 92% non-error-rate (NER)) in calibration and cross validation, respectively) in the whole period data set. Color or FT-NIR spectral data classified with PLSDA permitted relatively good classification of fruit (>83 % accuracy) since the 4th day of storage, while L, C, H^{o} color measurements or Vis-NIR hyperspectral imaging data combined with PLSDA generate trustworthy models only after the 6th day of storage. On the other hand, NIR hyperspectral imaging technique and kNN classification algorithm were incapable to separate the fruit either accurately or consistently. These results indicate a good potential of adapting selected protocols, in terms of technique, processing of the raw data and supervised classification algorithm, in order to minimize postharvest losses induced by the improper temperature management of chilling

sensitive fruit, such as the eggplants.

Keywords: chilling injury; supervised classification; Non-Error-Rate (NER); FT-NIR; hyperspectral image

1. Introduction

Eggplant (*Solanum melongena* L.) is a common annual vegetable crop grown in the sub-tropics and tropics, popular in Asia and some Mediterranean countries (Concellón et al., 2012).

Although, storage of fruit and vegetables at low temperatures is recommended as an effective means for preserving quality and nutritional value, plants originating in tropical and subtropical regions, such as eggplant, are prone to a physiological injury, known as chilling injury (CI), which is induced when they are exposed to temperatures below 12^{o}C but above their freezing point (Concellón et al., 2004; Megías et al., 2016).

Development of CI in eggplants can be perceived after slicing the fruit and revealing the internal flesh by detecting symptoms such as the darkening of seeds and pulp tissue (Concellón et al., 2005, 2004; Gao et al., 2015). Indeed, these symptoms become evident only after the produce is placed in warmer temperatures (ElMasry et al., 2009; Hashim et al., 2013). Some color changes may also appear in the skin, but they are barely perceived (Concellon et al., 2007). Simultaneously, other changes such as organic acids content ratio (Kozukue et al., 1978), or 1-aminocyclopropane-1-carboxylic acid (ACC) and 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC) and ethylene production increase (Concellón et al., 2005) may be considered as indication of CI development. Increases in PPO, POD, and PAL activities in eggplant pulp are also associated with damage caused by storage of eggplants at low, chilling injurious temperatures (Gao et al., 2015). As a result, the quality and shelf life of fruit are reduced and economic losses during transportation, storage and marketing occur (Carvajal et al., 2011; Hashim et al., 2013), while consumer acceptability is negatively affected due to unpleasant appearance and concomitant off-flavor development (Concellón et al., 2004; Lurie et al., 2011).

Therefore, it would be greatly advisable to develop rapid, reliable, and nondestructive tools for early detection of improperly stored fruit, so as to remove them as fast as possible from the marketing chain and increase consumer satisfaction and industry profitability (Lurie et al., 2011).

Nondestructive optical techniques are considered as the most promising for meeting these requirements. Optical techniques that are based on imaging and spectroscopy have been widely used for quality assessment and safety inspection of fresh agricultural commodities (Amodio et al., 2017; Francia et al., 2007; Huang et al., 2014; Li et al., 2016; Lorente et al., 2012). These techniques exploit the information that is returned after the exposure of commodities under light. In particular, the incident light induces interactions in which about 4 % of the photons will be reflected at the fruit surface while the remaining part enters the fruit tissue and is absorbed, transmitted, or scattered back (diffuse reflectance) from the region close to the incident point (Birth, 1976). Therefore, to some extent, the recording spectrum shows the chemical properties of the fruit and the spectra of fruit at different physiological statuses can thus be different (Sun et al., 2017). Unlike conventional spectroscopic or imaging systems, hyperspectral imaging obtains spectral information for each spatial pixel, producing hypercube image data which contains both spectral and spatial information, which are critical for reliable and comprehensive analysis of product properties or characteristics (Cen et al., 2016). According to the most recent results, hyperspectral reflectance imaging technique was successfully applied for the non-destructive optical detection of chilling injury on peaches, apples, and cucumbers (Cen et al., 2016; ElMasry et al., 2009; Hashim et al., 2013; Sun et al., 2017). Other techniques, such as time-resolved reflectance spectroscopy have also been used in identifying chilling injured nectarines by detecting internal woolliness and internal browning in fruit after storage at low temperatures (Lurie et al., 2011), but no studies regarding detection of CI on eggplant fruit have been already performed. Moreover, all these studies detect CI when the symptoms are already fully developed, while an early detection of CI would be much more valuable for both consumers and distributors.

The objective of this research was the development of an efficient non-destructive technique, in order to facilitate early and reliable detection of eggplant fruit stored at chilling injury temperatures during postharvest operations. In particular, the aim of the study was to test whether it is feasible to discriminate fruit according to their postharvest storage temperature by selection of the most accurate technique in terms of accuracy (% non-error-rate (NER) of classification) and reliability (repeatability within each storage day) and generate the best discriminant algorithm, in order to develop a complete method to be transferred to the market chain.

2. Material and methods

2.1. Plant material

Immature eggplant fruit (cv. Fantasy) were harvested from a commercial farm near Lecce and were transferred within 5 hours at the Postharvest facilities of the University of Foggia. Fruit were stored at 12^{o}C for 24 hours before being sorted, so that only sound ones were selected and divided into 11 groups with 12 fruit in each group.

One group represented Day 0 fruit while the rest 10 groups of eggplant fruit were divided into 2 sets. Each set was stored either at 2^{o}C (chilling injury conditions) or at 12^{o}C (safe conditions) for 2, 4, 6, 8 and 10 days. After each time of storage, fruit were left for 24 hours at 20^{o}C (shelf life), before measurement of external color and acquisition of hyperspectral images and FT-NIR spectra. Fruit were then used for destructive evaluation, as severity of CI and spectrophotometric reading of the peel methanolic extract. The extra day at 20 °C was required, in order to allow the symptoms to develop.

2.2. Color measurements

Color was measured on 3 sides of each fruit longwise (below the calyx, at the center and near the blossom-end) both in the CIE L*a*b* mode, as well as in reflectance scanning mode in the wavelength region 360-740 nm, with a CM-2600d Konica Minolta spectrophotometer and an average of the three readings was calculated. From primary color parameters Lightness (L*), a* and b*, Chroma (C*) and Hue angle (H^o) parameters were also calculated based on the equations: $C^* = (a^{*2} + b^{*2})^{1/2}$ and $H° = \arctan(b^{*2}/a^{*2})$.

2.3. Acquisition of the FT-NIR spectra

Fourier transformed- near infrared (FT-NIR) spectrometry was performed on the eggplant fruit at room temperature (20^{o}C). Three scans were acquired per fruit by manually displacing the fruit along its longwise axis (MPA Multi-Purpose FT-NIR Analyzer, Bruker Optics, Ettlingen, Germany), and an average of the three spectra was calculated. Reflectance mode (log(1/R)) was used during spectral acquisition over the absorbance range of 800-2777 nm (sphere macrosample resolution 1.71 nm, scanner velocity 10 kHz, sample scan time 64 scans, background scan time 64 scans). The instrument was equipped with a high-energy air-cooled NIR source (20 W tungsten-halogen lamp) and a permanently aligned and highly stable ROCKSOLID interferometer (Bruker).

2.4. Acquisition of the hyperspectral images

A hyperspectral line scan scanner (Version 1.4, DV srl, Padova, Italy) equipped with two spectrographs, one in the visible-near infrared (Vis-NIR) range of 400-1000 nm and the second in the near infrared (NIR) range of 900-1700 nm, with a spatial resolution of 1000×2000 pixels and a spectral resolution of 5 nm was used to acquire the images.

The images were calibrated using dark and white references by closing the shutter as a black reference, and using a 99% Spectralon reflectance standard as a white reference. This process has been done by the hyperspectral scanner software (Version 1.4, DV srl, Padova, Italy) using Eq. 1.

$$R = \frac{H - B}{W - B}$$

Where *H* is the raw hyperspectral image; and *B* and *W* are the acquired signals for both black and white references, respectively.

One fruit was taken for each replicate in a single image and self-developed MATLAB code was used for extracting the mean spectra of the fruit producing one spectrum per replicate. For the extraction of the mean spectrum, the original image was thresholded and the best contrast between the object and the background was found. Image thresholding was performed using the Otsu method, on the image depicting the best contrast between the foreground and background, corresponding to 795 nm for the Vis-NIR and 1495 nm for the NIR. A 2D binary image (mask) was obtained, with 0 value for the background and 1 for the fruit tissue. This mask was imposed to extract the mean spectra of the pixels corresponding to the fruit.

2.5. Chilling injury evaluation

After the acquisition of the above non-destructive optical data, fruit were halved for monitoring the chilling injury development during the storage time (Fig.1) Chilling injury was evaluated subjectively on a scale 1= no chilling injury and 2= chilling injured. This evaluation was performed according to either the presence of brown discoloration of the flesh or the blackening of the seeds (Concellón et al., 2004). Fruit obtaining spots of brown flesh and/ or black seeds were considered as a chilling injured one. The CI index was calculated according to the Eq 2:

$$CI = \sum \left(\frac{\text{Injury level } \times \text{No of fruit at that level}}{Total\ No\ of\ fruit} \right)$$

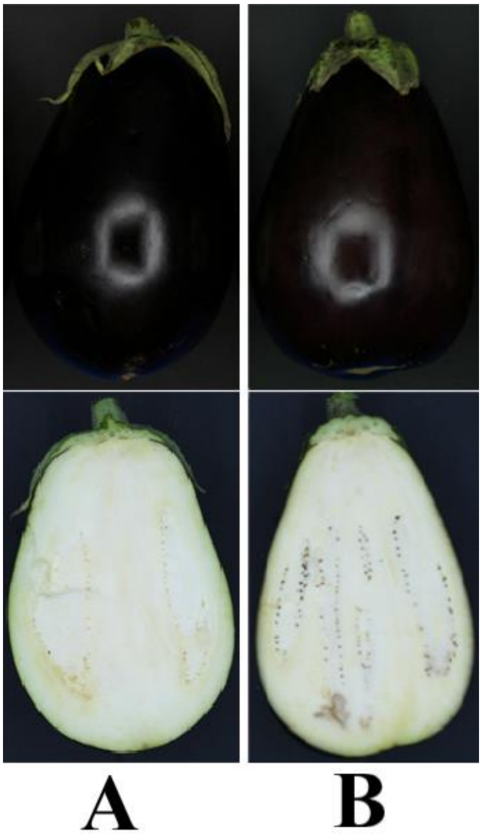


Fig. 1. Digital photos of the peel and the flesh of sound fruits (A), as well as with chilling injury symptoms (B).

2.6. Absorbance of the methanolic peel extract

One gram of peel was excised and extracted in 10 ml methanol 80 % acidified with 0.1 % (v/v) HCl, centrifuged at 10,000×g for 10 mins and the supernatant was placed in a cuvette and the absorbance was measured in the range 400- 700 nm, through scanning mode against a methanolic blank using a UV-1700 Shimadzu spectrophotometer (Jiangsu, China). The difference in the absorbance between 400 and 700 nm ($\Delta Abs_{400-700nm}$) is presented in the results, as a potential indication of the pigment content in the peel of the fruit.

2.7. Data analysis

All color, spectral and image data analyses were carried out using MATLAB (Version R2017, the Math-works Inc., and Natick, MA, USA) and PLS_TOOLBOX 8.6 (Eigenvector Research Inc., Manson, WA, USA). Color reflectance scanning, FT-NIR spectral and hyperspectral imaging data were initially preprocessed accordingly, in order to achieve the highest possible discrimination of fruit, combined with the minimal root mean square calibration and cross-validation errors.

Three supervised classification algorithms including partial least squares-discriminant analysis (PLS-DA), supervised vector machine (SVM), and k-nearest neighbor (kNN) were used for discriminating eggplants stored for one day at ambient temperature after storage at chilling and non-chilling conditions.

The partial least squares discriminant analysis (PLS-DA) model is an algorithm based on the relation between spectral intensity and sample characteristics. For the internal validation purpose, venetian blinds cross validation method was used with two splits of the data with 12 samples per blind. The discriminant analysis with this classifier is based on generated latent variables (LVs) with their number being dependent according to the lowest cross validation error and the highest possible non-error-rate, simultaneously.

SVM is a discriminant classifier based on finding the hyperplane that gives the largest minimum distance to the training data set and was implemented based on the quadratic programming optimization using a radial basis kernel (Cen, 2016).

kNN is a non-parametric instance-based learning algorithm based on a similarity measure such as distance function. The optimal value of k= 2 was chosen by first testing the data.

The validity of the three classifiers was evaluated by the overall accuracy in discriminating each class (fruit stored at chilling and non-chilling conditions) using pooled data from all storage days, but most importantly by the successive accuracy in each day of storage. In the figures, the mean value of calibration and cross-validation NER is presented ± standard error between the two classes (non-chilling and chilling conditions). Chilling injury scores and $\Delta Abs_{400-700nm}$ of peel extract are presented as the mean values of 12 fruit ± S.E.

3. Results

3.1. Chilling injury evaluation

The chilling injury started appearing on fruit only after the 4th day of storage at 2°C + 1 day of shelf life at 20°C. In particular, 8 out 12 fruit exhibited symptoms of either seeds blackening or flesh browning giving a score of 1.67, which indeed were aggravated since day 6 being apparent in up to 11 out of the 12 fruit, irrespectively of the severity of symptoms with a score of 1.92 (Fig. 2).

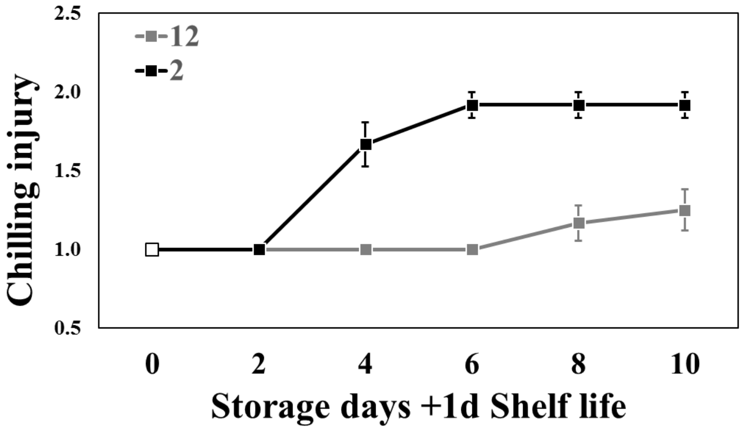


Fig. 2. Chilling injury evaluation of eggplant fruits stored at 2 or 12 °C. The CI on fruit was scored as 1 = no chilling on fruit and 2 = chilling injury on the inner flesh as assessed by the presence of black seeds and brown discoloration of the flesh, irrespectively of the intensity of the symptom. Each data point represents the mean of 12 fruit ± standard error (S.E.)

3.2. Absorbance of the methanolic peel extract

Scanning the methanolic peel extract revealed significant differences among fruit previously stored at 2 or 12°C (Fig. 3). Particularly, since day 4, the $\Delta Abs_{400-700nm}$ values in fruit stored at chilling injury temperature started declining until the end of storage, being lower hereinafter than fruit stored at 12°C, with the latter ones maintaining the initial levels during the whole storage period (Fig. 3).

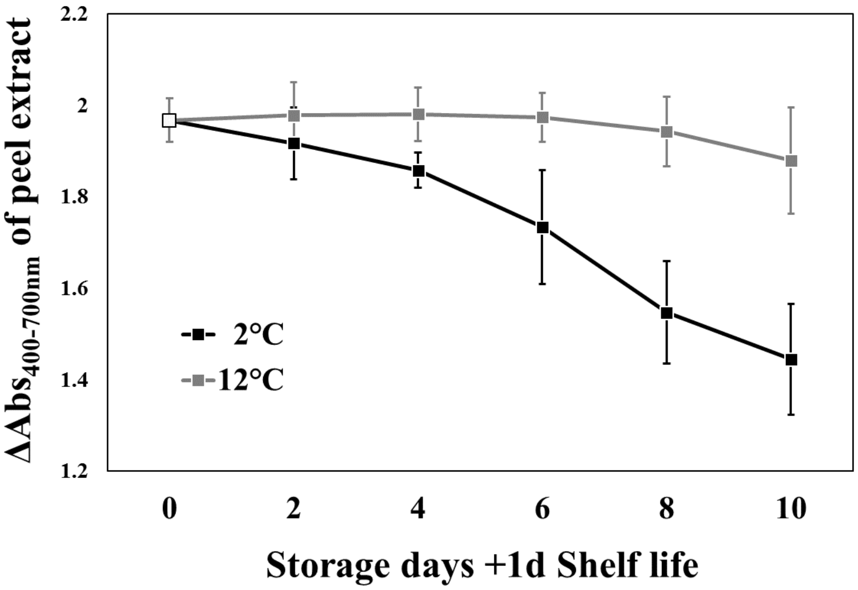


Fig. 3. Absorbance of the methanolic extract (ΔAbs400-700nm) of the eggplant's peel tissue. Each data point represents the mean of the ΔAbs of 12 fruit ± standard error (S.E.).

3.3. Color measurements

Among the color parameters, a* values followed a trend like the chilling injury development and indeed after day 4 there were significant differences among fruit that were stored in the two different temperatures (Fig. 4A). Although, a* on the peel of eggplant fruits that were stored at 12^{o}C remained constant during 10 days of storage, followed by one extra day at 20^{o}C, in the case of storage at 2^{o}C a significant increase was observed after day 4. In the same way, the ratio 740/660 nm that was acquired using the spectrophotometer also exhibited different pattern among fruit stored in the two temperatures (Fig. 4B), allowing their discrimination after the 4th day of storage.

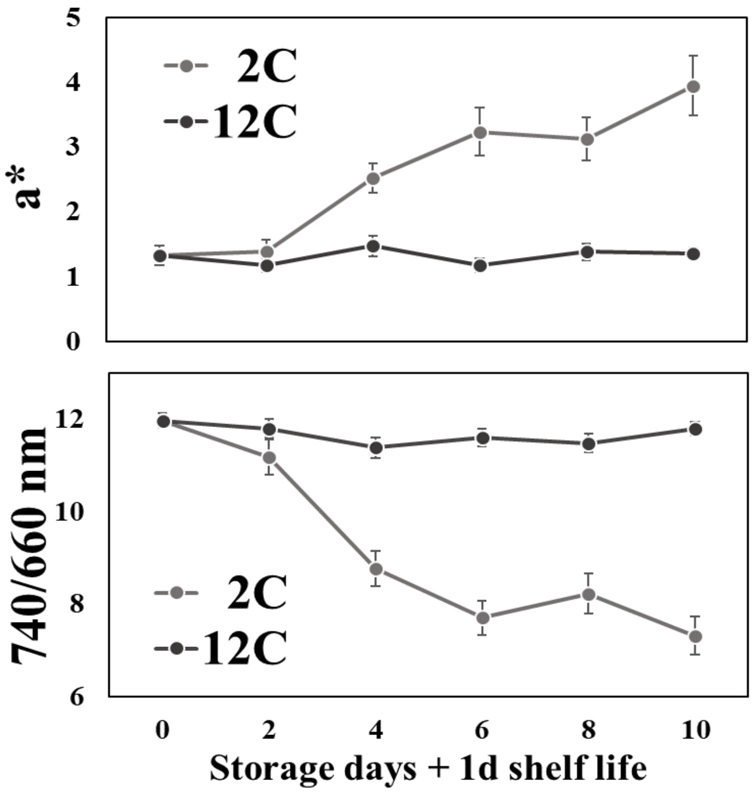


Fig. 4. a* color parameter (A) and the ratio 740/660 nm (B) that were collected from the fruit peel during storage at 2 and 12 °C followed by one day shelf life. Each data point represents the mean of 12 fruit ± standard error (S.E.)

When using color parameters, the optimal NER in calibration (CAL) and cross-validation (CV) was obtained using the PLSDA classifier with lightness, chroma and hue angle (L, C, H^{o}) variables of the whole data set (from 2 up to 10 days including 1day shelf life) reached 78 % of accuracy using 3 latent variables (LVs) (Table 1).

Table 1. Number of fruit correctly classified (over 60 fruits) for each of the 2 classes (storage temperatures of 2 and 12 °C), using PLSDA, SVM and kNN classifier for color parameters, color spectra, FT-NIR spectral, as well as visNIR and NIR hyperspectral imaging data of the whole storage period.

		Calibration			*Cross-validation*		
			12°C	2°C		12°C	2°C
Technique	Classifier	NER[a]	Number of fruit		NER	Number of fruit	
Color (L, C, H)	PLSDA	77.5	41	52	77.5	41	52
	SVM	87.5	51	54	80.0	41	52
	kNN	61.7	49	25	70.8	42	43
Color spectra	PLSDA	88.3	48	58	88.3	48	58
	SVM	88.3	49	57	89.2	48	58
	kNN	80.0	54	42	87.5	51	54
FT-NIR	PLSDA	81.7	51	47	82.5	51	48
	SVM	96.7	58	58	90.0	51	48
	kNN	78.3	55	39	82.5	48	51
VisNIR HIS	PLSDA	86.7	55	49	86.7	55	49
	SVM	80.0	54	42	78.3	55	49
	kNN	76.7	55	37	80.8	49	48
NIR HIS	PLSDA	60.8	38	35	59.2	38	33
	SVM	95.8	59	56	83.3	51	49
	kNN	73.3	53	35	75.8	43	48

[a] NER: Non-Error Rate= Accuracy of classification (%)

However, this discrimination was not consistent, as long as only after the 6th day the NER reached 83 % (Fig. 5A), with cross-validation NER among 75 and 91.7 % (data not shown), rendering this method not reliable. Results were somehow similar when the SVM classification was used, with the overall CAL and CV NER reaching 88 and 80 %, respectively (Table 1). Although mean CAL and CV NER for each storage time, after the 4th day, ranged from 79 to 96 %, there was a significant variation in discrimination between the two classes (stored in chilling and non-chilling temperatures) on the last day (10th), when only 9 out of the 12 fruit stored at

12°C were correctly classified (Fig. 5B). Even worst results were obtained when kNN was applied. In particular, overall mean CAL and CV NER were sustained below 71 % and significant variation and inconsistency was observed also during storage (Fig. 5C).

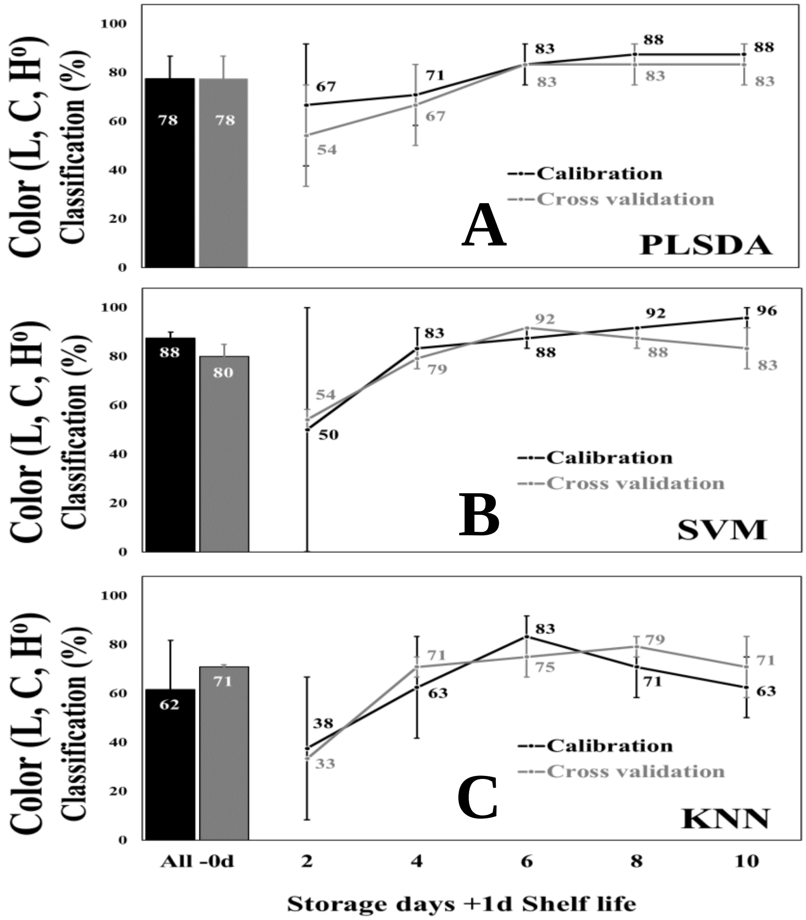


Fig. 5. Discriminant analysis results (% classification) for calibration and cross validation of fruit stored at 12 °C (non-chilling storage temperature) and 2 °C (chilling storage temperature) based on color data (L, C, H°) that were collected from the fruit peel and processed using PLSDA (A), SVM (B) and kNN algorithm. Each data point represents the mean of correctly classified fruit ± standard error (S.E.) either for the whole period of storage or within each storage day.

When using the reflectance scanning mode, the results were significantly improved applying PLSDA. In particular, color spectral data that were preprocessed by logarithm transformation and conversion to 1st derivative, reaching 88% CAL and

CV NER using the whole data set (beyond the 2nd day of storage), and were able to correctly classify more than 83 % of fruit in CV starting from the 4th day of storage (Fig. 6A). On the other hand, although the overall CAL and CV were higher after SVM model implementation (Table 1), meaning that 48 out of 60 fruit in each class were correctly classified, the consistency of discrimination was much worst as is evident by the low calibration NER (Fig. 6B). When processed data were classified using the kNN algorithm, neither overall accuracy, neither daily consistency was improved (Fig. 6C).

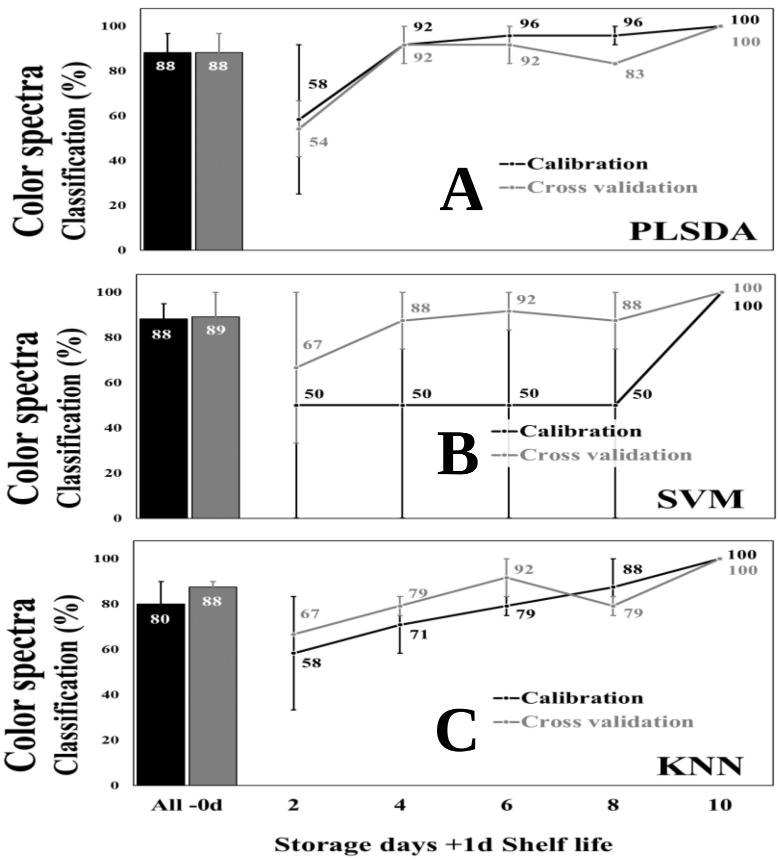


Fig. 6. Discriminant analysis results (% classification) for calibration and cross validation of fruit stored at 12 °C (non-chilling storage temperature) and 2 °C (chilling storage temperature) based on spectra reflectance data at the region 340–760 nm that were collected from the fruit peel and processed using PLSDA (A), SVM (B) and kNN algorithm. Each data point represents the mean of

correctly classified fruit ± standard error (S.E.) either for the whole period of storage or within each storage day.

3.4. FT-NIR spectral data

The raw data acquired from FT-NIR device for fruit stored at chilling temperature during the storage time is depicted in Fig. 7A. FT-NIR spectral data were initially preprocessed by conversion of reflectance to absorbance (Trans (log (1/R)), multiplicative signal correction (MSC), and detrend which allowed, among other transformations, to achieve the highest possible discrimination of fruit either for the whole storage period, as well as within each storage day (Fig 7B).

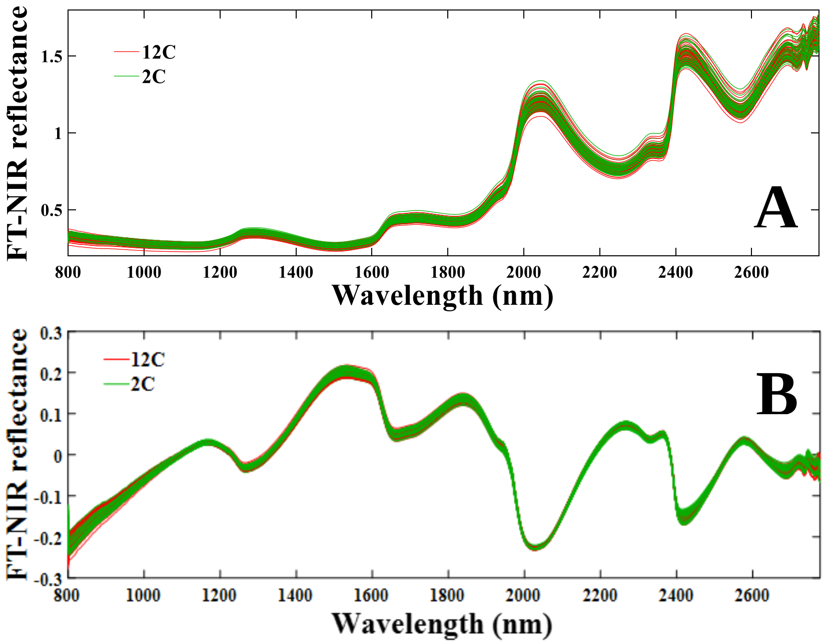


Fig. 7. Raw (A) and preprocessed (B) data acquired from FT-NIR reflectance spectra of fruit during storage at 2 or 12 °C. Each group (storage temperature) consists of 60 measurements.

The optimal overall calibration (CAL) and cross-validation (CV) NER for the whole storage period using the PLSDA classifier on spectra data was 82- 83 %, using 3 latent variables (LVs) (Table 1). Indeed, this discrimination was quite consistent, as long as since day 4 the mean NER were already shifted above 83 % (Fig. 8A), with cross-validation NER among 75 and 100 % (data not shown), making this method quite reliable for eggplant fruit classification. Results were substantially improved when the SVM classification was applied, with the overall CAL and CV NER climbing up to 97 and 90 %, respectively, resulting in > 51 fruit out of 60 correctly classified (Table 1) and a simultaneous constantly reliable discrimination of the two classes of fruit (stored at chilling and at non-chilling temperatures) starting from the 2nd day of storage, with at least 11 out of the 12 fruit being correctly classified in both CAL and CV process (Fig. 8B). By using the kNN algorithm, although the overall CV NER was as high as with PLSDA (83 %) remaining high (>88 %) within each storage day, there was a significant variation in CAL process that renders this model not trustworthy (Fig. 8C).

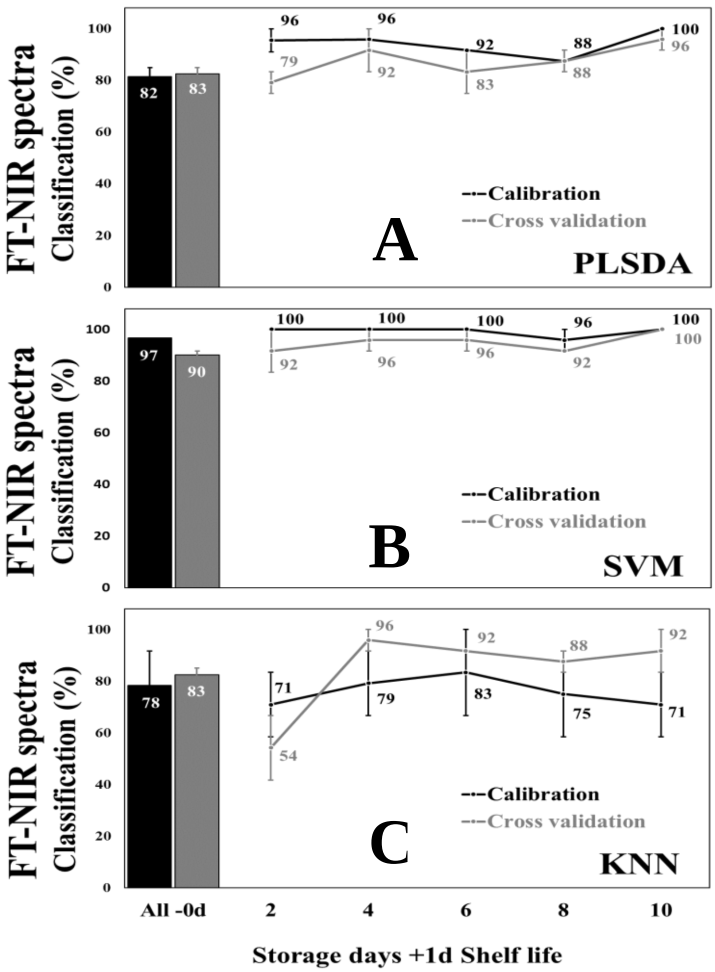


Fig. 8. Discriminant analysis results (% classification) for calibration and cross validation of fruit stored at 12 °C (non-chilling storage temperature) and 2 °C (chilling storage temperature) based on the FT-NIR reflectance data over the absorbance range of 800–2777 nm that were collected from the fruit peel and processed using PLSDA (A), SVM (B) and kNN algorithm. Each data point represents the mean of correctly classified fruit ± standard error (S.E.) either for the whole period of storage or within each storage day

3.5. Hyperspectral imaging data

The Vis-NIR hyperspectral imaging (HSI) data were initially preprocessed by detrend and 2nd derivative, before being used in the discrimination analysis of fruit. The highest overall calibration (CAL) and cross-validation (CV) NER for the whole storage period using the PLSDA classifier on Vis-NIR images data was for both 87 %, using 4 latent variables (LVs) (Table 1). Although, the consistency of fruit previously stored at chilling temperatures were 100 % correctly classified since day 6 (Fig. 9A), there were 3 out of 12 fruit stored at safe temperatures that were misclassified in the last day of storage (data not shown). The above results were even worse when the SVM classification was applied, with the overall CAL NER being as low as 50 %, and CV NER exhibiting significant variation, in contrast to the mean NER using the data of the whole storage period (80 and 78 %, respectively) (Table 1), with the most significant misclassifications observed in the 2^{o}C stored eggplants (Fig. 9B). By applying the kNN algorithm, although the overall CV NER was almost as accurate as the SVM algorithm (77-81 %), there was a significant variation in each storage day’s discrimination analysis, rendering once more, this model not suitable for use in fruit sorting (Fig. 9C).

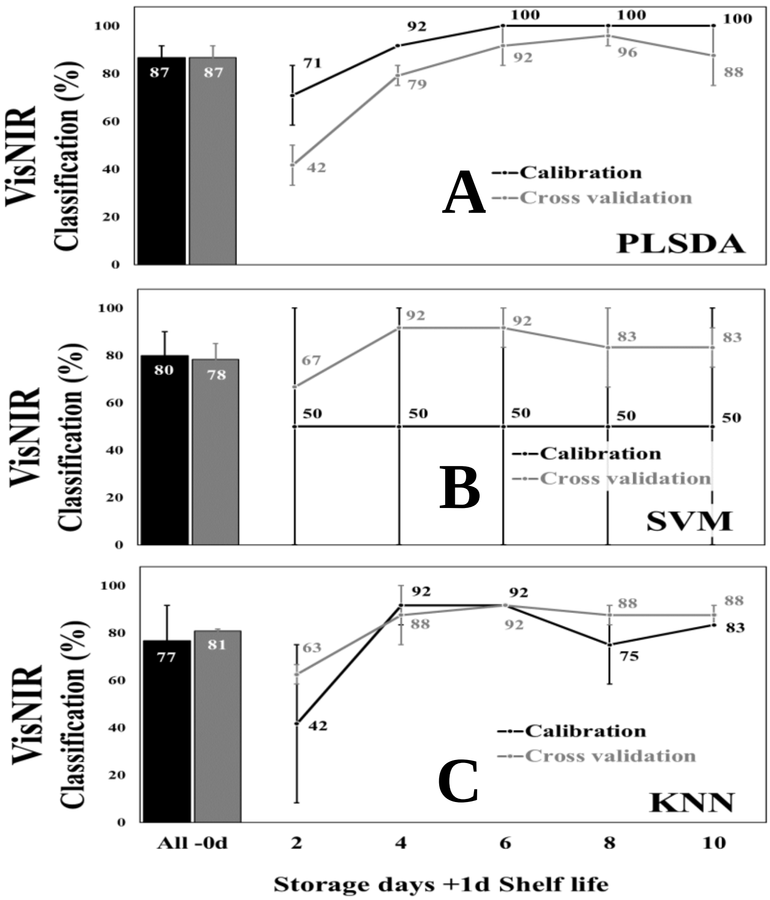


Fig. 9. Discriminant analysis results (% classification) for calibration and cross validation of fruit stored at 12 °C (non-chilling storage temperature) and 2 °C (chilling storage temperature) based on the imaging data acquired in the visible-near infrared (Vis-NIR) range of 400–1000 nm that were collected from the fruit peel and processed using PLSDA (A), SVM (B) and kNN algorithm. Each data point represents the mean of correctly classified fruit ± standard error (S.E.) either for the whole period of storage or within each storage day.

The NIR hyperspectral imaging data were initially preprocessed by 2nd derivative, standard normal variate (SNV) normalization and detrend, before being used in the discrimination analysis of fruit. Irrespectively of the algorithm adopted for the discrimination of fruit, the misclassification of fruit in the two classes was severe (Fig. 10A-C). In particular, the overall calibration (CAL) and cross-validation (CV) NER for the whole storage period using the PLSDA classifier on NIR image data were as low as 59 and 61 %, respectively, using 3 latent variables (LVs) (Fig. 10A) and only fruit on the last day of storage were 83-92 % correctly classified (data not shown). The above results were substantially improved when the SVM classification was applied, with the overall CAL and CV NER being as high as 96 and 83 %, meaning that > 51 out of 60 fruit were correctly classified in each of the two storage temperatures) (Table 1), but the model was consistently accurate only after the 6th of storage, reaching NER >92 % (Fig. 10B). By using the kNN algorithm, results were in-between the two above classifiers, in the sense that although the overall CAL and CV NER were 73 and 76 %, there was a significant variation in the discrimination analysis during the storage period, not allowing this model to be adopted for eggplant fruit discrimination (Fig. 10C).

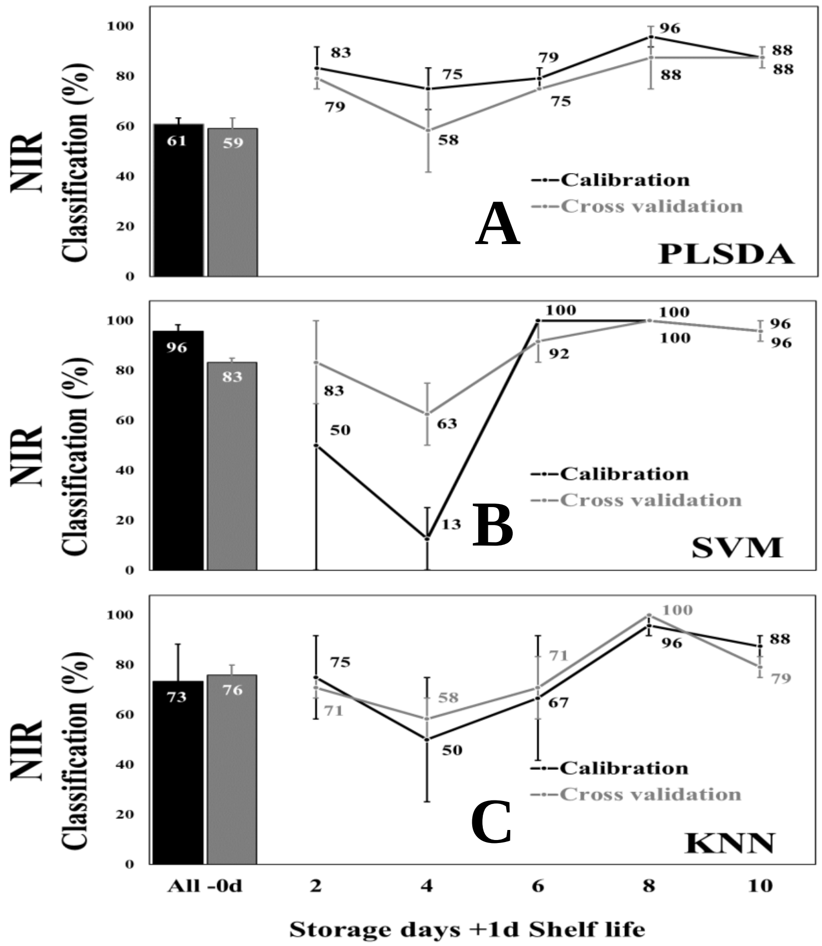


Fig. 10. Discriminant analysis results (% classification) for calibration and cross validation of fruit

stored at 12 °C (non-chilling storage temperature) and 2 °C (chilling storage temperature) based on the imaging data acquired in the near infrared (Vis-NIR) range of 900–1700 nm that were collected from the fruit peel and processed using PLSDA (A), SVM (B) and kNN algorithm. Each data point represents the mean of correctly classified fruit ± standard error (S.E.) either for the whole period of storage or within each storage day

4. Discussion

According to the sensory evaluation of chilling injury (CI) symptoms on the white inner flesh of the eggplant fruit, the deterioration of the fruit quality could be evident to the consumers when halving the eggplants, even since the 4th day of storage at low temperature, irrespectively to the intensity of the symptoms. This result is in agreement with Kozukue et al. (1978), which reported that eggplants previously stored at 1 °C, developed surface pitting of peel, as well as browning of seeds and of vascular bundles after 4 days at 1°C, but is in contrast to other studies when CI was apparent only after 6 to 10 days at 0^{o}C (Concellón et al., 2012, 2005). Apart from the different degrees of chilling temperatures in the above studies, the variety and the growing stage of the fruit may be also responsible for the development of CI symptoms (Zaro et al., 2014).

It has been suggested that chilling injury detection can be confirmed by other destructive techniques, such as the increases of the malic to citric acid content ratio in fruit flesh, of respiration and ethylene production rates (Kozukue et al., 1978), of 1-aminocyclopropane-1-carboxylic acid (ACC) and 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC) contents (Concellón et al., 2005) or of catalase activity and malondialdehyde and H_2O_2 content (Carvajal et al., 2011), which were not evaluated in this study.

Nonetheless, the CI symptoms since the 4th day of storage (Fig. 2) were probably accompanied with compositional changes on the eggplant fruit skin, as implied by scanning the methanolic peel extract spectrophotometrically (Fig. 3). Differences among fruit previously stored at 2 or 12^{o}C regarding the absorbance of the skin extract probably reflect compositional alteration of pigments, mainly anthocyanins, a subgroup of flavonoids that are responsible for the pericarp color of eggplant fruit with delphinidin-3-(p-coumaroylrutinoside)-5-glucoside (nasunin), 3-caffeoylrutinoside-5-glucoside, delphinidin- 3-glucoside, delphinidin-3-glucosyl-rhamnoside and petunidin being considered as the major ones in eggplant cultivars (Concellón et al., 2007; Dranca and Oroian, 2017).

Indeed, consistent and reliable discrimination results based on color parameters were only observed after the 6^{th} day of storage using only partial least square (PLS) or supervised vector machine (SVM) algorithms, as long as kNN algorithm was not efficient enough to classify fruit correctly according to their postharvest storage temperature. According to our results, L, C and H^o color data permitted fruit discrimination only after chilling injury initiation (Fig. 5), in contrast to Concellón et al. (2007) who showed that color changes are concomitant to chilling injury development. The same authors also state that color changes during storage are not following the same pattern but are dependent on the position of the measurement on fruit longwise axis, occurring faster near the calyx than the center of the fruit. In our study, only a* parameter revealed differences on fruit peel after the CI induction (Fig. 4A), whereas neither lightness, chroma or hue angle allowed a safe discrimination (data not shown). Similarly to our study, neither the color parameters of the external surface (peel) of apple fruit were able to classify them in two classes; normal and chilling injured ones, because of the absence of significant differences between them (ElMasry et al., 2009).

However, the use of Konica Minolta spectrophotometer, apart from the L, C, H^o parameters, permitted the recording of spectra reflectance of the peel in the range 340-760 nm which produced significantly better discrimination NER. Indeed, the ratio 740/660 nm exhibited significant differences among fruit after day 4 (Fig. 4B), while even more interesting results were obtained when this method was coupled with PLSDA classifier (Fig. 5A) and can therefore be safely adapted during the sorting of fruit, given that eggplants are previously stored for at least 2 days in low, chilling temperatures.

Recording of FT-NIR spectral data using multipurpose analyzer turned out to be the most suitable technique, in order to achieve the earliest possible and consistent detection of eggplants fruit stored at chilling injurious conditions. However, it should be noted that the optimal discrimination requires appropriate preprocessing of the spectral data, such as transmission of reflectance to absorbance, multiplicative signal correction (MSC) and detrend combined with SVM algorithm analysis. In this way the overall mean CAL and CV NERs reached 97 and 90%, being higher than 92%, already from the second day of storage. PLSDA algorithm performance was inferior to SVM in classifying fruit in terms of accuracy, while kNN classifier was substantially worst and therefore unsuitable to be implemented for such sorting purposes.

Among the Vis-NIR (400-1000nm) and NIR (900-1700nm) hyperspectral imaging data, better results were obtained in the visible range. In terms of screening eggplant fruit according to their postharvest storage temperature regimes, Vis-NIR image data required preprocessing by detrend and 2^{nd} derivative, before using the PLSDA, SVN and kNN classifiers (Figs. 9A-C). Indeed, PLSDA was superior in accuracy and consistency than SVM, contrary to the FT-NIR spectral, while kNN was once more inappropriate to be used.

The preprocessed NIR hyperspectral imaging data (2^{nd} derivative + standard normal variate (SNV) normalization + detrend), turned out to be extremely efficient (>92 %) in discriminating fruit only after the 6^{th} day of storage, when the SVM supervised classification model was applied (Fig. 10B). Neither PLSDA nor kNN algorithm, were able to improve NIR image data acquisition efficiency in separating fruit (Figs. 10A, C).

Apart from accuracy and consistency, the choice of a particular method depends on other factors as well, such as the nature of the problem, the size of the data set, the ease of implementation and the economic feasibility (ElMasry et al., 2009). For instance, the major disadvantage of the hyperspectral imaging technique is that handling the huge amount of data extracted from hyperspectral images requires extra time and resources, although hyperspectral imaging is considered advantageous relatively to spectroscopic techniques, which acquire the spectral data from a single point or from an integration of a small region on the tested fruit (ElMasry et al., 2009; Li et al., 2016). Nonetheless, in this case HSI gave lower performance than FT-NIR may be due to the lower spectral range (maximum 1800 nm for HSI versus 2700 nm), the lower spectral resolution and the acquisition method which requires a longer acquisition time from a higher distance between the object and the detector. Several studies on hyperspectral imaging or FT-NIR spectral acquisition confirm the need to follow various preprocessing steps of the raw extracted data, as well as various statistical analysis and classifiers, in order to reach promising results. Similarly to our study, Cen et al. (2016) applied various supervised classification algorithms, such as with naïve Bayes (NB), supervised vector machine (SVM), and k-nearest neighbor (kNN) combined with feature selection techniques, such as mutual information feature selection (MIFS), max-relevance min-redundancy (MRMR) and sequential forward selection (SFS) for optimal wavebands selection of Vis-NIR hyperspectral imaging data, in order to find the most robust model that could permit the identification and classification of fresh and chilling injured cucumber fruit in an online sorting system. According to their results, the SVM classifier combined with the SFS spectral feature subset was proven to achieve the best classification performance, similarly to our study where SVM excelled in consistency and accuracy in comparison to PLSDA and kNN, in both vis NIR hyperspectral or FT NIR data. In another recent research on chilling injury detection of peach fruit, Vis-NIR hyperspectral reflectance imaging data partial least were processed by partial least squares-discriminant analysis (PLSDA), artificial neural networks (ANN), and supervised vector machine algorithms (SVM), classified fruit according to the severity of CI symptoms (Sun et al., 2017). Interestingly, high classification NER were obtained only among chilled and non-chilled peach fruit, while the accuracy was reduced in calibration and cross validation NER of the three or four classes.

Apparently, the selection of the proper non-destructive protocol, in terms of technique, preprocessing data method and classification algorithm is the resultant of rapidness, consistency, repeatability and apparently is species specific.

5. Conclusion

Eggplant fruit developed chilling injury symptoms since the 4th day of storage at 2^{o}C, as revealed destructively by internal flesh browning and seed blackening and confirmed by measuring the absorbance of the peel extract in a photometer. In order to develop a non-destructive method for the earliest possible detection of stored eggplants at chilling injurious conditions, several techniques were applied. The earliest (since the 2nd day of storage at 2^{o}C), and most consistent results (92-100 %, throughout the storage period) were obtained using the FT-NIR spectral data, generated after several preprocessing steps and classified using the SVM algorithm. Color or FT NIR spectral data classified with PLSDA permitted relatively good classification of fruit (>83 % accuracy) since the 4th day of storage, while L, C, H^{o} color measurements or Vis-NIR hyperspectral images combined with PLSDA generate trustworthy models only after the 6th day of storage. NIR hyperspectral imaging technique and kNN classification algorithm are incapable of separating the fruit neither accurately, neither consistently.

Apparently, specific FT NIR wavelengths should be identified out of the whole region captured (800- 2777 nm), in order to optimize the models and increase speed of discrimination process before implementing the method in quality control during retail market of eggplant fruit or even developing a prototype hand held spectral acquisition equipment for that purpose.

Acknowledgements

P.T. acknowledges the financial support provided by the University of Foggia, during a short period contract as a visiting professor in the Department of Science of Agriculture, Food, and Environment (SAFE).

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Amodio, M.L., Ceglie, F., Chaudhry, M.M.A., Piazzolla, F., Colelli, G., 2017. Potential of NIR spectroscopy for predicting internal quality and discriminating among strawberry fruit from different production systems. Postharvest Biol. Technol. 125, 112–121. https://doi.org/10.1016/j.postharvbio.2016.11.013

Birth, G.S., 1976. How light interacts with foods, In: Gaffney, J.J. Jr. (Ed.), Quality Detection in Foods. American Society for Agricultural Engineering, St. Joseph, MI, pp. 6-11.

Carvajal, F., Martinez, C., Jamilena, M., Garrido, D., 2011. Differential response of zucchini varieties to low storage temperature. Sci. Hortic. (Amsterdam). 130, 90-96. https://doi.org/10.1016/j.scienta.2011.06.016

Carvajal, F., Palma, F., Jamilena, M., Garrido, D., 2015. Cell wall metabolism and chilling injury during postharvest cold storage in zucchini fruit. Postharvest Biol. Technol. 108, 68–77. https://doi.org/10.1016/j.postharvbio.2015.05.013

Cen, H., Lu, R., Zhu, Q., Mendoza, F., 2016. Nondestructive detection of chilling injury in cucumber fruit using hyperspectral imaging with feature selection and supervised classification. Postharvest Biol. Technol. 111, 352–361. https://doi.org/10.1016/j.postharvbio.2015.09.027

Concellón, A., Añón, M.C., Chaves, A.R., 2007. Effect of low temperature storage on physical and physiological characteristics of eggplant fruit (*Solanum melongena* L.). LWT - Food Sci. Technol. 40, 389–396. https://doi.org/10.1016/j.lwt.2006.02.004

Concellón, A., Añón, M.C., Chaves, A.R., 2005. Effect of chilling on ethylene production in eggplant fruit. Food Chem. 92, 63–69. https://doi.org/10.1016/j.foodchem.2004.04.048

Concellón, A., Añón, M.C., Chaves, A.R., 2004. Characterization and changes in polyphenol oxidase from eggplant fruit (*Solanum melongena* L.) during storage at low temperature. Food Chem. 88, 17–24. https://doi.org/10.1016/j.foodchem.2004.01.017

Concellón, A., Zaro, M.J., Chaves, A.R., Vicente, A.R., 2012. Changes in quality and phenolic antioxidants in dark purple American eggplant (*Solanum melongena* L. cv. Lucía) as affected by storage at 0°C and 10°C. Postharvest Biol. Technol. 66, 35–41. https://doi.org/10.1016/j.postharvbio.2011.12.003

Dranca, F., Oroian, M., 2017. Total Monomeric Anthocyanin, Total Phenolic Content and Antioxidant Activity of Extracts from Eggplant (*Solanum Melongena* L.) Peel Using Ultrasonic Treatments. J. Food Process Eng. 40, n/a-n/a. https://doi.org/10.1111/jfpe.12312

ElMasry, G., Wang, N., Vigneault, C., 2009. Detecting chilling injury in Red Delicious apple using hyperspectral imaging and neural networks. Postharvest Biol. Technol. 52, 1–8. https://doi.org/10.1016/j.postharvbio.2008.11.008

Francia, D., Demaria, D., Calderini, O., Ferraris, L., Valentino, D., Arcioni, S., Tamietti, G., Cardinale, F., 2007. Wounding induces resistance to pathogens with different lifestyles in tomato: Role of ethylene in cross-protection. Plant,

Cell Environ. 30, 1357–1365. https://doi.org/10.1111/j.1365-3040.2007.01709.x
Gao, H., Kang, L.N., Liu, Q., Cheng, N., Wang, B.N., Cao, W., 2015. Effect of 24-epibrassinolide treatment on the metabolism of eggplant fruit in relation to development of pulp browning under chilling stress. J. Food Sci. Technol. 52, 3394–3401. https://doi.org/10.1007/s13197-014-1402-y
Hashim, N., Pflanz, M., Regen, C., Janius, R.B., Abdul Rahman, R., Osman, A., Shitan, M., Zude, M., 2013. An approach for monitoring the chilling injury appearance in bananas by means of backscattering imaging. J. Food Eng. 116, 28–36. https://doi.org/10.1016/j.jfoodeng.2012.11.018
Huang, H., Liu, L., Ngadi, M., 2014. Recent Developments in Hyperspectral Imaging for Assessment of Food Quality and Safety. Sensors 14, 7248–7276. https://doi.org/10.3390/s140407248
Kozukue, N., Kozukue, E., Kishiguchi, M., Lee, S.W., 1978. Studies on keeping-quality of vegetables and fruit. III. Changes in sugar and organic acid contents accompanying the chilling-injury of eggplant fruit. Sci. Hortic. (Amsterdam). 8, 19–26. https://doi.org/10.1016/0304-4238(78)90065-1
Li, J., Huang, W., Tian, X., Wang, C., Fan, S., Zhao, C., 2016. Fast detection and visualization of early decay in citrus using Vis-NIR hyperspectral imaging. Comput. Electron. Agric. 127, 582–592. https://doi.org/10.1016/j.compag.2016.07.016
Lorente, D., Aleixos, N., Gómez-Sanchis, J., Cubero, S., García-Navarrete, O.L., Blasco, J., 2012. Recent Advances and Applications of Hyperspectral Imaging for Fruit and Vegetable Quality Assessment. Food Bioprocess Technol. 5, 1121–1142. https://doi.org/10.1007/s11947-011-0725-1
Lurie, S., Vanoli, M., Dagar, A., Weksler, A., Lovati, F., Eccher Zerbini, P., Spinelli, L., Torricelli, A., Feng, J., Rizzolo, A., 2011. Chilling injury in stored nectarines and its detection by time-resolved reflectance spectroscopy. Postharvest Biol. Technol. 59, 211–218. https://doi.org/10.1016/j.postharvbio.2010.10.005
Megías, Z., Martínez, C., Manzano, S., Barrera, A., Rosales, R., Valenzuela, J.L., Garrido, D., Jamilena, M., 2014. Cold-induced ethylene in relation to chilling injury and chilling sensitivity in the non-climacteric fruit of zucchini (*Cucurbita pepo* L.). LWT - Food Sci. Technol. 57, 194–199. https://doi.org/10.1016/j.lwt.2013.12.044
Megías, Z., Martínez, C., Manzano, S., García, A., del Mar Rebolloso-Fuentes, M., Valenzuela, J.L., Garrido, D., Jamilena, M., 2016. Ethylene biosynthesis and signaling elements involved in chilling injury and other postharvest quality traits in the non-climacteric fruit of zucchini (*Cucurbita pepo*). Postharvest Biol. Technol. 113, 48–57. https://doi.org/10.1016/j.postharvbio.2015.11.001
Sun, Y., Gu, X., Sun, K., Hu, H., Xu, M., Wang, Z., Tu, K., Pan, L., 2017. Hyperspectral reflectance imaging combined with chemometrics and successive projections algorithm for chilling injury classification in peaches.

LWT - Food Sci. Technol. 75, 557–564. https://doi.org/10.1016/j.lwt.2016.10.006

Zaro, M.J., Keunchkarian, S., Chaves, A.R., Vicente, A.R., Concellón, A., 2014. Changes in bioactive compounds and response to postharvest storage conditions in purple eggplants as affected by fruit developmental stage. Postharvest Biol. Technol. 96, 110–117. https://doi.org/10.1016/j.postharvbio.2014.05.012

EARLY DETECTION OF CHILLING INJURY IN GREEN BELL PEPPERS BY HYPERSPECTRAL IMAGING AND CHEMOMETRICS

Farahmand Babellahi[1], Jitendra Paliwal[2], Chyngyz Erkinbaev[2], Maria Luisa Amodio[1*], Muhammad Mudassir Arif Chaudhry[1], Giancarlo Colelli[1]

[1] Dip.to di Scienze Agrarie, degli Alimenti e dell'Ambiente, Università di Foggia, Via Napoli 25, 71122 Foggia Italy

[2]Department of Biosystems Engineering, University of Manitoba, Canada

Published in the *Postharvest Biology and Technology* Journal (2020)

DOI: 10.1016/j.postharvbio.2019.111100

ABSTRACT

The feasibility of using hyperspectral imaging in the combined wavelength regions comprising of visible to near infrared (VIS-NIR) (400-1000 nm) and short wave infrared (SWIR) (1000-2500 nm) was investigated for discriminating fresh bell peppers from those stored under refrigeration. In addition, the technique was used for early detection of chilling injury (CI) in mature fruit. Supervised classification models were developed using Partial Least Square Discriminant Analysis (PLS-DA) for raw and pre-processed spectra followed by wavelength selection using VIP scores. Reliable classification of fresh and stored fruit was achieved using pre-processed data in VIS-NIR range by 88 % and 84 % non-error-rate (NER) for calibration (Cal) and cross-validation (CV), respectively, but a slightly higher classification accuracy was manifested in the SWIR range using raw spectra; in this case, wavelength selection resulted in six wavelengths in VIS-NIR reaching to 87 %, 83 NER for Cal, CV, respectively, and four wavelengths in SWIR range yielding to NER of 84 % for Cal and 81 % to satisfy the aforementioned objective. Secondly, classification of fruit based on days of cold storage was achieved using preprocessed data both for VIS-NIR and SWIR whole ranges where wavelength reduction resulted in 12 wavelengths in the VIS-NIR and 13 wavelengths in SWIR range without impressive varying model performance in case of VISNIR and decreasing model accuracy in SWIR range. In addition, Partial Least Square Regression (PLSR) was conducted on the data extracted from VIS-NIR HSI, to predict days of cold storage both in full spectral range and selected wavelengths obtained from VIP scores. PLSR models based on full range spectra yielded R^2_{CV} = 0.92, while for the PLSR model based on selected wavelengths R^2_{CV} = 0.79 was obtained, along with reasonable RMSEC and RMSECV. Conclusively, based on the results, VIS-NIR hyperspectral imaging is a reliable option for on-line classification of fresh versus refrigerated fruit and for identifying early incidence of CI.

Key words: Green bell peppers, chilling injury, hyperspectral imaging, classification, PLSR, VIP scores

1. Introduction

Bell pepper (*Capsicum annuum L.*) is an important commercial product that is consumed throughout the world due to its pleasant flavor. Moreover, it is a rich source of essential vitamins (i.e. A, B, C, and E) and many other antioxidant phytochemicals (Marín et al. 2004). As bell peppers are delicate and perishable, they require careful postharvest handling. At storage temperatures below 7 °C, bell peppers are particularly sensitive to chilling injury (CI), the extent of which can depend on cultivar and maturity (S Meir et al. 1995; Paull 1990). The first expression of CI is damaged membranes, which reduce the membrane function (Concellón et al. 2005). CI symptoms of bell peppers include discoloration of calyx and seed cavity, surface pitting, and shriveling owing to moisture loss. The severity of these symptoms increases when the fruit transitions to ambient temperatures after cold storages (Cantwell 1999). CI is a major indicator of improper postharvest storage and results in 25-35 % production losses of the fruit (Cheema et al. 2018). Therefore, development of a rapid and reliable method for the early detection of CI would be valuable for the industry.

A variety of techniques have been investigated for detecting of CI. Liu et al. (2015) identified pectin content, polygalacturonase (PG) and pectin methyl esterase (PME) activity, transmission electron microscopy and light microscopy, aroma volatiles, and main fatty acid content for evaluating quality degradation in bell peppers. Shi et al. (2018) identified sepal browning, black lesions, skin wrinkling, and malondialdehyde, anthocyanin, and phenolic contents as factors. Yang et al. (2011) measured membrane permeability, lipid peroxidation and antioxidant enzyme activity to assess CI in cucumbers. All of these procedures are time-consuming, expensive, require sample preparation and need to be done by trained personnel.

Non-destructive techniques for evaluation of quality in agricultural products are gaining popularity, as recent advancements in sensor technology have made these techniques suitable for online inspection, reliable, and relatively inexpensive. Several researchers have investigated near infrared (NIR) spectroscopy for the evaluation of quality attributes of fruit and vegetables including prediction of solid soluble content (SSC) and firmness in pears (Nicolaï et al. 2008), measurement of acidity, firmness, and SSC in mandarins (Gómez et al., 2006), assessment of internal quality of strawberries (Amodio et al. 2017) and prediction of sugar content in sweet cherries (Lu 2001).

Additionally, optical techniques based on imaging and spectroscopy are also being used for quality inspection of fruit and vegetables, especially during storage (Cen et al. 2016a). Hyperspectral imaging (HSI) allows simultaneous spectral and spatial information of samples under consideration. A single HS image cube comprises of multiple sub-images, each of which is related to the intensity information at a particular wavelength (ElMasry et al. 2009). As light is incident on a sample surface, around 4 % is reflected back causing specular reflectance, and the rest of the incident energy is transmitted through the surface into the cellular structure of the fruit where it is scattered via small interfaces within the tissue or absorbed by cellular constituents (Birth 1976). Each pixel spectrum of the sample presents a special composition characteristic as a fingerprint. The advantage of using this technique over spectroscopy is that the chemical composition of a sample can be determined, mapped and visualized.

HSI has been tested for detection of deformities such as bruises and mechanical damage at early stages in pears, apples, and peaches (Lee et al. 2014; Li et al. 2018; Di Natale et al. 2001; Xing et al. 2005). HSI has been used to evaluate of capsaicin and dihydrocapsaicin concentrations and water content in chili peppers (Jiang et al., 2018), and chlorophyll and carotenoid, total soluble solid, and ascorbic acid contents in bell peppers Schmilovitch et al., (2014).

ElMasry et al. (2009) used HSI in the range of VIS-NIR (400-1000 nm) combined with the use of feed-forward backpropagation artificial neural networks (ANN) for CI detection in ‘Red Delicious’ apples. Pan et al. (2016) studied the detection of CI in peaches using HSI and ANN. Sun et al. (2017) analyzed peaches by HSI combined with chemometrics and successive projections algorithm. They classified different levels of CI based on severity using Partial Least Square-Discriminant Analysis (PLS-DA), Support Vector Machine (SVM), and ANN. Cen et al. (2016) investigated the potential of HSI in reflectance (500–675 nm) and transmittance (675–1000 nm) modes followed by supervised classification for the detection of CI in cucumbers.

Despite all these efforts to detect chilling injuries in fruit and vegetables, there is a lack of literature regarding the development of non-destructive techniques and methodologies for the early detection of such damage in bell peppers. Thus, the objective of this study was to discriminate fruit for temperature and storage time and to predict days of cold storage. To this aim, the most effective wavelengths were selected in order to arrive at simple computationally inexpensive models that are efficient for real-time implementation.

2. Materials and methods

2.1 Experimental design

A total of 150 mature green bell peppers were purchased from a local fresh retailer market located in Winnipeg (Manitoba, Canada), just few hours after harvesting. All fruit were washed and visually inspected to select those free from defects and damage, with 126 fruit chosen for further use. Eighteen fruit were selected as fresh (day 0) and the rest were divided into two groups and kept in two different temperature and humidity controlled environmental chambers (Conviron, Controlled Environments Ltd., Winnipeg, MB, Canada). The first chamber was set at 4 °C as the chilling temperature (Paull 1990) and 90 % relative humidity. A second chamber was set at 12 °C as a safe storage temperature for bell peppers (Paull, 1990) and 90 % relative humidity. Eighteen fruit were removed from each chamber at intervals of six days as shown in Fig. 1 and scanned. Prior to scanning, the fruit were left at room temperature (~20 °C) for 24 h to eliminate the temperature effect and to allow the symptoms to further develop. All HS images were acquired in the Image Processing Lab of the Department of Biosystems Engineering at the University of Manitoba, Canada.

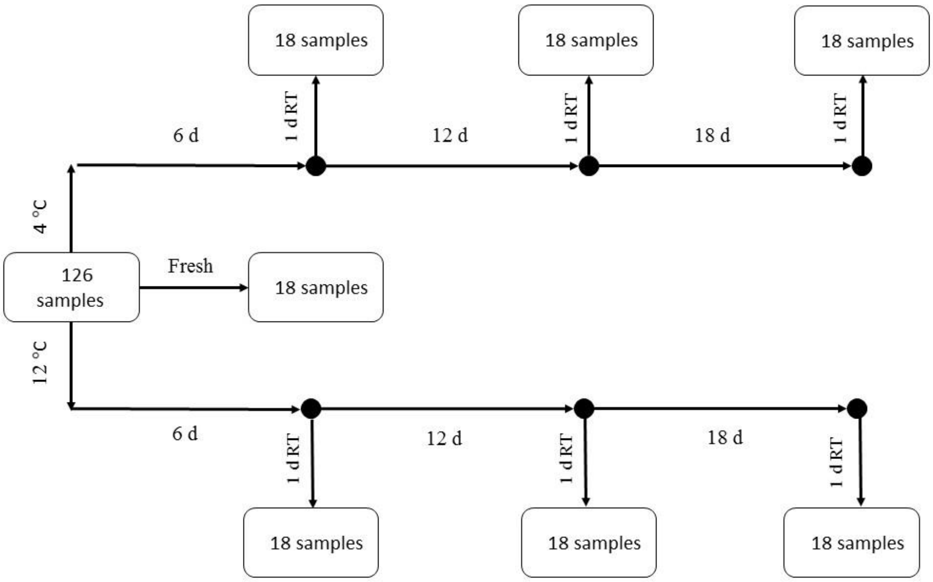


Fig. 1. Scheme of experimental design for dividing the fruit into fresh, and for fruit stored at 4 ˚C or12 ˚C followed by 1 d at room temperature (RT)

2.2 Hyperspectral imaging system

In this study, VIS-NIR and SWIR line-scanning HSI systems (SPECIM Spectral Imaging Ltd., Oulu, Finland) were used for image acquisition of the fruit. The VIS-NIR system comprised of a charge coupled device (CCD) camera with 1024×896 pixels equipped with a spectrograph (SPECIM V10E, 397.66-1003.81 nm, 2.6 nm spectral resolution), and a focusing lens (SPECIM OLET 15). The lighting unit consisted of two 150 W tungsten lamps (3900-ER, Illumination Technology, Inc., USA) which were located on the two sides of sample at 45°. The SWIR HSI system (Fig.2) included a spectrograph (SPECIM N25E, 953.36-2567.37 nm with a spectral resolution 5.6 nm) and a 30 mm focusing lens (SPECIM OLES 30) that allowed it to obtain hypercubes of 300×384×288 pixels. The system had a cryogenically cooled mercury-cadmium-telluride (MCT) detector array.

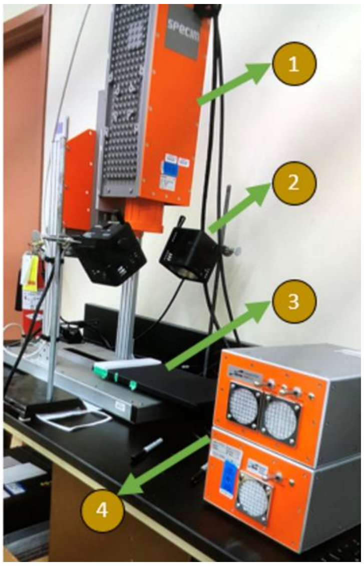


Fig. 2. An integrated SWIR hyperspectral imaging system; 1) spectrograph 2) lighting unit 3) moving platform 4) control unit

2.3 Image acquisition and correction

To achieve thermal and temporal stability of the lighting system and camera, the system was switched on 30 min. prior to image acquisition based on previous research (Castorena et al. 2015; Erkinbaev et al. 2017) using the two camera systems. The frame rate for both the cameras was set as 20 frame per second (fps). The exposure time and platform speed for the VIS-NIR system were 20 ms and 7 mm/s, respectively, and for the SWIR system 8 ms and 7 mm/s, respectively. The speed of the moving stage of 7 mm/s was chosen based on best aspect ratio of the frame rate and exposure time (Erkinbaev et al. 2019). After acquisition of images, the black and white reference correction was applied. The black reference was measured by closing the shutter automatically while for the white reference, a 99 % Spectralon reflectance standard (Labsphere, North Sutton, NH), was positioned at the top of each image.

The following equation was used to calculate the relative reflectance, R:

$$R = \frac{H-D}{W-D} \qquad \text{Eq. 1}$$

Where H represents the original hyperspectral image; and D and W are the acquired signals for both black and white references, respectively.

2.4 Image Processing

An image processing algorithm was developed to extract spectral information from the images. Bell peppers have a highly glossy skin, which results in sections within a HS image, where the incident light is dominant, creating regions where image becomes saturated. Such saturated regions affect the spectral profiles and consequently the results; therefore, elimination of these saturated regions is essential. It was observed, that images at wavelengths of 692 nm and 827 nm in the VIS-NIR range best for separated the saturated regions and whole fruit from the background, respectively. For each sample, grayscale images were binarized at these two wavelengths corresponding to the saturated regions and whole fruit, respectively. The algorithm also identified and removed small regions where signal-to-noise ratio was very low. After combining the two binary images representing the whole fruit and saturated regions, a logical AND operator was implemented to create one binary image that was without background or saturated regions. This image was considered as a reference to be applied for all waveband images, imposing zero values to all 224 channels and keep only the non-saturated pixel values of the grayscale sub-images. Finally, the algorithm averaged all spectra from each waveband image and saved these mean spectral values to an excel file for further analysis as depicted in Fig. 3. The same technique was applied for SWIR hyperspectral images.

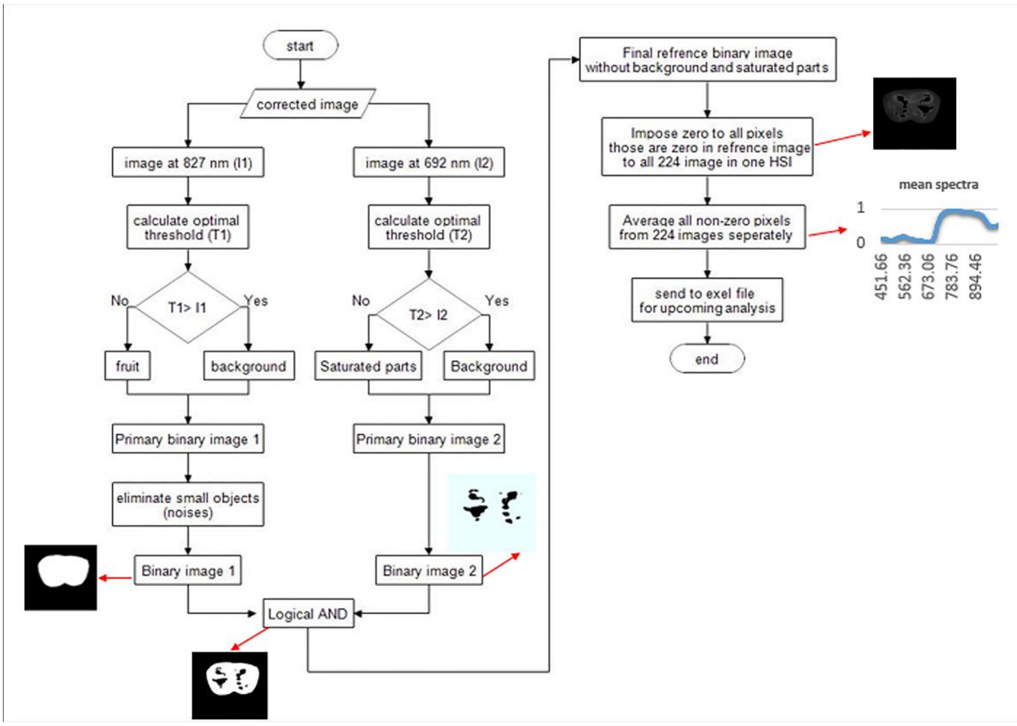


Fig. 3. Image processing flowchart for removing background, saturated parts and extracting data from images (for VIS-NIR images)

2.5 Chilling evaluation

The external appearance of fruit (shriveling and pitting) were assessed. Fruit were then cut one fifth from calyx and then longitudinally, and categorized into four classes according to the area of discolored seeds which was evaluated by a panel of 7 experts (4 females and 3 males, personnel of the department, not affiliated with the experiment, semi-trained for the experiment). CI indices are shown in Fig. 4, where, 0 = no chilling (0 % area of discolored seeds), 1= slight chilling (≤25 % area of discolored seeds), 2 = moderate chilling (> 25 % and ≤50 % area of discolored seeds), and 3 = severe chilling (> 50 % area of discolored seeds)

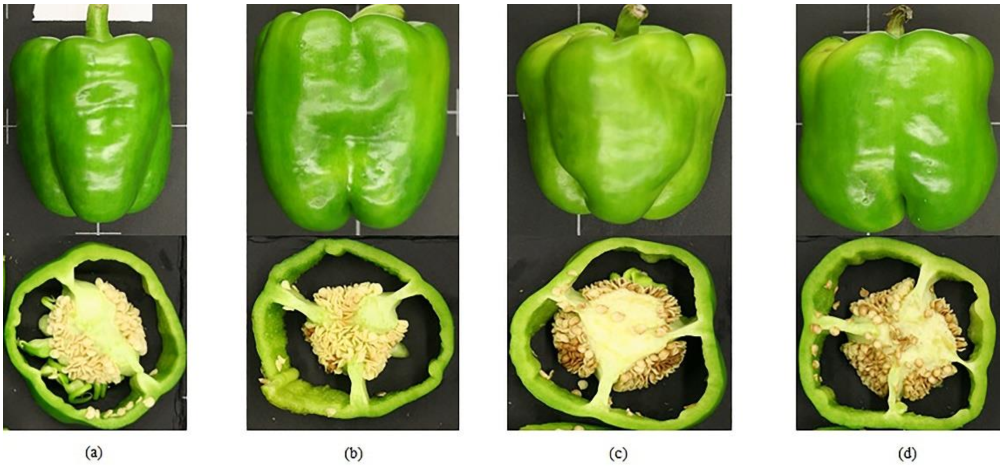


Fig.4. CI indices based on visual evaluation. A. CI 0, B. CI 1, C. CI 2, and D. CI 3

2.6 Multivariate data analysis

Most HSI systems have poor signal-to-noise ratios (SNR) at the two extreme regions of wavelengths. Therefore, spectral regions of poor SNR were eliminated leaving 204 wavebands in the VIS-NIR range and 250 wavebands in SWIR range for model development. Multivariate and chemometric methods were applied for the development of calibration models on the extracted datasets (De Jong 1990) .

Prior to the application of classification and regression models, principal component analysis (PCA) was used as a preliminary operation to exclude outliers. The technique transforms a set of correlated original variables into fewer uncorrelated orthogonal variables called PCs, which are the linear combinations of the original variables. The established PCA loadings on the other hand, represent the relationship among variables (Eriksson et al. 2013). Combination of variables describe major trends in the data, and can be a used as a tool for detecting outliers (Bro and Smilde 2014).

As a classifier, PLS-DA was selected. The PLS-DA classification algorithm works on the basis of interaction of spectral intensity and sample characteristics by maximizing the covariance between variables (Barker and Rayens 2003). Firstly, PLS-DA classification models were developed with the aim of discriminating fruit stored at 12 $^{\circ}$C and 4 $^{\circ}$C. In the second step, PLS-DA models were developed to classify fruit based on the number of days at each temperature. The performance of the developed PLS-DA model was evaluated on the basis of specificity, sensitivity, and non-error-rate (NER), which represented true negative rate, true positive rate, and total correctly classified samples, respectively.

$$Sensitivity\ (SEN) = \frac{true\ positives}{(true\ positives + false\ negatives)} \qquad \text{Eq. 2}$$

$$Specificity\ (SPEC) = \frac{true\ negatives}{(true\ negarives + false\ positives)} \qquad \text{Eq. 3}$$

$$NER = \frac{\sum_{i=1}^{n} Sens}{n} \qquad \text{Eq. 4}$$

Where, *n* is the number of samples. The accuracy of the PLS-DA models can be enhanced by carefully selecting the number of latent variables (LVs) which is determined on the basis of minimum root mean square error of cross-validation (RMSECV).

Additionally, a Partial Least Square Regression (PLSR) model was developed estimate the days of storage at 4 °C. PLSR is a verified multivariate calibration method for quantitative analysis that overcomes problems associated with overlapping spectral bands and collinearity of data (Barboza and Poppi 2003). To keep the regression models in line with the classification models and preventing complexity, the same number of LVs obtained from classification models was also selected for PLSR models. The calibration models were evaluated using coefficient of determination (R^2) and the root mean squared error (RMSE) for both calibration and cross-validation

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y}_i)^2} \qquad \text{Eq. 5}$$

$$RMSE = \sqrt{\frac{1}{n}} \sum_{i=1}^{n}(\hat{y}_i - y_i)^2 \qquad \text{Eq. 6}$$

where n is the number of samples, y is the storage days at chilling temperature, $\bar{y}$ is the mean values of y, and $\hat{y}$ represent days of storage predicted by HSI spectra.

2.7 Data Pre-processing

Even though the data extracted from the HS images of bell peppers were affected by baseline shift and non-linearities, such influences could be removed using appropriate pre-processing techniques. Commonly, these issues occur due to the fact that agricultural products are biological materials and light scattering occurs depending on their particle sizes (Rinnan et al. 2009). The spectral data in this case was further confounded by the shiny surface of bell peppers and unwanted noise. Therefore, pre-processing techniques such as mean-centering (MC), multiplicative scatter correction (MSC), 1^{st} derivative (1^{st} D), and normalization (Norm) were explored on the data as stand-alone methods or in different combinations.

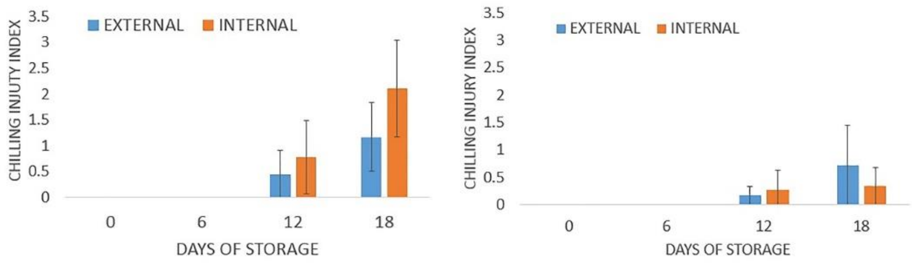
2.8 Wavelength selection

To keep PLS-DA and PLSR models simple, efficient, and fast for on-line industrial implementation, it is imperative to select the variables with the highest weights. In other words, HS images can be reduced to a handful of multi-spectral images by selecting the most appropriate variables. Variable/wavelength selection was performed by calculating variable importance in projection (VIP) scores of the regression models(Wold, Johansson, and Cocchi 1993). Typically, VIP scores>1.0 represent highly dominant variables, while VIP scores<0.8 represent variables that are less effective (Wold, Johansson, and Cocchi 1993). All the chemometric techniques were implemented in PLS-Toolbox software (Eigenvector Research Inc., USA) within the MATLAB computational environment.

3. Results and discussion

3.1 Chilling injury evaluation

Fig. 5 shows the indices of CI over time for bell peppers stored at 4 °C and 12 °C. CI symptoms were not detected until day 12 of storage (plus RT). A shelf-life period results in faster manifestation of CI (Park et al. 2018; Yao et al. 2018). By day 12 of storage at 4 °C, external CI symptoms started to appear, and more importantly internally. On the last day of storage, a significant increase in the incidence of internal symptoms was observed even though the external t CI remained at 1. Some symptoms similar to CI were also detected at 12 °C, where it is possible that some pitting occurred due to accelerated senescence. Therefore, based on external inspection alone, chilling symptoms were not easily detectable without cutting the fruit even after the prolonged storage period of 12 d. Similar conclusions were deduced by Lim et al. (2007), where it was reported that CI symptoms in bell peppers started to reveal after two weeks of storage at 1 °C.

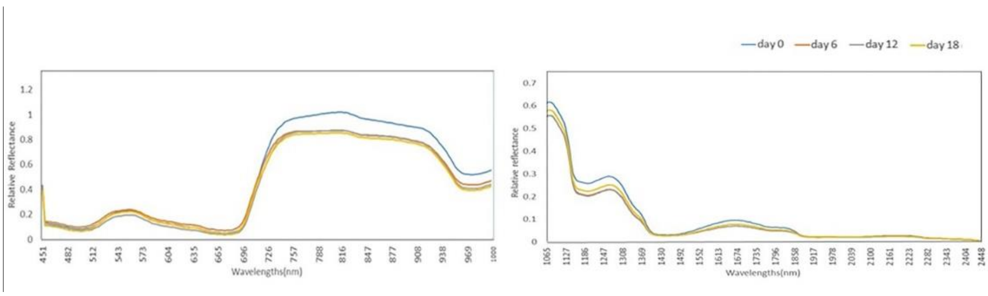


A B

Fig. 5. CI index for bell peppers stored A. 12 °C and B. 4 °C for up to 18 d.

3.2 Characteristics of hyperspectral images

The reflectance spectra in the VIS-NIR and SWIR wavelength ranges for fresh bell peppers and for fruit stored for 6, 12, and 18 d at 4 °C are shown in Fig. 6.



A B

Fig. 6. Spectra of fruit stored at 4 °C in A.VIS-NIR range and B. SWIR range

It is speculated that cells undergo structural changes depending on CI severity and storage length, thereby affecting the reflectance spectra. No big differences in the 400-700 nm range were seen between fresh and stored fruit (Fig. 6A). This indicates that visible spectrum of light cannot be used to develop a precise classification model for CI. However, in the NIR wavelength range of 700-1000 nm, a clear distinction was observed between the spectra of fresh and stored fruit, and also among fruit stored for different time periods. Such changes may be related to the second and third overtone of O-H stretching vibrations associated with water, plus degradation of phenolic content (Siedliska et al. 2018a), which ultimately caused the reflectance of fruit to decrease over time. Similarly, in case of the SWIR mean spectra (Fig. 6B), high differences among spectra were apparent at 1140-1270 nm and 1550-1750 nm ranges. At these wavelengths the vibration of the first overtones of C–H and O-H bond stretching is reported (T. Ignat et al. 2012).

3.3 Classification models based on the full wavelength range

PLS-DA classification models were developed to classify bell peppers stored at 4 °C, 12 °C and fresh fruit. A second classification approach was adopted to classify fruit based on storage time (6, 12, and 18 d) at 4 °C. Accordingly, classification models based on full wavelength ranges of VIS-NIR and SWIR modes were developed and compared to evaluate their performance. For industrial application purposes, data pre-treatments are not preferred, with model development based on raw data, but in the current research, different methods of data pre-processing were explored to ensure model reliability. The most effective data pre-processing combination for all VIS-NIR dataset was found to be MSC followed by MC. This combination of pre-processing techniques eliminated the effect of scattering in spectra and reduced multi-collinearity of the data (Rinnan et al. 2009). For SWIR HSI data, two different combinations of pre-processing methods were applied to optimize the best pre-processing technique that yielded superior classification results for each discrimination objective. To discriminate between stored and fresh fruit, normalization followed by mean-centering (Norm + MC) yielded the best results, while the 1st derivative followed by MSC and then mean-centering (1st D + MSC + MC) was selected for classification of storage time.

Table 1 shows the confusion matrices for the classification performance of fresh and stored fruit in the VIS-NIR and SWIR wavelength ranges. In addition to correctly classified samples, the confusion matrix also shows percentage of misclassified samples, using both raw and pre-processed data.

Table1. Confusion matrices for discrimination of fruit stored at 4 °C and 12 °C and fresh fruit based on the raw and pre-processed data in A. VIS-NIR and B. SWIR wavelength ranges

A

			Confusion matrix (%) Actual				Global (%)		Non-Error-Rate (%)
				Fresh	4 °C	12 °C	SENS	SPEC	
Raw data (No-Preprocessing)	Cal	predicted	Fresh	89	4	8	88	96	78.33
			4 °C	0	63	23	63	86	
			12 °C	11	33	69	84	72	
				Fresh	4 °C	12 °C			
	CV	predicted	Fresh	89	0	8	89	98	74.63
			4 °C	0	63	15.5	63	88	
			12 °C	11	37	76.5	73	63	
			Confusion matrix (%) Actual				Global (%)		Non-Error-Rate (%)
				Fresh	4 °C	12 °C	SENS	SPEC	
Preprocessed data	Cal	predicted	Fresh	100	0	0	100	100	88
			4 °C	0	89	15.5	81	85	
			12 °C	0	11	84.5	84	89	
				Fresh	4 °C	12 °C			
	CV	predicted	Fresh	100	0	0	100	100	84.33
			4 °C	0	74	23	78	74	
			12 °C	0	26	77	77	78	

B

			Confusion matrix (%) Actual	Global (%)	Non-Error-Rate (%)
Raw	C	pr			

Data	Set		Predicted	Fresh	4 °C	12 °C	SENS	SPEC	Non-Error-Rate (%)
				Fresh	4 °C	12 °C	SENS	SPEC	
			Fresh	100	0	0	100	100	
			4 °C	0	81.5	37	85	60	82.66
			12 °C	0	18.5	63	63	83	
				Fresh	4 °C	12 °C			
	CV	predicted	Fresh	100	3.4	0	100	98	
			4 °C	0	81.48	40	85	60	81.66
			12 °C	0	15.12	60	60	85	
				Confusion matrix (%)			Global (%)		Non-Error-Rate (%)
				Actual					
				Fresh	4 °C	12 °C	SENS	SPEC	
Preprocessed data	Cal	predicted	Fresh	100	0	0	100	98	
			4 °C	0	81.5	37	81	60	81.33
			12 °C	0	18.5	63	63	83	
				Fresh	4 °C	12 °C			
	CV	predicted	Fresh	100	7.4	0	100	98	
			4 °C	0	70.37	40	78	60	80.33
			12 °C	0	22.22	60	63	90	

In case of VIS-NIR range, pre-processing (MSC + MC) of the data enhanced model performance, but no significant differences were observed in the model performance after data pre-processing (Norm + MC) in the SWIR range. Confusion matrices in both VIS-NIR and SWIR ranges indicate that fresh fruit were properly classified. In VIS-NIR they were discriminated from stored fruit by 88 % and 96 % sensitivity and specificity for calibration and 89 % and 98 % for cross-validation sets, respectively. The sensitivity and specificity improved to 100 % for both calibration and cross-validation sets after pre-treatment. The classification model based on raw data in VIS-NIR did not effectively discriminate among fruit stored at 4 °C and 12 °C, but after pre-processing, sensitivity and specificity reached to almost 100 % for the cross-validation set. As mentioned earlier, another parameter for evaluation of classifier performance is NER, which is the average of all sensitivities of different classes. In pre-processed data for VIS-NIR range, NER was 88 % and 84.33 % for calibration and cross-validation sets, respectively. In case of SWIR range, the PLS-DA model after data pre-processing yielded an NER of 81.33 % and 80.33 % for calibration and cross-validation sets, respectively.

Results of discrimination for days of storage of fruit kept at 4 °C are depicted in Table 2.

Table 2. Confusion matrices for discrimination of fruit stored at 4 °C for 6, 12, and 18 d based on the raw and pre-processed data in A. VIS NIR and B. SWIR ranges

A

				Confusion matrix (%)			Global (%)		Non-Error-Rate (%)
				Actual					
Raw data(No-Preprocessing)	Cal	predicted		d6	d12	d18	SENS	SPEC	
			d6	100	0	0	100	94	
			d12	0	100	0	100	100	100
			d18	0	0	100	100	100	
	CV	predicted		d6	d12	d18			
			d6	87.5	0	12.5	100	94	
			d12	0	72	0	78	94	88
			d18	12.5	28	87.5	89	89	
				Confusion matrix (%)			Global (%)		Non-Error-Rate (%)
				actual					
Preprocessed data	Cal	predicted		d6	d12	d18	SENS	SPEC	
			d6	100	0	0	100	100	
			d12	0	100	0	100	100	100
			d18	0	0	100	100	100	
	CV	predicted		d6	d12	d18			
			d6	100	0	0	100	100	
			d12	0	100	0	89	94	96
			d18	0	0	100	100	100	

B

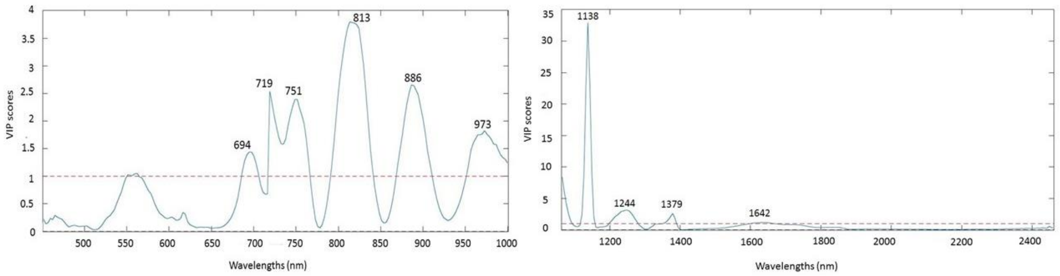
Data	Set	predicted	actual d6	actual d12	actual d18	SENS	SPEC	Non-Error-Rate (%)
Raw data(No-Preprocessing)	Cal	d6	66.7	22.2	11.1	89	83	74
		d12	0	55.5	22.3	55	78	
		d18	33.3	33.3	66.6	78	39	
	CV	d6	44.5	22.2	44.5	55	72	55
		d12	22.2	33.3	33.3	55	72	
		d18	33.3	44.5	22.2	55	44	
Preprocessed data	Cal	d6	88.9	0	0	78	100	85.33
		d12	11.1	88.9	11.1	89	89	
		d18	0	11.1	88.9	89	94	
	CV	d6	66.6	11.1	0	78	83	81.66
		d12	22.3	66.6	11.1	78	72	
		d18	11.1	22.3	88.9	89	72	

Confusion matrix (%) columns are actual classes; SENS and SPEC are Global (%).

In the VIS-NIR range, even without any pre-processing of data, the sensitivity and specificity were quite high, and further improved after pre-processing (i.e. applying MSC+MC). For both raw and pre-processed data in the VIS-NIR range, all periods of storage were perfectly classified with the calibration model. For the cross-validation, pre-processing of data resulted in an increase in NER value from 88 % to 96 %. For the raw data in the SWIR range, lower performance of the PLS-DA classification was found, resulting in an overall NER of 85.33 % and 81.66 % after pre-processing (i.e. 1st D + MSC + MC), for the calibration and cross-validation sets, respectively.

3.4 Classification models based on the selected wavelengths

To reduce the number of wavelengths in the model classifier for on-line and real-time purposes, the VIP method was employed. Fig. 7 shows that the most effective wavelengths in PLS-DA models for classification of stored and fresh fruit in VIS-NIR and SWIR ranges. Wavelengths in the VIS-NIR range were 694, 719, 751, 813, 886, and 973 nm, which are associated with C-H stretch and chlorophyll b, texture, and internal chemical composition (T. Ignat et al. 2012; Nordey et al. 2017) .Chlorophyll is a key factor for evaluation of chilling sensitivity specially in green vegetable (Lim, Kang, and Cho 2007; Shimon Meir et al. 1997; Smillie et al. 1987). On the other hand, in the SWIR range 1138, 1244, 1379, 1642 nm are considered as the most effective wavelengths that infer the presence of ascorbic acid, water and sugar (Golic et al. 2003; T. Ignat et al. 2012; Timea Ignat et al. 2013). The first point above '1' in VIP score plot of SWIR was neglected since it fell in the poor SNR region. After variable selection for both VIS-NIR and SWIR using the VIP scores, the number of variables decreased from a total of 204 to seven in the VIS-NIR range and from 250 to four in the SWIR range.



A B

Fig. 7. Variable importance in projection (VIP) scores for A. VIS NIR and B. SWIR PLS-DA models for discriminating the different lengths of storage (d) among bell peppers stored at 4 °C

Table 3 shows the confusion matrices of the model (with selected wavelengths) and their ability to discriminate stored and fresh fruit for the two wavelength ranges.

Table 3. Confusion matrices of stored and fresh fruit classification based on selected wavelengths and pre-

processed data in A. VIS-NIR and B. SWIR ranges

A

Preprocessed Data				Confusion matrix (%) Actual			Global (%)		Non-Error-Rate (%)
				Fresh	4 °C	12 °C	SENS	SPEC	
	Cal	predicted	Fresh	100	0	0	100	100	
			4 °C	0	85	23	81	80	87
			12 °C	0	15	77	80	86	
				Fresh	4 °C	12 °C			
	CV	predicted	Fresh	100	0	0	100	100	
			4 °C	0	74	27	78	78	83.66
			12 °C	0	26	73	80	78	

B

Preprocessed Data				Confusion matrix (%) Actual			Global (%)		Non-Error-Rate (%)
				Fresh	4 °C	12 °C	SENS	SPEC	
	Cal	predicted	Fresh	100	11.1	3.7	100	94	
			4 °C	0	63	37	74	61	84
			12 °C	0	25.9	59.2	78	58	
				Fresh	4 °C	12 °C			
	CV	predicted	Fresh	88.11	11.1	3.7	100	90	
			4 °C	0	63	37	70	55	81.33
			12 °C	11.12	25.9	59.2	74	61	

After using selected wavelengths in VIS-NIR spectra with MSC+MC pre-processing, the NER yielded to 87 % and 83 % for the calibration and cross-validation sets, respectively. For the SWIR dataset, after pre-processing with Norm+MSC+MC, and using four selected wavelengths in this range, the NER reached to 84 % for calibration, and 81% for cross-validation, set, respectively for discriminating fresh and stored fruit.

Similar procedure using the VIP scores was used for variable reduction in PLS-DA classification models for fruit stored at 4 °C based on storage time. For the PLS-DA model in the VIS-NIR range, the VIP scores revealed 12 peaks which were most effective in the model corresponding to 457, 473, 481, 697, 700, 706, 716, 719, 727, 748, 997, 999 nm. These wavelengths are related to carotenoid and pigment absorption band, carbohydrate absorption band, and second overtone O-H vibration association with water (Kačuráková and Mathlouthi 1996; Kodad et al. 1994; Siedliska et al. 2018b). Moreover, in SWIR range, 13 wavelengths (i.e. 1110, 1166, 1233, 1312, 1390, 1676, 1905, 1967, 2051, 2247, 2252, 2308, 2420 nm) were also selected. Upon exploration these wavelengths were found to be related to carbohydrate absorption bond, C-H stretching bond, O-H stretching and O-H deformation linked to water, combination of the aromatic C-H and ring C=C stretching vibration.(Kačuráková and Mathlouthi 1996; Kodad et al. 1994).

Table 4. Confusion matrices for discrimination among fruit stored at 4 °C for 6, 12, and 18 d based on selected wavelengths and pre-processed data in A. VIS NIR and B. SWIR ranges.

A

				Confusion matrix (%)			Global (%)		Non-Error-Rate (%)
				Actual					
				d6	d12	d18	SENS	SPEC	
Preprocessed data	Cal	predicted	d6	100	0	0	100	100	
			d12	0	100	0	100	100	100
			d18	0	0	100	100	100	
				d6	d12	d18			
	CV	predicted	d6	100	0	0	89	100	
			d12	0	100	0	100	100	96.33
			d18	0	0	100	100	100	

B

				Confusion matrix (%) Actual			Global (%)		Non Error-Rate(%)
				d6	d12	d18	SENS	SPEC	
Preprocessed data	Cal	predicted	d6	88.11	0	12	89	89	
			d12	0	88.11	11	89	89	77.66
			d18	11.2	11.2	77	55	89	
				d6	d12	d18			
	CV	predicted	d6	77	11	44.4	78	83	
			d12	11	77	33.3	78	78	63
			d18	12	12	22.3	33	83	

The results of the PLS-DA model for the SWIR range after wavelength reduction show diminished performance in comparison to the whole dataset analysis; whereas the model in the VIS-NIR maintained the same performance level as of the full wavelength range that is 100 % for calibration and 96 % for cross-validation.

3.5 Regression models

PLSR calibration models for the prediction of days of storage at 4 °C were developed using the pre-processed data of VIS-NIR HSI, both in full spectra range and for selected wavelengths. VIS-NIR HSI data were preferred for regression model development, since this technique gave better results in comparison to SWIR HSI classification models. Prior to developing calibration models, the number of LVs was optimized during cross-validation of PLSR models. Six and five LVs were used for full range and for selected wavelength models, respectively. The results of the best PLSR models for predicting days of storage at 4 °C are presented in Table 5.

Table 5. PLSR model results for prediction of days of storage at 4°C

Spectral range	***No. of Wavelengths***	***LVs***	R^2_{Cal}	R^2_{CV}	***RMSEC***	***RMSECV***
Full range	204	6	0.95	0.92	0.23	0.30
Selected wavelengths	12	5	0.87	0.79	0.39	0.51

The PLSR models yielded R^2_{Cal}= 0.95, R^2_{CV} = 0.92, RMSEC=0.23, and RMSECV=0.30 for full range spectra, while lower accuracy was obtained by reducing the number of wavelengths to 12 (R^2_{Cal}= 0.87, R^2_{CV} = 0.79, RMSEC=0.39, and RMSECV=0.5). Nonetheless even in this case error in CV was equal to half day which can be more than acceptable for fruit sorting proposal.

Sun et al. (2017) successfully discriminated chilled peaches at different stages from non-chilled peaches by using VIS-NIR spectra and three different classifiers (PLS-DA, ANN, and SVM). Moreover, they considered first derivative spectrum for selection of the most effective wavelengths. In the current study, VIP scores were applied as a feature selection, since VIP score of a spectrum is considered as a weighted sum of the squared correlations between the PLS-DA components and the original spectrum (Banerjee et al. 2013) that could be more reliable compared with processed spectra. On the other hand, Cen et al. (2016) used both reflectance (500-675 nm) and transmittance (675-1000 nm) mode combined with texture features for detection of CI in cucumbers. They also explored three different classifiers, support vector machine(SVM), artificial neural network (ANN), and Naive Bayes (NB), all of which are non-linear and time-consuming methods compared with PLS-DA classifier was used in this study. For detection of CI in peaches using HS reflectance imaging and ANN, Pan et al. (2016) used the normalized importance (NI) derived from ANN model for variable selection. No pre-processing was performed in that study while in the present study different pre-treatment methods and their combinations were investigated to see the effect on the performance of calibration models (only the best one was shown). ElMasry et al. (2009) employed HSI (400-1000 nm) coupled with ANN classifier to identify CI in 'Red Delicious' apples. They used Importance of measure values for wavelength selection, which is a complicated method when compared with VIP scores. Also, they did not report the effect of pre-processing on their models. Moreover, all of the previously conducted studies have dealt with the discrimination of fruit with and without CI. Our study is the first attempt aimed at early prediction of the CI, being able to discriminate cold stored fruit and to predict storage days at low temperature, so that corrective action can be taken to avoid further spoilage. In addition, the current study explores the entire visible to near-infrared spectrum from 400 nm to 2500 nm, and not only the Vis-NIR part. SWIR spectra allowed when used raw, better discrimination compared to SWIR for discrimination of fresh and stored fruit at the two temperatures, and this can be useful information for further transfer to industrial application. Although the experiment was conducted offline and under controlled lab conditions, the information can be easily transferred to industrial application.

4. Conclusion

This work used HSI in VIS-NIR (400-1000 nm) and SWIR (100-2500 nm) ranges combined with chemometric tools for early detection of CI in green bell peppers. No externally observable CI symptoms were detected even after 18 d of storage at 4 °C, but results of PLS-DA classifications discriminated cold stored fruit from fresh fruit and from fruit stored at higher temperature by using 6 variables in the VIS-NIR or 4 variables in the NIR range. In addition, using 12 variables in the VIS-NIR was possible to detect fruit stored at 4 °C early. The results of this study demonstrate the feasibility of HSI, in particular VIS-NIR range, for early detection of CI of green bell pepper. Moreover, it was proved that it is quite possible to apply these results as multispectral imaging for industrial purposes.

Acknowledgment

This research was financially supported by the Natural Sciences and Engineering Research Council of Canada at the Department of Biosystems Engineering, University of Manitoba. Università di Foggia, Italy, provided financial support for travelling costs of the visiting student.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Reference

Amodio, Maria Luisa et al. 2017. "Potential of NIR Spectroscopy for Predicting Internal Quality and Discriminating among Strawberry Fruits from Different Production Systems." *Postharvest Biology and Technology* 125: 112–21. http://dx.doi.org/10.1016/j.postharvbio.2016.11.013.

Banerjee, Priyanka et al. 2013. "Identification of Key Contributory Factors Responsible for Vascular Dysfunction in Idiopathic Recurrent Spontaneous Miscarriage." *PLoS ONE* 8(11).

Barboza, Fernando D, and Ronei J Poppi. 2003. "Determination of Alcohol Content in Beverages Using Short-Wave near-Infrared Spectroscopy and Temperature Correction by Transfer Calibration Procedures." *Analytical and bioanalytical chemistry* 377(4): 695–701.

Barker, Matthew, and William Rayens. 2003. "Partial Least Squares for Discrimination." *Journal of Chemometrics* 17(3): 166–73. https://doi.org/10.1002/cem.785.

Birth, G.S. 1976. "How Light Interacts with Foods." *Quality Detection in Foods,ASAE*.

Bro, Rasmus, and Age K Smilde. 2014. "Principal Component Analysis." *Analytical Methods* 6(9): 2812–31. http://dx.doi.org/10.1039/C3AY41907J.

Cantwell, Marita. 1999. "Bell Pepper: Recommendations for Maintaining Postharvest Quality." *Perishables Handling* 87: 8–10.

Castorena, Juan, Jason Morrison, Jitendra Paliwal, and Chyngyz Erkinbaev. 2015. "Non-Uniform System Response Detection for Hyperspectral Imaging Systems." *Infrared Physics & Technology* 73: 263–68. http://www.sciencedirect.com/science/article/pii/S1350449515002510.

Cen, Haiyan, Renfu Lu, Qibing Zhu, and Fernando Mendoza. 2016a. "Nondestructive Detection of Chilling Injury in Cucumber Fruit Using Hyperspectral Imaging with Feature Selection and Supervised Classification." *Postharvest Biology and Technology* 111: 352–61. https://www.sciencedirect.com/science/article/pii/S0925521415301320 (February 26, 2018).

———. 2016b. "Nondestructive Detection of Chilling Injury in Cucumber Fruit Using Hyperspectral Imaging with Feature Selection and Supervised Classification." *Postharvest Biology and Technology* 111: 352–61. http://dx.doi.org/10.1016/j.postharvbio.2015.09.027.

Cheema, Amer et al. 2018. "Postharvest Hexanal Vapor Treatment Delays Ripening and Enhances Shelf Life of Greenhouse Grown Sweet Bell Pepper (Capsicum Annum L.)." *Postharvest Biology and Technology* 136(September 2017): 80–89. http://dx.doi.org/10.1016/j.postharvbio.2017.10.006.

Concellón, Analía, María C. Añón, and Alicia R. Chaves. 2005. "Effect of Chilling on Ethylene Production in Eggplant Fruit." *Food Chemistry* 92(1): 63–69.

ElMasry, Gamal, Ning Wang, and Clément Vigneault. 2009. "Detecting Chilling Injury in Red Delicious Apple Using Hyperspectral Imaging and Neural Networks." *Postharvest Biology and Technology* 52(1): 1–8.

Eriksson, Lennart et al. 2013. 1 *Multi-and Megavariate Data Analysis Basic Principles and Applications*. Umetrics Academy.

Erkinbaev, Chyngyz, Kieran Derksen, and Jitendra Paliwal. 2019. "Single Kernel Wheat Hardness Estimation Using near Infrared Hyperspectral Imaging." *Infrared Physics & Technology* 98: 250–55. https://www.sciencedirect.com/science/article/pii/S1350449519300106 (June 26, 2019).

Erkinbaev, Chyngyz, Kelly Henderson, and Jitendra Paliwal. 2017. "Discrimination of Gluten-Free Oats from Contaminants Using near Infrared Hyperspectral Imaging Technique." *Food Control* 80: 197–203. http://www.sciencedirect.com/science/article/pii/S0956713517302232.

Golic, Mirta, Kerry Walsh, and Peter Lawson. 2003. "Short-Wavelength Near-Infrared Spectra of Sucrose, Glucose, and Fructose with Respect to Sugar Concentration and Temperature." *Applied Spectroscopy* 57(2): 139–45. https://doi.org/10.1366/000370203321535033.

Gómez, Antihus Hernández, Yong He, and Annia García Pereira. 2006. "Non-Destructive Measurement of Acidity, Soluble Solids and Firmness of Satsuma Mandarin Using Vis/NIR-Spectroscopy Techniques." *Journal of Food Engineering* 77(2): 313–19. http://www.sciencedirect.com/science/article/pii/S0260877405004449.

Ignat, T. et al. 2012. "Non-Destructive Measurement of Ascorbic Acid Content in Bell Peppers by VIS-NIR and SWIR Spectrometry." *Postharvest Biology and Technology* 74: 91–99. http://dx.doi.org/10.1016/j.postharvbio.2012.06.010.

Ignat, Timea et al. 2013. "Nonlinear Methods for Estimation of Maturity Stage, Total Chlorophyll, and Carotenoid Content in Intact Bell Peppers." *Biosystems Engineering* 114(4): 414–25. http://www.sciencedirect.com/science/article/pii/S1537511012001675.

De Jong, Sijmen. 1990. "Multivariate Calibration, H. Martens and T. Naes, Wiley, New York, 1989. ISBN 0 471 90979 3. Price: £75.00, US$138.00. No. of Pages: 504." *Journal of Chemometrics* 4(6): 441. https://doi.org/10.1002/cem.1180040607.

Kačuráková, Marta, and Mohamed Mathlouthi. 1996. "FTIR and Laser-Raman Spectra of Oligosaccharides in Water: Characterization of the Glycosidic Bond." *Carbohydrate Research* 284(2): 145–57. https://www.sciencedirect.com/science/article/pii/0008621595004122 (July 1, 2019).

Kodad, H, R Mokhlisse, E Davin, and G Mille. 1994. "FTIR Analysis of Sugars in Aqueous-Solution Using Attenuated Total-Reflection." *Canadian Journal of Applied Spectroscopy* 39(4): 107–12.

Lee, Wang Hee et al. 2014. "Hyperspectral Near-Infrared Imaging for the Detection of Physical Damages of Pear." *Journal of Food Engineering* 130: 1–7. http://dx.doi.org/10.1016/j.jfoodeng.2013.12.032.

Li, Jiangbo, Liping Chen, and Wenqian Huang. 2018. "Detection of Early Bruises on Peaches (Amygdalus Persica L.) Using Hyperspectral Imaging Coupled with Improved Watershed Segmentation Algorithm." *Postharvest Biology and Technology* 135(September 2017): 104–13. http://dx.doi.org/10.1016/j.postharvbio.2017.09.007.

Lim, CS, SM Kang, and JL Cho. 2007. "Bell Pepper (<i>Capsicum Annuum<i> L.) Fruits Are Susceptible to Chilling Injury at the Breaker Stage of Ripeness." *HortScience* 42(7): 1659–64. http://hortsci.ashspublications.org/content/42/7/1659.short.

Liu, Ling et al. 2015. "Intermittent Warming Improves Postharvest Quality of Bell Peppers and Reduces Chilling Injury." *Postharvest Biology and Technology* 101: 18–25.

Lu, R. 2001. "PREDICTING FIRMNESS AND SUGAR CONTENT OF SWEET CHERRIES USING NEAR–INFRARED DIFFUSE REFLECTANCE SPECTROSCOPY." *Transactions of the ASAE* 44(5): 1265. http://elibrary.asabe.org/abstract.asp?aid=6421&t=3.

Marín, Alicia, Federico Ferreres, Francisco A Tomás-Barberán, and María I Gil. 2004. "Characterization and Quantitation of Antioxidant Constituents of Sweet Pepper (Capsicum Annuum L.)." *Journal of Agricultural and Food Chemistry* 52(12): 3861–69. https://doi.org/10.1021/jf0497915.

Meir, S et al. 1995. "Improvement of the Postharvest Keeping Quality and Colour Development of Bell Pepper (Cv. 'Maor') by Packaging with Polyethylene Bags at a Reduced Temperature." *Postharvest Biology and Technology* 5(4): 303–9. http://www.sciencedirect.com/science/article/pii/092552149400035Q.

Meir, Shimon, Reuven Ronen, Susan Lurie, and Sonia Philosoph-Hadas. 1997. "Assessment of Chilling Injury during Storage: Chlorophyll Fluorescence Characteristics of Chilling-Susceptible and Triazole-Induced Chilling Tolerant Basil Leaves." *Postharvest Biology and Technology* 10(3): 213–20. https://www.sciencedirect.com/science/article/pii/S0925521497014105 (September 9, 2019).

Di Natale, Corrado et al. 2001. "The Evaluation of Quality of Post-Harvest Oranges and Apples by Means of an Electronic Nose." *Sensors and Actuators, B: Chemical* 78(1–3): 26–31.

Nicolaï, Bart M et al. 2008. "Time-Resolved and Continuous Wave NIR Reflectance Spectroscopy to Predict Soluble Solids Content and Firmness of Pear." *Postharvest Biology and Technology* 47(1): 68–74. http://www.sciencedirect.com/science/article/pii/S0925521407002050.

Nordey, Thibault et al. 2017. "Robust NIRS Models for Non-Destructive Prediction of Mango Internal Quality." *Scientia Horticulturae* 216: 51–57. http://dx.doi.org/10.1016/j.scienta.2016.12.023.

Pan, Leiqing et al. 2016. "Detection of Cold Injury in Peaches by Hyperspectral

Reflectance Imaging and Artificial Neural Network.” *Food Chemistry* 192: 134–41. http://dx.doi.org/10.1016/j.foodchem.2015.06.106.
Park, Me-Hea, Peerapong Sangwanangkul, and Ji-Weon Choi. 2018. “Reduced Chilling Injury and Delayed Fruit Ripening in Tomatoes with Modified Atmosphere and Humidity Packaging.” *Scientia Horticulturae* 231: 66–72. https://www.sciencedirect.com/science/article/pii/S0304423817307446 (July 1, 2019).
Paull, R.E. 1990. “Chilling Injury of Crops of Tropical and Subtropical Origin.” In *Chilling Injury of Horticultural Crops*, , 17–36.
Rinnan, Åsmund, Frans van den Berg, and Søren Balling Engelsen. 2009. “Review of the Most Common Pre-Processing Techniques for near-Infrared Spectra.” *TrAC Trends in Analytical Chemistry* 28(10): 1201–22. https://www.sciencedirect.com/science/article/pii/S0165993609001629#aep-section-id29 (January 24, 2019).
Schmilovitch, Ze’ev et al. 2014. “Hyperspectral Imaging of Intact Bell Peppers.” *Biosystems Engineering* 117: 83–93. http://www.sciencedirect.com/science/article/pii/S1537511013001104.
Shi, Junyan et al. 2018. “Low-Temperature Conditioning Enhances Chilling Tolerance and Reduces Damage in Cold-Stored Eggplant (Solanum Melongena L.) Fruit.” *Postharvest Biology and Technology* 141(March): 33–38. https://doi.org/10.1016/j.postharvbio.2018.03.007.
Siedliska, Anna et al. 2018a. “Detection of Fungal Infections in Strawberry Fruit by VNIR/SWIR Hyperspectral Imaging.” *Postharvest Biology and Technology* 139(September 2017): 115–26.
———. 2018b. “Detection of Fungal Infections in Strawberry Fruit by VNIR/SWIR Hyperspectral Imaging.” *Postharvest Biology and Technology* 139(January): 115–26.
Smillie, Robert M, Robyn Nott, Suzan E Hetherington, and Gunnar Öquist. 1987. “Chilling Injury and Recovery in Detached and Attached Leaves Measured by Chlorophyll Fluorescence.” *Physiologia Plantarum* 69(3): 419–28. https://doi.org/10.1111/j.1399-3054.1987.tb09219.x.
Sun, Ye et al. 2017. “Hyperspectral Reflectance Imaging Combined with Chemometrics and Successive Projections Algorithm for Chilling Injury Classification in Peaches.” *LWT - Food Science and Technology* 75: 557–64. http://dx.doi.org/10.1016/j.lwt.2016.10.006.
Wold, S., E. Johansson, and M. Cocchi. 1993. “PLS - Partial Least-Squares Projections to Latent Structures.” In *3D QSAR in Drug Design*, ed. Hugo Kubinyi. ESCOM Science, 523–50.
Xing, Juan et al. 2005. “Detecting Bruises on ‘Golden Delicious’ Apples Using Hyperspectral Imaging with Multiple Wavebands.” *Biosystems Engineering* 90(1): 27–36.
Yang, Huqing, Fenghua Wu, and Jiyu Cheng. 2011. “Reduced Chilling Injury in Cucumber by Nitric Oxide and the Antioxidant Response.” *Food Chemistry*

127(3): 1237–42. http://www.sciencedirect.com/science/article/pii/S0308814611002548.

Yao, Wensi et al. 2018. "Glycine Betaine Treatment Alleviates Chilling Injury in Zucchini Fruit (Cucurbita Pepo L.) by Modulating Antioxidant Enzymes and Membrane Fatty Acid Metabolism." *Postharvest Biology and Technology* 144: 20–28. https://www.sciencedirect.com/science/article/pii/S0925521418302047 (July 1, 2019).

ASSESSMENT OF EGGPLANT FRUIT FRESHNESS USING NON-DESTRUCTIVE TECHNIQUES

Babellahi F. [1] , Tsouvaltzis.P[2] , Amodio M.L.[1], Colelli G[1].

[1]*Department of Science of Agriculture, Food and Environment, University of Foggia, Via Napoli 25, 71122 Foggia, Italy*

[2]*Department of Horticulture, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece*

Submitted in *Acta Horticulturae* Journal- ***In Press (2019)***

Abstract

Eggplant fruit is a chilling injury sensitive vegetable, which should be stored at temperature of 12°C; however, at this temperature, the metabolism of the fruit is still intensively active and therefore significant quality deterioration may be induced. Since these quality losses can be difficult to detect by eyes, objective of this study was to develop a novel non-destructive method to estimate freshness of eggplants. Eggplant fruits (cv. Fantasy) were harvested from a commercial farm in Lecce, Italy, during July 2017. Fruits were stored at 12°C for 10 days. Every 2 days, fruits from were sampled and left at room temperature (20°C), for one additional day, simulating one-day shelf life at the market. Color spectra (360-740nm), Fourier Transform (FT)-NIR spectra (800- 2777 nm) and hyperspectral images in the Vis-NIR range (400-1000 nm) were also acquired on each fruit. Partial least square regression analyses were carried out between the data collected and the storage days and appropriate models were built, allowing safe assessment of the freshness of the fruits. According to the results based on whole wavelength ranges, storage days correlated very well with both the FT-NIR spectra and the hyperspectral data extracted from the Vis-NIR imaging system (R_C> 0.98, R_{CV}> 0.94, $RMSE_C$ < 0.4 and $RMSE_{CV}$< 0.8), in contrast to the color measurements with low R_C and R_{CV} values (0.78 and 0.71, respectively) and significantly high root means square errors (1.5 and 1.8, respectively). Moreover, after conducting SPA as a variable selection method, classification models could almost keep the performance. The results of this study may set the basis to develop a protocol allowing a rapid screening and sorting of eggplants according to their postharvest freshness either upon handling in a distribution center or even upon the reception in the retail market.

Keywords: Eggplant fruits, freshness, non-destructive, PLS-R, SPA

Introduction

Freshness of fruits and vegetables is playing a key role in relation to consumer’s acceptability, particularly for species that with short shelf life. Eggplants (Solanum melongena L.) is an annual and popular crop that can be grown in the sub-tropics and tropics area such as Mediterranean countries and Asia(Concellón et al. 2012). This produce is also an economically important and has been ranked the 25th global commodity crop(Shi et al. 2018).

Due to inappropriate transportation systems from the field to the market and also problems in post-harvest handling, eggplant fruit suffer from different quality deteriorations such as shrinkage in skin, decreasing color indices, and pedicel dryness (Concellón et al. 2012). In addition being chilling-sensitive they may manifest various chilling injury (CI) symptoms if they are stored at a temperature below 12 °C(Concellón, Añón, and Chaves 2005). On the other side, storing them for a prolonged time at the safe temperature (i.e. 12°C) induce a rapid deterioration of its quality. Thus, detection of freshness of eggplant fruits is crucial.

Non-destructive optical techniques have been used with different purposes for agricultural commodities(Amodio et al. 2017; Chaudhry et al. 2018; Giovanelli et al. 2014; Piazzolla et al. 2013). Infrared spectroscopy technique is suitable for the measurement of compounds comprising polar functional groups such as -OH, C-O, and N-H (Blanco and Villarroya 2002) and provides substitute tool for predicting the presence and/or concentration of specific chemical constituents in fruit and vegetables without prior sample preparation, moreover, can be exploited in different ranges such as Near-Infra-Red (NIR), Middle-Infra-red (MIR), and Far-Infra-Red(FIR)(Bureau, Cozzolino, and Clark 2019). Hyperspectral imaging, on the other hand, is one of the high efficiency computer vision system which can provide both spectral and spatial information of an object. One hyperspectral image is composed by multiple sub-images, each of them related to individual wavelength (ElMasry et al. 2007). As light is incident on a sample surface, around 4% of it is reflected back causing specular reflectance, and the rest of the incident energy is transmitted through the surface into the cellular structure of the fruit where it is scattered via small interfaces within the tissue or absorbed by cellular constituents(Birth 1976)

This study aimed to evaluate the potential of three different non-destructive optical techniques (i.e. Fourier transform-NIR spectroscopy (FT-NIR), hyperspectral imaging in VIS-NIR range (400-1000 nm) and color measurement (360-740nm)) for assessment of freshness of eggplant fruits. Partial Least Square Regression (PLSR) as a quantitative model, as well as, Successive Projections Algorithm (SPA) as a variable (wavelength) selection method were applied for this purpose.

2.Materials and Methods

2.1. Plant material

Immature eggplant fruit (cv. Fantasy) were harvested from a commercial farm near Lecce (Italy) and then transported within few hours at the Postharvest Laboratory of the University of Foggia. Fruits were divided into six groups of 12 samples. The first group was used for the determination on the fresh sample and the rest were stored at 12°C for 2, 4, 6, 8, and 10 days. Each group after storage was removed from cold room and was left at room temperature (~20 °C) for one additional day to simulate the market situation, before measurement of external color and acquisition of hyperspectral images and FT-NIR spectra.

2.2. Color measurements

Color was measured on 3 sides of each fruit longwise (below the calyx, at the center and near the blossom-end) in reflectance scanning mode in the wavelength region 360-740 nm, with a CM-2600d Konica Minolta spectrophotometer and an average of the three readings was calculated

2.3. Acquisition of the FT-NIR spectra

Fourier transformed- near infrared (FT-NIR) spectrometry was conducted on the eggplant fruit at room temperature (20 °C). Three points were scanned per fruit by manually displacing the fruit along its longwise axis (MPA Multi-Purpose FT-NIR Analyzer, Bruker Optics, Ettlingen, Germany), and an average of the three spectra was calculated. Reflectance mode was used during spectral acquisition over the absorbance range of 800- 2777 nm at an interval of 1.7 nm (sphere macro sample resolution, scanner velocity 10 kHz, sample scan time 64 scans, background scan time 64 scans). The instrument was equipped with a high-energy air-cooled NIR source (20 W tungsten-halogen lamp) and a permanently aligned and highly stable ROCKSOLID interferometer (Bruker).

2.4. Acquisition of the hyperspectral images

A hyperspectral line scan scanner (Version 1.4, DV srl, Padova, Italy) equipped with a spectrograph, working in the visible-near infrared (Vis-NIR) range (i.e. 400-1000 nm) with a spatial resolution of 1000×2000 pixels and a spectral resolution of 5 nm was employed to acquire the images. One fruit was taken for each replicate in a single image and self-developed MATLAB code was used for extracting the mean spectra of the fruit producing one spectrum per replicate. For the extraction of the mean spectrum, the original image was thresholded. Image thresholding was performed using the Otsu method, on the image depicting the best contrast between the foreground and background, corresponding to 795 nm. A 2D binary image (mask) was obtained, with 0 value for the background and 1 for the fruit tissue. This mask was imposed to extract the mean spectra of the pixels corresponding to the fruit(Tsouvaltzis et al. 2020) (Fig.1).

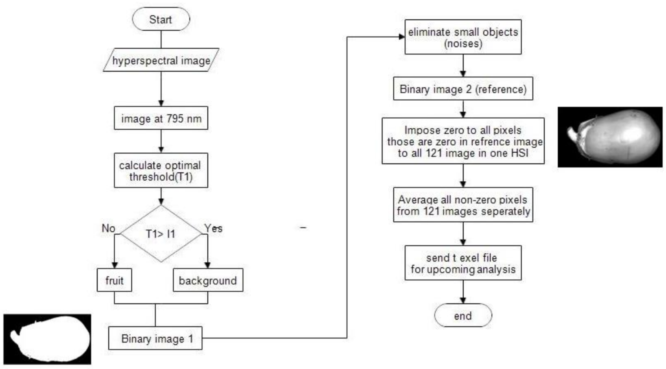


Fig.1. Data extraction flowchart for hyperspectral images

2.5. Multivariate data analysis

Multivariate and chemometric methods were applied for the development of calibration models on the extracted datasets (De Jong 1990). As the first step of data analysis, principal component analysis (PCA) was performed on data as a preliminary technique to reduce data redundancy. The technique transforms a set of correlated original variables into fewer uncorrelated orthogonal variables called Principal Components (PCs), which are the linear combinations of the original variables. The established PCA loadings on the other hand, represent the relationship among variables (Eriksson et al. 2013). Combination of variables describe major trends in the data, and can be a used as a tool to detecti outliers (Bro and Smilde 2014).

For developing the predictive models to assess the freshness of eggplant fruits, Partial Least Square Regression (PLS-R) model were used. In this case, the pre-processed data were considered as independent variables (X) and days of storage as dependent variables (Y). Generally, the PLS-R models amend the covariance between X and Y by decomposing them at the same time. For evaluation of the developed predictive models, root mean square error for calibration (RMSEC) and the root mean square error for cross-validation (RMSECV) were analyzed based on Eq.1.

$$\text{Eq.1: RMSEC=RMSECV=}\frac{\sqrt{\sum_{i=1}^{n_p}(\hat{y}_i-y_i)^2}}{n_p}$$

Where, $\hat{y}_i$ is the predicted value of an attribute in fruit number i, y_i is the measured value of an attribute in fruit number i, and $n_p$is the number of validated cases. The accuracy of PLSR models can be enhanced by carefully selecting the number of latent variables (LVs) which is determined on the basis of the minimum root mean square error of cross-validation (RMSECV).

2.6. Data Pre-processing

Even though the data extracted from the hyperspectral images of eggplants was affected by baseline shift and non-linearities, these kind of influences can be removed by conducting proper pre-processing. Commonly, these issues occur due to the fact that agricultural products are biological materials and the wavelengths in the short-wave NIR region are comparable to their particle sizes (Rinnan, Berg, and Engelsen 2009). The spectral data in this case was further confounded by the shiny surface of eggplants and unwanted noise. Therefore, pre-processing techniques such as mean-centering (MC) and multiplicative scatter correction (MSC), were applied, after transforming reflectance to absorbance, for all data sets acquired from the hyperspectral images, FT-NIR spectra, and spectra taken with the colorimeter.

2.7. Wavelength selection technique

Since the data acquired from spectroscopy and hyperspectral imaging devices are always redundant, selection of the most effective variables/wavelengths could be a way to reduce the number of wavelengths and make models simpler for on-line applications. In this study Successive Projections Algorithm (SPA) was applied on the data in this regard. SPA is a forward selection method that employs simple operations in a vector space to minimize variable collinearity(Araújo et al. 2001).

3. Results and discussions

3.1. Spectral analysis

Raw and average data extracted from FT-NIR spectroscopy technique is depicted in Fig.2.

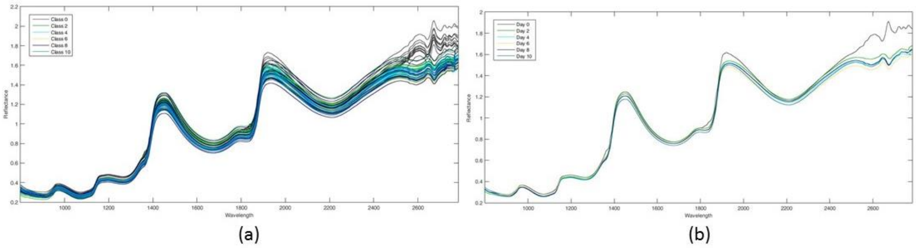


Fig.2 spectra of FT-NIR spectroscopy (a)raw spectra (b) mean spectra

As it is noticeable in Fig.2 there are clear distinction among days of storage in the area of the 2 peaks 1400- 1470 nm, 1900- 2020 nm, and in the last part of the spectra corresponding to 2400- 2700 nm which could be related to the first overtone of O-H stretching vibrations associated with water, combinations of the aromatic C-H and ring C=C stretching vibration, and C-H stretching bond, respectively (Siedliska et al. 2018). In case of Vis-NIR HSI data (not shown), a difference can be observed in the range of 815-910 nm that may be related to chemical composition (C-H stretch) and texture of the samples, and in the ranges of 960-990 nm that could be related to second overtone O-H stretching vibrations associated with water (Ignat et al. 2012; Siedliska et al. 2018). Regarding the data extracted from colorimeter (not shown), there was no perceptible difference between data extracted from the samples stored in different days.

3.2. PLS-R models for freshness assessment

PLS-R models were conducted on the whole spectra ranges of FT-NIR, Vis-NIR HSI, and colorimeter to predict the days of storage (freshness). To this aim, the spectra were correlated to storage days (i.e. 0, 2,4,6,8, and 10 days). Table 1 shows the optimum models chosen according to optimal number of Latent Variables (LVs), allowing to have high $R^2$and low RMSEC and RMSECV.

Table 1. Assessment of freshness using PLS-R models by various optical techniques

Non-destructive technique	Samples No.	Wavelengths No.	LVs	R^2 Calibration	RMSEC	R^2 Cross-Validation	RMSECV
Vis-NIR HSI	60	121	13	0.98	0.40	0.96	0.60
FT-NIR spectroscopy	60	1154	12	0.98	0.40	0.94	0.80
Colorimeter	60	34	7	0.78	1.5	0.71	1.8

Using whole treated spectra range of Vis-NIR hyperspectral image and 13 LVs, allowed to obtain PLS-R models with R^2_C 0.98 and RMSEC 0.40, as well as, R^2_{CV} 0.96 and RMSECV 0.60. Similarly, model developed based on the FT-NIR spectroscopy utilizing 12 LVs showed R^2 0.98 and 0.94 for calibration and cross-validation, respectively along with RMSEC 0.40 and RMSECV 0.80. In contrast, model of colorimeter technique didn't reveal an acceptable performance if compared to the other techniques. (R^2_{CV} 0.71 and 1.8 RMSECV with 7 LV). In other studies, different non-destructive methods were applied also for quality and freshness prediction of eggplants. N. Jha and Matsuoka (2002) used relative spectral reflectance and computerized spectral radiometer system to correlate surface gloss, stiffness, and density for the evaluation of freshness the in eggplant fruit. In their case, measurement of different quality indices was considered making the evaluation more time consuming. In another study, Ngadi, Martínez, and Schwinghamer (2016) applied nonlinear functions model against weight loss, peel gloss loss, surface stiffness loss, density ratio minus one, and storage period and they could predict very accurately stiffness loss (R^2_{adj} =0.98) , but not the days of storage.

3.3. PLS models based on selected wavelengths

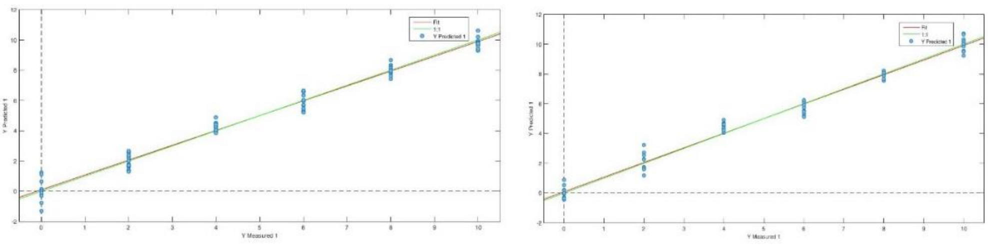
FT-NIR spectroscopy and hyperspectral images contain a big quantity of data and variable which make the time of analysis not appropriate for industrial purposes. So as to decrease the redundant information and enhance speed processing of HSI and FT-NIR spectroscopy data, different techniques are often applied to eliminate pointlessly data for selecting the most effective wavelengths. Due to this fact, SPA algorithm was used here. Among HIS data, 24 out of 121 wavelengths were selected (i.e.535, 590, 685, 710, 740, 750, 755, 765, 775, 820 855 ,870, 875 ,885, 895, 910, 925, 930, 945, 950 ,970, 975 ,995, and 1000 nm), as well as, 10 out of 1154 wavelengths (i.e. 1395, 1887, 2025, 2197, 2474, 2592, 2645, 2673, 2683, and 2723 nm) were chosen for FT-NIR data.

Table 2. Assessment of freshness using PLS-R-SPA models

Non-destructive technique	Samples No.	Wavelengths No.	LVs	R² Calibration	RMSEC	R² Cross-Validation	RMSECV
Vis-NIR HSI	60	24	6	0.96	0.65	0.94	0.72
FT-NIR	60	10	8	0.96	0.63	0.95	0.73

spectroscop y							

As shown in Table 2, the PLS-R models by means of selected wavelengths retained almost analogous performance PLS-R models generated from the full spectra ranges (Table 1), considering a slight increasing of the RMSE for calibration and cross-validation in Vis-NIR HSI, and a decrease of RMSECV in FT-NIR spectroscopy technique. Fig.3 shows the performance of the models based on the selected wavelengths graphically.



(a) (b)

Fig.3. Actual storage day (Y measured) vs Predicted storage days (Y predicted) for FT-NIR spectroscopy (a) and Vis-NIR HIS (b) techniques

4. Conclusion

The results demonstrated the applicability of the integrated VIS-NIR hyperspectral imaging and FT-NIR spectroscopy for freshness assessment of eggplant fruits, whereas, the colorimeter didn’t manifest an acceptable performance. Moreover, Successive Projection Algorithm (SPA) was also a trustworthy technique for variable selection to reduce the number of wavelengths and make the models simpler in order to be transferred for upcoming industrial purposes.

Reference

Amodio, Maria Luisa et al. 2017. "Potential of NIR Spectroscopy for Predicting Internal Quality and Discriminating among Strawberry Fruits from Different Production Systems." *Postharvest Biology and Technology* 125: 112–21. http://dx.doi.org/10.1016/j.postharvbio.2016.11.013.

Araújo, Mário César Ugulino et al. 2001. "The Successive Projections Algorithm for Variable Selection in Spectroscopic Multicomponent Analysis." *Chemometrics and Intelligent Laboratory Systems* 57(2): 65–73. https://www.sciencedirect.com/science/article/pii/S0169743901001198 (July 11, 2019).

Birth, G.S. 1976. "How Light Interacts with Foods." *Quality Detection in Foods,ASAE*.

Blanco, M., and I. Villarroya. 2002. "NIR Spectroscopy: A Rapid-Response Analytical Tool." *TrAC Trends in Analytical Chemistry* 21(4): 240–50. https://www.sciencedirect.com/science/article/pii/S0165993602004041 (September 30, 2019).

Bureau, Sylvie, Daniel Cozzolino, and Christopher J. Clark. 2019. "Contributions of Fourier-Transform Mid Infrared (FT-MIR) Spectroscopy to the Study of Fruit and Vegetables: A Review." *Postharvest Biology and Technology* 148(May 2018): 1–14. https://doi.org/10.1016/j.postharvbio.2018.10.003.

Chaudhry, Muahmmad M.A. et al. 2018. "Hyperspectral Imaging and Multivariate Accelerated Shelf Life Testing (MASLT) Approach for Determining Shelf Life of Rocket Leaves." *Journal of Food Engineering* 238: 122–33. https://linkinghub.elsevier.com/retrieve/pii/S026087741830267X (October 25, 2018).

Concellón, Analía, María C. Añón, and Alicia R. Chaves. 2005. "Effect of Chilling on Ethylene Production in Eggplant Fruit." *Food Chemistry* 92(1): 63–69.

Concellón, Analía, María J. Zaro, Alicia R. Chaves, and Ariel R. Vicente. 2012. "Changes in Quality and Phenolic Antioxidants in Dark Purple American Eggplant (Solanum Melongena L. Cv. Lucía) as Affected by Storage at 0°C and 10°C." *Postharvest Biology and Technology* 66: 35–41. http://dx.doi.org/10.1016/j.postharvbio.2011.12.003.

ElMasry, Gamal, Ning Wang, Adel ElSayed, and Michael Ngadi. 2007. "Hyperspectral Imaging for Nondestructive Determination of Some Quality Attributes for Strawberry." *Journal of Food Engineering* 81(1): 98–107.

Giovanelli, G. et al. 2014. “NIR Spectroscopy for the Optimization of Postharvest Apple Management.” *Postharvest Biology and Technology* 87: 13–20. http://dx.doi.org/10.1016/j.postharvbio.2013.07.041.

Ignat, T et al. 2012. “Non-Destructive Measurement of Ascorbic Acid Content in Bell Peppers by VIS-NIR and SWIR Spectrometry.” *Postharvest Biology and Technology* 74: 91–99. http://www.sciencedirect.com/science/article/pii/S0925521412001524.

N. Jha, S, and T Matsuoka. 2002. “DEVELOPMENT OF FRESHNESS INDEX OF EGGPLANT.” *Applied Engineering in Agriculture* 18(5): 555. http://elibrary.asabe.org/abstract.asp?aid=10142&t=3.

Ngadi, Michael O, Arturo A Mayorga Martínez, and Timothy D Schwinghamer. 2016. “Development of an Objective Freshness Index for a Variety of Mediterranean Eggplant.” *International Journal of Postharvest Technology and Innovation* 5(3): 231–50. https://www.inderscienceonline.com/doi/abs/10.1504/IJPTI.2016.083690.

Piazzolla, Francesca et al. 2013. “To Discriminate between Table Grapes Harvested at Different Times on Co m m Er Ci Al Us e on l on Co m m Er Ci Al E.” XLIV.

Shi, Junyan et al. 2018. “Low-Temperature Conditioning Enhances Chilling Tolerance and Reduces Damage in Cold-Stored Eggplant (Solanum Melongena L.) Fruit.” *Postharvest Biology and Technology* 141: 33–38. https://www.sciencedirect.com/science/article/pii/S0925521417311638 (July 10, 2019).

Siedliska, Anna et al. 2018. “Detection of Fungal Infections in Strawberry Fruit by VNIR/SWIR Hyperspectral Imaging.” *Postharvest Biology and Technology* 139(January): 115–26.

Tsouvaltzis, Pavlos, Farahmand Babellahi, Maria Luisa Amodio, and Giancarlo Colelli. 2020. “Early Detection of Eggplant Fruit Stored at Chilling Temperature Using Different Non-Destructive Optical Techniques and Supervised Classification Algorithms.” *Postharvest Biology and Technology* 159: 111001. https://www.sciencedirect.com/science/article/pii/S0925521419305447 (September 24, 2019).

CHARACTERIZATION OF THE STORAGE TIME AND TEMPERATURE ON CHILLING INJURY PROCESS IN EGGPLANT FRUIT BY NON DESTRUCTIVE OPTICAL-BASED TECHNIQUE AND CHEMOMETRICS

Farahmand Babellahi[1], Maria Luisa Amodio[1], Federico Marini[2] , Muhammad Mudassir Arif Chaudhry[1] , Leonarda Mastrandrea[1], Giancarlo Colelli[1]

[1]Dip.to di Scienze Agrarie, degli Alimenti e dell'Ambiente, Università di Foggia, Via Napoli 25, 71122 Foggia Italy

[2]Department of Chemistry, University of Rome "La Sapienza", P.le Aldo Moro 5, I-00185 Rome, Italy

ABSTRACT

The objective of the study was to non-destructively assess the early detection of chilling injury (CI) which is a physiological disorder occurring in the eggplant fruit subjected to temperatures lower than 12°C. Reference measurements of CI were acquired by conducting visual appearances analysis, measuring electrolyte leakage (EL), weight loss and firmness evaluation resulting in the fact that even before three days of storage at 2°C, the CI process initiated. ANOVA-simultaneous component analysis (ASCA) was used for the investigation of the effect of temperature and storage time also on the FT-NIR spectral fingerprints. The ASCA model depicted that temperature, duration of storage, and their interaction had a significant effect on the spectra. In addition, it was possible to highlight the main variations in the experimental profiles referable to the effects of the main factors and particularly in the case of storage time, to discover a major longitudinal monotonic trend. In addition to ASCA, partial least square- discriminant analysis (PLS-DA) was used as a supervised classification method to discriminate fruit based on the chilling and safe temperatures. In this case, only the spectral wavelengths which were found to be significantly influenced by the effect of temperature based on ASCA were utilized. PLS-DA prediction accuracy resulted to be 87.4±2.7% as estimated by a repeated double-cross-validation procedure (50 runs) and the significance of the observed discrimination was further proved by means of permutation tests. The outcomes of this study manifested a promising potential of near infrared spectroscopy for non–invasive, rapid and reliable detection of CI in eggplants.

Keywords: Eggplant, Chilling injury, ASCA, optical-technique, PLS-DA, rDCV

1 Introduction

Various factors influence the quality of the stored fruits and vegetables among which temperature and time of storage hold significant importance in terms of chilling injury (CI) occurrence (Kader 2013). Conventionally, most of the fruits and vegetables are recommended to be stored at low temperature for shelf life enhancement and nutritional quality retention but chilling sensitive produce is prone to CI when exposed to low temperatures (Fallik et al. 1995). Basically, CI induces damage to the cell membranes resulting in the solute diffusion and increased tissue permeability (Concellón, Añón, and Chaves 2005).

Eggplant (*Solanum melongena L.*) is an economical and a worldwide popular fruit which is non-climacteric, and which suffers severe CI when stored at temperatures below 12˚C (Concellón, Añón, and Chaves 2005). CI in eggplants is a physiological disorder which leads to pitting in peel, flesh browning, blackening of the seeds and increased decay, particularly in the calyx, which can be more severe when the fruit shifts to market temperature after being exposed to chilling temperature (Fallik et al. 1995; Shi et al. 2018).

Conventionally, phenolic content, anthocyanin content, malondialdehyde content, polyphenol oxidase, peroxidase, catalase activity, and electrolyte leakage are considered to be the most significant parameters related to CI (Concellón, Añón, and Chaves 2005; Fan et al. 2016; Shi et al. 2018).

Due to fact that all aforementioned conventional techniques are destructive, time consuming, and comparatively expensive, the possibility of having non-invasive, accurate, and rapid techniques for evaluation and detection of the CI would be completely worthwhile.

A variety of non-destructive optical techniques have been successfully applied for quality assessment of agricultural and horticultural commodities in the past few years (Amodio et al. 2017; Chaudhry et al. 2018; Cortés et al. 2017; Erkinbaev, Henderson, and Paliwal 2017; Munera et al. 2018).

In this regard, near infrared spectroscopy (NIRS) has proved to be effective for the estimation of compounds comprising polar functional groups such as -OH, C-O, and N-H (Blanco and Villarroya 2002) and serves as a substitute for predicting the presence of specific chemical constituents in fruit and vegetables without prior sample preparation.

Recently, Cen et al. (2016) used hyperspectral imaging for detection of CI in cucumbers utilizing supervised classifiers and feature selection techniques. Similar research objectives have successfully been pursued using hyperspectral imaging on Red Delicious apples and peaches (ElMasry, Wang, and Vigneault 2009; Pan et al. 2016). Moreover, Moomkesh et al. (2017) have investigated detection of freeze-damaged sweet lemons using reflectance, half-transmittance, and full-transmittance VIS/SWIR spectroscopy combined with various machine learning techniques.

Tsouvaltzis et al. (2020) successfully reported the possibility of classifying eggplant fruit based on temperature of storage (2°C and 12°C) using different optical-based techniques, and showed that Fourier-transform-near infrared (FT-NIR) spectroscopy was the most efficient technique to this aim. Nonetheless, in this paper the effect caused by temperature and duration of storage on the spectral response was not investigated. Therefore, objective of this study was to characterize the effect of storage time and temperature (together with their possible interaction) on FT-NIR spectra of eggplants stored at chilling and not chilling temperature, applying ANOVA-simultaneous component analysis (ASCA), in order to evaluate whether these effects could be considered statistically significant and, if so, to associate the changes of the instrumental signals to the progress of the CI. Moreover, a second objective was to use these results to discriminate fruit stored at the different temperatures.

2 Materials and methods

2.1 Sampling

Eggplant fruit (cv. Fantasy) were hand-harvested from a commercial farm located in Molfetta, Italy (41° 12' 0'' North, 16° 36' 0'' East) in July 2019. After inspection for absence of any defects and uniformity in terms of size, 87 fruits were transported to Postharvest Lab of the University of Foggia within 2 hours. Upon arrival at the laboratory, the fruit were placed at room temperature for temperature regulation, after which they were divided into three groups. The first group including 15 fruit was categorized as fresh eggplants for initial measurements, the second group comprised of 36 eggplants was stored at chilling temperature and the third group of 36 fruit at safe temperature (i.e., 2°C and 12°C, respectively). Fruits were removed from cold storage after 3, 6 and 10 days and were left at ambient temperature for five hours for temperature regulation prior to acquisition of spectra.

2.2 Electrolytic Leakage measurement

Electrolytic leakage (EL) was measured according to the method described by Fuchs et al. (1989), on six randomly selected fruit from each group. Seven discs (5 g) of each eggplant pulp with a thickness of 10mm each were removed from the equatorial region of every sample using a 10-mm diameter cork-borer. The discs were incubated in 25 mL solution 0.3 M of mannitol at 20°C. The conductivity of the solution was measured using conductivity meter (CM35, Crison, Carpi, Italy) at time zero (C1) and after 2 hours (C2) of incubation with orbital shaking (DAS12500, Intercontinental equipment, Roma, Italy) at a speed of 60 cycles min^{-1}. In case of the last measurement (C3), the tube including the sample and the solution was frozen and then defrosted after being kept at -20°C for 24h. Results were stated as a percentage of total electrolytes leaking out of the tissue as shown in the Eq1. Determinations were performed in duplicate and the results were averaged.

$$\text{Electrolytic Leakage (\%)} = \frac{C2-C1}{C3} \times 100 \qquad \text{Eq.1.}$$

2.3 Chilling injury evaluation

CI symptoms in eggplant fruit appear both internally and externally. Accordingly, the evaluation was done based on a checklist of four external (i.e. calyx browning, peel discoloration, pitting, and firmness) and two internal indicators (i.e. pulp browning and seek blackening) by four trained panelists. For each fruit, each CI symptom had a score based on the severity (i.e., 0=no chilling symptoms (0% of indices), 1= moderate chilling symptoms (<50% of indices), and 2= severe chilling symptoms (>50% of the indices)).

2.4 Firmness and weight loss evaluation

The firmness of each fruit was measured using a Texture Analyzer (TA.XT2, Stable Micro Systems Ltd., England, UK) equipped with a 5-mm diameter probe which was used to penetrate the eggplant pulp with a loading speed of 50 mm/min in three positions on the equator, consequently averaged. The maximum force (N) obtained from the force-deformation curve was used as an indication of the fruit firmness. The average maximum force was used as the firmness index of the eggplants.

Weight loss was measured for each fruit using an electronic balance (EU-C 7500 DR, GIBERTINI, Italy) as percentage loss between the day 0 and the end of each cold storage period.

2.5 FT-NIR spectroscopy

After fruit removal from each cold storage, fruit were kept at room temperature for five hours for temperature regulation, prior to FT-NIR spectra acquisition. A Multi-Purpose FT-NIR Analyzer (MPA, Bruker Optics, Ettlingen, Germany) was used to acquire three scans per sample taken along its longitudinal direction, which were averaged to formulate a representative spectrum for that particular sample. Reflectance mode was utilized during spectral acquisition over the absorbance range of 3600-12500 cm^{-1} at an interval of 3.8 cm^{-1}. The instrument was equipped with a high-energy air-cooled NIR source (20 W tungsten-halogen lamp) and a permanently aligned and highly stable ROCKSOLID interferometer (Bruker).

2.6 Chemometrics

2.6.1 ANOVA-simultaneous component analysis (ASCA)

To evaluate whether one or more controlled factors (and their interactions) have a significant effect on a multivariate signal, multivariate analysis of variance (MANOVA) is conventionally used as the generalization of ANOVA; however, this approach is not effective when the number of variables/wavelengths are higher than the number of measured samples and/or when the multivariate descriptors are highly correlated among one another and breaks down because it cannot handle singular covariance matrices (Stahle and Wold 1990). Hence, analysis of variance simultaneous component analysis, ANOVA-SCA or ASCA was designed as a multivariate exploratory technique to cope with data matrices resulting from an experimental design (Jansen et al. 2005; Smilde et al. 2005). In fact, ASCA combines a partitioning of the variability in the original data matrix X consistent with the scheme of the ANOVA, to the bilinear modelling of the effect sub-matrices attained utilizing simultaneous component analysis; a method which, under the constraints of the ANOVA scheme, is mathematically identical to principal component analysis (Smilde et al. 2005). In particular, in the case of the present study, where two factors, namely “temperature” and “storage time”, were controlled and, hence, the effect of three terms (the two factors plus their binary interaction) has to be investigated, the first step of ASCA involves partitioning the centered matrix X_c according to:

$$Xc = X\text{-}1m^T = X_{temperature} + X_{storage\ time} + X_{temperature\times\ storage\ time} + X_{res} \quad \text{Eq.2}$$

where 1 is a vector of ones, m is the mean spectrum of the samples, $X_{temperature}$ and $X_{storage\ time}$ are the matrices accounting for the effect of the main factors, $X_{temperature\times\ storage\ time}$ is the effect matrix for the interaction and X_{res} is the residual matrix, assembling the variability which has not been accounted for any of the previous factors. Each of the effect matrices X_i is built as follows: all the rows corresponding to a level of the specific factor/interaction contain identical copies of the mean spectrum of all the observations collected at that level.

Successively, significance of the observed effect is evaluated by permutation testing and interpretation of the design terms identified as significant is carried out by SCA of the corresponding effect matrix.

2.6.2 Partial Least Square- Discriminant Analysis (PLS-DA)

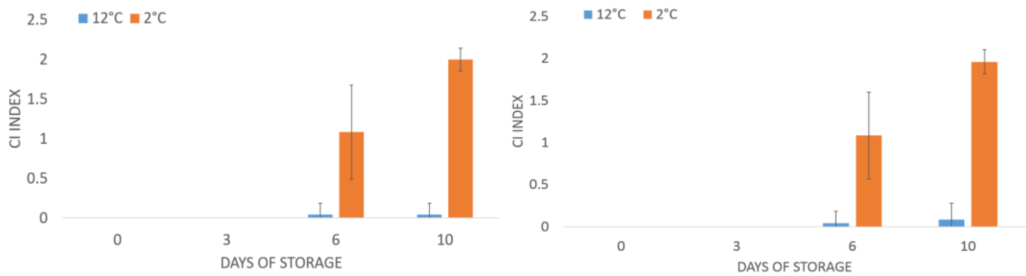
PLS-DA was applied on the wavelengths identified by ASCA model to discriminate between fruit stored at the 2 temperatures. PLS-DA, as a supervised classification technique, results from PLS regression (PLSR) and includes forming a regression model between the X (data acquired from instrument) and *Y* (dummy binary vector for coded samples). Classification of the samples is then accomplished based on the values of the predicted Y which, differently than those of the dummy matrix used for model building, are real-valued. (Brereton and Lloyd 2014).

Given the number of samples, to guarantee that validation of the predictive model could be carried out on external samples (i.e., individuals not used neither for model building nor for model selection), and, at the same time, to ensure that enough specimen could be used for model building and validation, a repeated double cross-validation (rDCV) strategy was adopted. Double cross-validation (DCV) consists of two cross-validation loops (an inner and an outer loop) nested in one another. The inner cross-validation loop is used for model selection (i.e., for choosing the optimal preprocessing and number of latent variables), whereas the outer loop contains the samples which are in turn treated as external validation set (i.e., which do not take part in model selection). To avoid that the estimated be biased by a specific division of samples into the different cancelation groups, the whole procedure is repeated a certain number of times (in the present study, 50), hence the name repeated DCV. More details can be found in Filzmoser et al. (2009). In particular, different preprocessing methods, i.e., SNV, derivatives calculating with different number of points and orders of the interpolating polynomial, and their combinations were tested on the data. As said, for each cancelation group in the outer cross-validation loop, selection of the optimal model (in terms of optimal pretreatment and number of latent variables) to predict the validation samples was carried out based on the minimum classification error in the inner CV loop. The best pretreatment was consistently found to be SNV + first derivative (second order polynomial and 11 points interpolating window) while the optimal model complexity was always selected to be 3 latent variables.

3 Results and discussion

3.1 Evaluation of chilling injury

The CI indices during storage are shown in Fig.1. It can be clearly observed that the fruit stored at 12°C almost did not manifest chilling symptoms until the end of storage; however, fruits stored at 2°C, started to show chilling symptoms on six days of storage that were mostly internal. Afterwards, chilling indices continued to increase, depicting severe CI symptoms, including browning, wrinkles, and scalds in the peel and pulp browning. Observed CI indices were in agreement with the result reported by Tsouvaltzis et al. (2020). In that study, the CI indices initiated to appear after four days, but it was quite possible also in the current work to be same, since in the second sampling (six days of storage) the CI indices reached almost 1.



(A) (B)

Fig.1. CI evaluation of eggplant fruit externally (A) and internally (B). The CI on fruit was scored as 0 = no chilling on fruit, 1 = slight CI symptoms and 2 = severe CI symptoms. Each point represents the mean of 12 fruit ± standard error (S.E.)

3.2 Evaluation of electrolytic leakage

The results of EL are shown in Fig.2. Starting from about 9 % as the initial value, there was a slight EL alteration for fruit stored at 12°C. On the other hand, after three days of storage at 2°C, EL started to increase and reached to 12% at the end of the storage period.

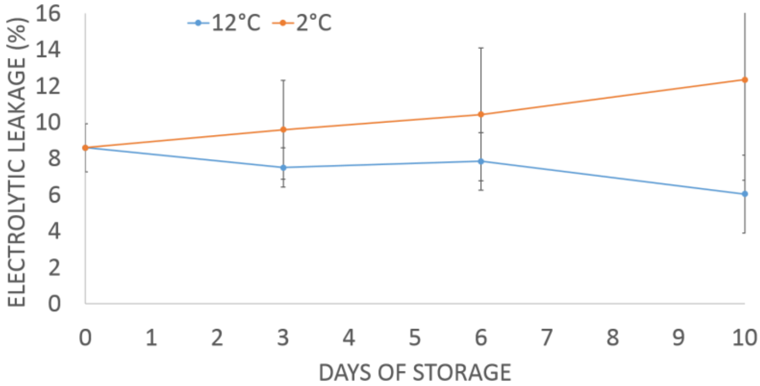
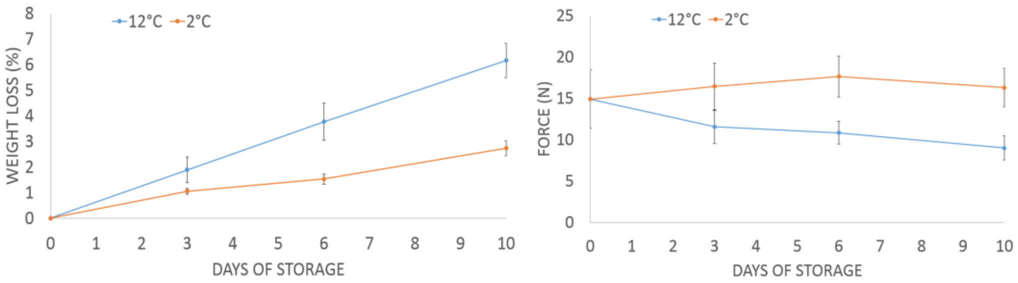


Fig.2. EL percentage from pulp tissue of eggplant during storage at 10°C (blue line) and 2°C (orange line). Each value is the mean of six replicates

3.3 Evaluation of weight loss and firmness

Weight loss increased during storage at both storage temperatures, and particularly for fruit stored at 12°C, as expected since the metabolism is higher with the increase of the temperature. As Fig. 3A depicts, at the end of storage period, the weight loss of fruit at safe temperature was 2.2 times higher than fruit stored at 2°C. Regarding firmness of eggplants, samples stored at 2°C nearly maintained the initial firmness, however, fruit at 12°C lost 33% of the firmness (Fig. 3B), and this can be directly related to firmness loss as also to the higher enzymatic activity at the higher temperature (Fan et al. 2016). In fact, even if recommended storage temperature is 12°C, by storing fruit at no-chilling temperature, quality degradation is unavoidably faster than at low temperature, but on the other side, the occurrence of CI make the chilled fruit not-marketable.



(A) (B)

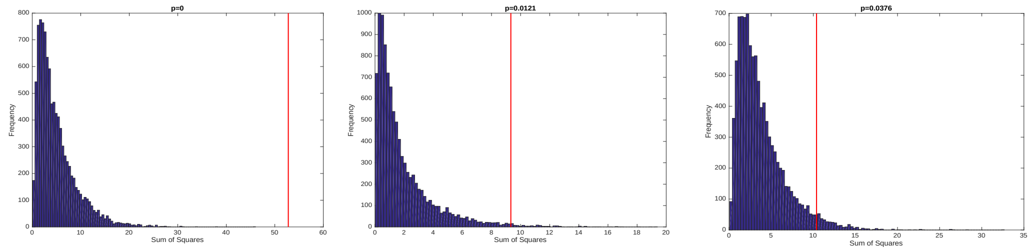
Fig.3. Firmness (A) and weight loss (B) of eggplant fruit stored at 12 °C (blue line) and 2 °C (orange line). Data presented are the means ± SE of 12 replicate samples

3.4 ASCA on FT-NIR data

This experiment was conducted based on a full factorial design, comprising two main factors (i.e. temperature and storage time). The temperature included two levels (2°C and 12°C) and storage time comprised of three levels (i.e. 3, 6, and 10 days). For investigating the effect of main factors and their interaction, a multivariate data analysis using ANOVA simultaneous component analysis (ASCA) was conducted on the data extracted from FT-NIR instrument as described at section 2.6.1. ASCA is a method which links the ANOVA variance separating pattern to evaluate whether any of the terms in the experimental design contributes significantly with bilinear modeling for the interpretation of the observed effects.

The ASCA modelling was applied on the data after preprocessing to interpret the effects of any of main factors and their interaction on the data.

Consequently, the mean-centered data matrix was partitioned according to the ANOVA scheme into the effect matrices for the three design terms and the residual matrix. Then, the multivariate effect of each design term was estimated by the sum of squares of the elements of the corresponding matrix: to evaluate whether the effect of each term could be considered as statistically significant, the value of the corresponding sum of squares was compared to its distribution under the null hypothesis, which was estimated non-parametrically by a permutation test (with10,000 randomizations), as shown in Fig.4. It can be clearly observed that all the effects were statistically significant, revealing that the spectral changes of eggplant fruit were affected by both temperature and storage time and that there was a non-negligible interaction between the two factors.



(A) (B) (C)

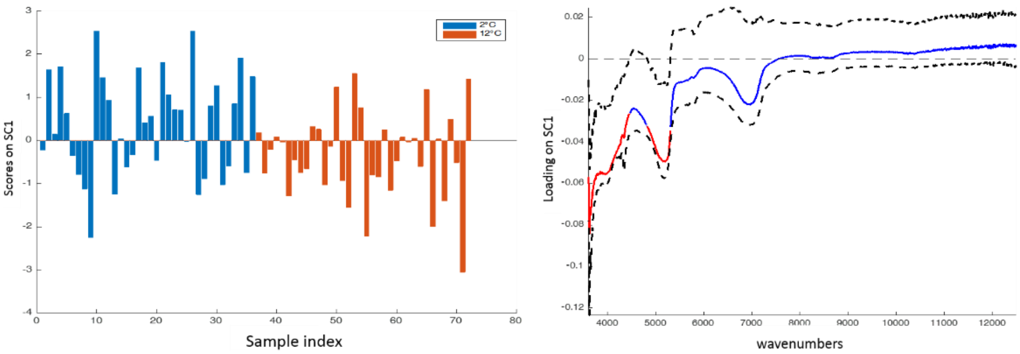
Fig.4. Assessment of the significance of the observed effects by comparing the experimental sum of squares (vertical red line) to its distribution under the null hypothesis, non-parametrically estimated via permutation tests (blue histogram). (A) Effect of temperature; (b) effect of storage time; (c) effect of temperature×storage time interaction

After proving that both the main factors and the interaction have a significant effect on the spectra, the next step regarding ASCA modelling is to interpret the observed variation using simultaneous component analysis (SCA) on the individual effect matrices. Initially, the effect of the temperature was explored, by computing a SCA model of the temperature effect matrix in which, as briefly explained in Section 2.6.1, half of the rows contain identical copies of the mean spectrum of the samples stored at low temperature and the other half are made of identical copies of the mean spectrum of the fruit stored at high temperature, after centering.

In order to have a visual hint for the variability related to the effect of a certain factor in ASCA model, residuals can be projected on the simultaneous component (SC) space of that design factor; in the case of temperature, for which, due to the reasons illustrated above, a one component model explains 100% variance in the effect matrix), this can be accomplished by calculating the score vector, reported below (Zwanenburg et al. 2011) :

$$\mathbf{t}_{temperature+res} = (X_{temperature}+X_{res}) \times \mathbf{p}_{tepmprature} \qquad \text{Eq.3.}$$

Where $\mathbf{p}_{tepmprature}$ is the loading vector of the SC model for the effect of the factor "temperature". The corresponding scores plot, reported in Fig.5A, shows how (also considering the variability associated to the effect of the factor "temperature") the difference between the scores of 2°C and 12°C can be considered statistically significant, as already evaluated by means of permutation tests. Fig 5 B shows the loadings of all the variables on SC1, together with their 95% confidence interval, indicating the spectral regions mostly affected by the temperature (in red). As can be noticed, only a reduced part of the spectral range was significantly affected by the temperature of storage (i.e. 3600-4555 cm^{-1} and 4740-5490 cm^{-1}).



(A) (B)

Fig.5. ASCA analysis on FT-NIR data: SCA model of the temperature effect. (A) Scores plot for the effect with projected residuals; (B) Variable loadings for SC1 (continuous line) together with their 95% confidence interval (black dashed lines): red and blue colors indicate whether the corresponding wavelength contributes significantly or not to the bilinear model, respectively

Due to the fact that positive and negative scores on SC1 indicate samples stored at 2°C and 12 °C, respectively, investigation of the loadings plot in Fig, 5B highlights that the significant bands (in red) are more intense (have higher pseudo-absorbance) when storage takes place at higher temperatures.

On the other hand, when considering the main effect of time, since the factor was controlled at three levels, the first two SC explain jointly 100% of the spectral variance related to the design term. Scores (after projection of the residuals) and loadings for SC1 are reported in fig. 6A and B, respectively; in particular, to account for the longitudinal trend, in panel 6A, for each level of the factor time the scores along SC1 of the corresponding observations are reported as mean (i.e., the score which would result without projection of the residuals) ± standard deviation. As it can be noticed, score increased with time, and almost all the range was significantly affected by the time of storage (3600-10000 cm^{-1}). Nonetheless, since temperature and time of storage also showed a significant interaction, it may be better to investigate and interpret jointly the main effect of storage time and the storage$_{\text{time} \times \text{temperature}}$ interaction, so to highlight not only the average effect of storage time on the extracted spectra, but also how the two temperatures differently affected the longitudinal behavior.

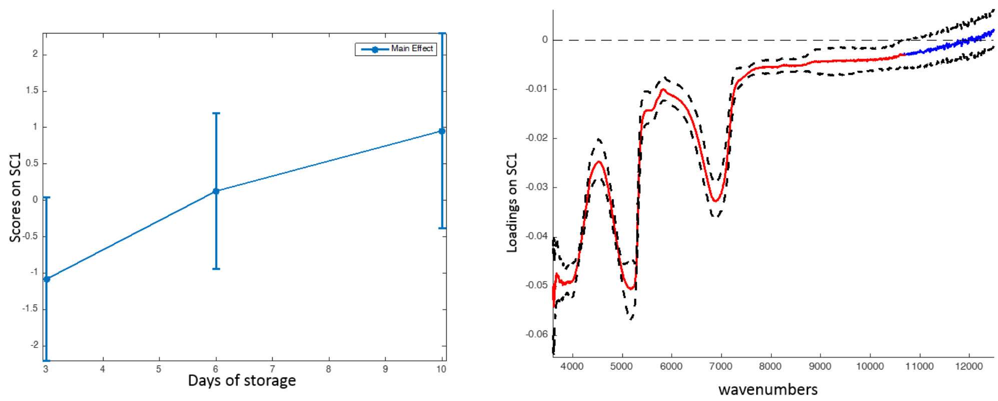


Fig.6. ASCA analysis on FT-NIR data: SCA model of the storage time effect. (A) Longitudinal plot of the scores along SC1 (expressed as mean± standard deviation after projection of the residuals) vs time; (B); Variable loadings for SC1 (continuous line) together with their 95% confidence interval (black dashed lines): red and blue colors indicate whether the corresponding wavelength contributes significantly or not to the bilinear model, respectively

To this aim, instead of analyzing each factor separately, SCA was carried out on the matrix $X_{\text{storage time + storage time × temperature}}$ (resulting from the addition of $X_{\text{storage time}}$ and $X_{\text{storage time × temperature}}$). Accordingly, since the matrix $X_{\text{storage time + storage time × temperature}}$ contains identical copies of six mean spectra only, corresponding to the six different possibilities coming out from the combination of factor levels (3d at 2°C, 6d at 2°C, 10d at 2°C, 3d at 12°C, 6d at 12°C, 10d at 12°C), for each component there will only be six different score values identifying the design cells. On the other hand, also in this case it is possible also to project the residual matrix onto the SC subspace defined by the effect matrix for visualizing the variability associated with the factor + interaction levels and, consequently, to have a visual idea about the significance of the design terms.

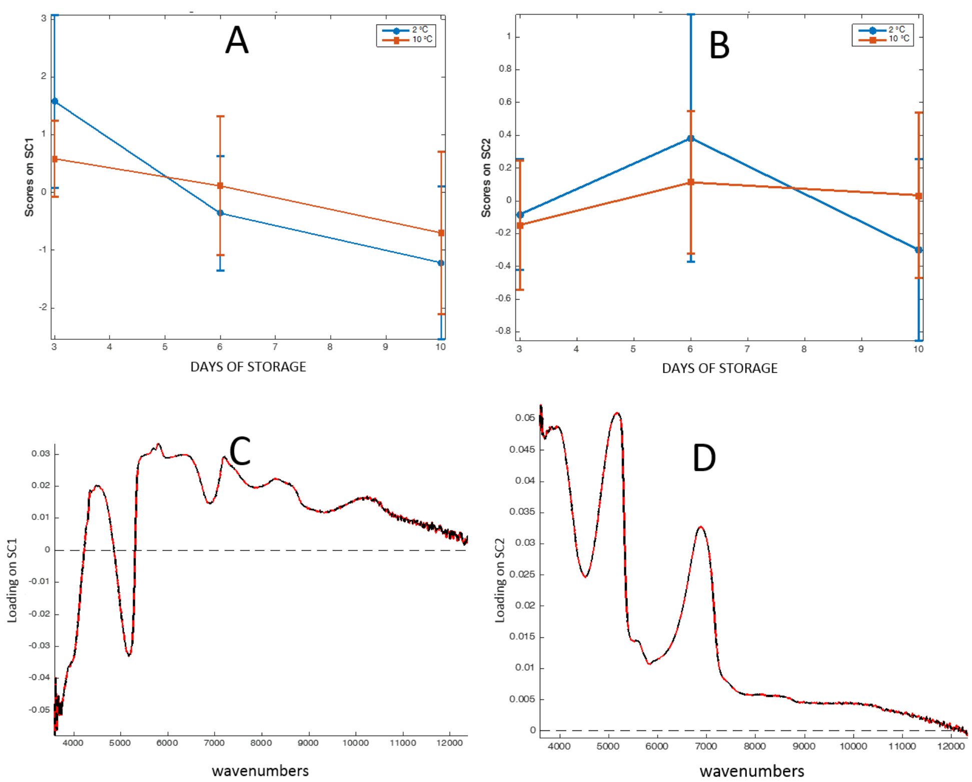


Fig.7. ASCA analysis on FT-NIR data: SCA model of the effect of storage time + temperatures × time interaction (A) Longitudinal plot of the scores along SC1 (expressed as mean± standard deviation after projection of the residuals) vs storage time: eggplant fruit stored at 2°C and 12°C are indicated as blue circles and line or red circles and line, respectively; (B) Longitudinal plot of the scores along SC2 (expressed as mean± standard deviation after projection of the residuals) vs storage time: eggplant fruit stored at 2°C and 12°C are indicated as blue circles and line or red circles and line, respectively; (C) Variable loadings for SC1 (continuous line) together with their 95% confidence interval (black dashed lines); (D) Variable loadings for SC2 (continuous line) together with their 95% confidence interval (black dashed lines). In panels (C) and (D), red and blue colors indicate whether the corresponding wavelength contributes significantly or not to the bilinear model, respectively

So as to have a better perception for the storage time effects and the changes in longitudinal behavior allied to the storage temperatures, the scores along SC1 and SC2 (together with error bars representing the projected residuals) have been plotted as a function of time in Fig. 7A and Fig. 7B.

By looking at Fig. 7A and 7B, it is observable how there are two main trends linked to the effect of storage time. Component 1 accounted for a constant decrease of the score but as it is perceivable there is an inversion at day 6 which might be related to chemical transformation caused by CI. Therefore, the spectral bands with negative loadings on this component will decline their pseudo-absorbance with time and the contrary will happen for the bands having positive loadings. Instead, scores along component 2 showed a maximum in correspondence of the sixth day of storage and again decreasing by following storage day. This alternation in the trend could be recognized as a development of CI (as it was described in the first component as well) in the fruit, which are then further transformed as time progresses. In this regard, given the sign of the scores, it can be confirmed that the spectral regions having positive loadings along SC2 would reach their minimum pseudo-absorbance at sixth day of storage and later on increase to their primary values at longer times, while the opposite occurred for variables with negative loadings.

It is noticeable (Fig. 7C and 7D) that storage temperature and time of storage (and its interaction with storage temperature) significantly affected all the FT-NIR spectra (all the loadings are statistically different than zero). Then, according to the phenomena described already for SC1, it could be confirmed that by increasing the storage time, there is an increase in the pseudo-absorbance of all the bands in possessing positive values in the loading on SC1, while the remaining variables decreased their signal.

3.5 Classification model discriminating fruit stored at chilling temperature

As ASCA model proved that temperature had a significant effect on the FT-NIR spectra, a classification model using the PLS-DA algorithm was conducted on the data for classifying the fruit stored at chilling and safe temperature. In order to check the validity of the ASCA model, as well as to reduce the complexity of the classification model, only the wavebands identified by ASCA were used to discriminate between fruit stored at different temperature. In this regard, 36 samples stored at chilling temperature (3, 6, and 10 days of storage) and 36 samples stored at safe temperature (3, 6, and 10 days of storage) were labeled as chilled and healthy fruit, respectively. The final dataset resulted in a matrix including 72 samples × 369 effective wavebands (those identified by ASCA and showed in Fig. 5B; the whole range of FT-NIR spectra comprised 2307 wavebands).

The use of rDCV allows to repeat the external validation of the PLS-DA model on the outer loop samples as many times as the number of DCV runs: this allows to obtain not only a point estimate of the predictive ability of the model, but also a corresponding confidence interval, so that the classification accuracy can be more solidly evaluated. In particular, when considering the outer DCV loop, i.e., the one mimicking an external validation set, it was found that 90.2 ± 4.0 % of the healthy and 84.7±3.1% of the chilled fruits were correctly predicted, leading to an overall accuracy of 87.4±2.7% (corresponding to a value of the area under the ROC curve of 0.941±0.016). These results indicate a very good discrimination between the fruit according to the storage temperature. Moreover, to rule out the possibility that these outcomes could result from chance correlation, the observed values of classification accuracy and of the area under the ROC curve were compared to their distributions under the null hypothesis, which were non-parametrically estimated by means of a permutation test with 1000 randomization. For both classification figures of merit, an empirical p-value <0.001 was obtained, thus confirming that the observed discrimination between the classes can be considered highly statistically significant.

The validity of PLS-DA results based on the spectral region introduced by ASCA were endorsed by comparing with the results of work performed by Tsouvaltzis et al. (2020). However, in their study they used full range FT-NIR spectra (3600-12500 cm^{-1}) of eggplant fruit for discriminating fruit stored at chilling temperature, but still the accuracy of the classifier (PLS-DA) was less than the current study.

The method ASCA is getting attention nowadays for analysis of hyperspectral data, but still few applications can be found for postharvest handling of fruit and vegetable. For instance, Leisso et al. (2016) explored ASCA for gene expression and metabolism preceding for soft scald, a CI of 'Honeycrisp' apple fruit, but this method is time- consuming and compared to spectroscopy needs expertise even for sample preparation. In the field of food research such as coffee analysis (De Luca et al. 2016), this method was utilized on HPLC-DAD, NIR data but after realizing the effect of varieties and roasting time, the effective wavelengths were not used as an input of classifier while in the current study it was tried to verify the ability of ASCA by putting the output of it as an input of PLS-DA classifier.

Our study designed at to use ASCA modelling as an additional investigation tool to previous research (Tsouvaltzis et al. 2020) to gather information on the effect of temperature and time of storage on the spectral data extracted from non-invasive instrument.

4 Conclusion

As the chilling injury (CI) in subtropical fruit such as eggplant is a critical disorder, it is important to understand how the temperature and duration of storage may impact its quality. To this aim non-destructive techniques such FT-NIR spectra can be used. For the first time, in this kind of study, the effect of temperature and storage time on fruit spectral response was investigated. By applying ANOVA-simultaneous component analysis (ASCA), it was found that both the "temperature" and the "storage time" factors, as also their interaction significantly affected the spectral profile of eggplant fruit over storage. For each factor the most significant wavelengths were individuated. This information could be used to build a partial least square-discriminant analysis (PLS-DA) for discriminating eggplant fruit based on the temperature. The PLS-DA model could classify fruit with high accuracy 87.4±2.7% (90.2±4.0% for healthy and 84.7±3.1% for chilled fruit; evaluated on the outer loop of a repeated double cross-validation procedure). It is worth stressing that the proposed approach allowed the classification of chilled and healthy fruit by a rapid, comparatively cheap and non-invasive technique (FT-NIR), without requiring any sample pretreatment and irrespectively of the days of storage. In this viewpoint, the results are provided very promising tool for the future industrial approach for discarding the unhealthy fruit and decreasing the food losses.

Reference

Amodio, Maria Luisa et al. 2017. "Potential of NIR Spectroscopy for Predicting Internal Quality and Discriminating among Strawberry Fruits from Different Production Systems." *Postharvest Biology and Technology* 125: 112–21. http://dx.doi.org/10.1016/j.postharvbio.2016.11.013.

Blanco, M., and I. Villarroya. 2002. "NIR Spectroscopy: A Rapid-Response Analytical Tool." *TrAC Trends in Analytical Chemistry* 21(4): 240–50. https://www.sciencedirect.com/science/article/pii/S0165993602004041 (September 30, 2019).

Brereton, Richard G., and Gavin R. Lloyd. 2014. "Partial Least Squares Discriminant Analysis: Taking the Magic Away." *Journal of Chemometrics* 28(4): 213–25.

Cen, Haiyan, Renfu Lu, Qibing Zhu, and Fernando Mendoza. 2016. "Nondestructive Detection of Chilling Injury in Cucumber Fruit Using Hyperspectral Imaging with Feature Selection and Supervised Classification." *Postharvest Biology and Technology* 111: 352–61. http://dx.doi.org/10.1016/j.postharvbio.2015.09.027.

Chaudhry, M.M.A. et al. 2018. "Hyperspectral Imaging and Multivariate Accelerated Shelf Life Testing (MASLT) Approach for Determining Shelf Life of Rocket Leaves." *Journal of Food Engineering* 238.

Concellón, Analía, María C. Añón, and Alicia R. Chaves. 2005. "Effect of Chilling on Ethylene Production in Eggplant Fruit." *Food Chemistry* 92(1): 63–69.

Cortés, Victoria et al. 2017. "Prediction of the Level of Astringency in Persimmon Using Visible and Near-Infrared Spectroscopy." *Journal of Food Engineering* 204: 27–37.

ElMasry, Gamal, Ning Wang, and Clément Vigneault. 2009. "Detecting Chilling Injury in Red Delicious Apple Using Hyperspectral Imaging and Neural Networks." *Postharvest Biology and Technology* 52(1): 1–8.

Erkinbaev, Chyngyz, Kelly Henderson, and Jitendra Paliwal. 2017. "Discrimination of Gluten-Free Oats from Contaminants Using near Infrared Hyperspectral Imaging Technique." *Food Control* 80: 197–203. http://www.sciencedirect.com/science/article/pii/S0956713517302232.

Fallik, E., Naomi Temkin-Gorodeiski, Shoshana Grinberg, and H. Davidson. 1995. "Prolonged Low-Temperature Storage of Eggplants in Polyethylene Bags." *Postharvest Biology and Technology* 5(1–2): 83–89.

Fan, Linlin et al. 2016. "Methyl Jasmonate Delays Postharvest Ripening and Senescence in the Non-Climacteric Eggplant (Solanum Melongena L.) Fruit."

Postharvest Biology and Technology 120: 76–83. https://www.sciencedirect.com/science/article/pii/S0925521416301041 (October 16, 2019).

Fuchs, Y., G. Zauberman, I. Rot, and A. Weksler. 1989. “Chilling Injury and Electrolyte Leakage in Cold Stored Mango and Avocado Fruits.” *Acta Horticulturae* (258): 303–8.

Jansen, Jeroen J. et al. 2005. “ASCA: Analysis of Multivariate Data Obtained from an Experimental Design.” *Journal of Chemometrics* 19(9): 469–81.

Kader, Adel A. 2013. “Postharvest Technology of Horticultural Crops - An Overview from Farm to Fork.” *Journal of Applied Sciences and Technology* 1(1): 1–8.

Leisso, Rachel S. et al. 2016. “Gene Expression and Metabolism Preceding Soft Scald, a Chilling Injury of ‘Honeycrisp’ Apple Fruit.” *BMC Genomics* 17(1). http://dx.doi.org/10.1186/s12864-016-3019-1.

De Luca, Silvia et al. 2016. “Characterization of the Effects of Different Roasting Conditions on Coffee Samples of Different Geographical Origins by HPLC-DAD, NIR and Chemometrics.” *Microchemical Journal* 129: 348–61. http://dx.doi.org/10.1016/j.microc.2016.07.021.

Moomkesh, Shahram, Seyed Ahmad Mireei, Morteza Sadeghi, and Majid Nazeri. 2017. “Early Detection of Freezing Damage in Sweet Lemons Using Vis/SWNIR Spectroscopy.” *Biosystems Engineering* 164(31): 157–70. https://doi.org/10.1016/j.biosystemseng.2017.10.009.

Munera, Sandra et al. 2018. “Potential of VIS-NIR Hyperspectral Imaging and Chemometric Methods to Identify Similar Cultivars of Nectarine.” *Food Control* 86: 1–10.

Pan, Leiqing et al. 2016. “Detection of Cold Injury in Peaches by Hyperspectral Reflectance Imaging and Artificial Neural Network.” *Food Chemistry* 192: 134–41. http://dx.doi.org/10.1016/j.foodchem.2015.06.106.

Shi, Junyan et al. 2018. “Low-Temperature Conditioning Enhances Chilling Tolerance and Reduces Damage in Cold-Stored Eggplant (Solanum Melongena L.) Fruit.” *Postharvest Biology and Technology* 141(March): 33–38. https://doi.org/10.1016/j.postharvbio.2018.03.007.

Smilde, Age K. et al. 2005. “ANOVA-Simultaneous Component Analysis (ASCA): A New Tool for Analyzing Designed Metabolomics Data.” *Bioinformatics* 21(13): 3043–48.

St»hle, Lars, and Svante Wold. 1990. “Multivariate Analysis of Variance (MANOVA).” *Chemometrics and Intelligent Laboratory Systems* 9(2): 127–

41. https://www.sciencedirect.com/science/article/abs/pii/016974399080094M (October 24, 2019).

Tsouvaltzis, Pavlos, Farahmand Babellahi, Maria Luisa Amodio, and Giancarlo Colelli. 2020. "Early Detection of Eggplant Fruit Stored at Chilling Temperature Using Different Non-Destructive Optical Techniques and Supervised Classification Algorithms." *Postharvest Biology and Technology* 159: 111001. https://www.sciencedirect.com/science/article/pii/S0925521419305447 (September 24, 2019).

Zwanenburg, Gooitzen et al. 2011. "ANOVA–Principal Component Analysis and ANOVA–Simultaneous Component Analysis: A Comparison." *Journal of Chemometrics* 25(10): 561–67.

GENERAL CONCLUSIONS

The general objective of this thesis was to study the potentiality of non-destructive optical techniques based on spectral information for the early detection of CI of bell pepper and eggplant fruit, as crop models. To this aim different instruments and chemiometric methods were applied to discriminate between fruit stored at chilling and safe temperature. This objective was reached by using eggplant and bell peppers as crop models.

Both FT-NIR spectra and hyperspectral images gave promising results in term of possible online implementation of these techniques, allowing to make the following conclusions:

-For eggplant fruit, which developed chilling injury symptoms after 4th day of storage at 2°C, in the first experiment, the earliest, and most consistent result (92–100 %, throughout the storage period) was obtained using the FT-NIR spectral data, and SVM algorithm. In this case, a good discrimination of chilled fruit was possible since the 2nd day of storage at 2°C. Color or FT-NIR spectral data classified with PLSDA permitted relatively good classification of fruit (>83% accuracy) since the 4th day of storage;

-In case of bell peppers, CI was less severe, occurring after 12 days of storage. HSI in VIS-NIR and NIR ranges and wavelength selection techniques were applied. Results of PLS-DA classifications discriminated cold stored fruit from fresh fruit and from fruit stored at higher temperature by using 6 variables in the VIS-NIR or 4 variables in the NIR range.

-Using 12 variables in the VIS-NIR range, was also possible to correctly classify bell peppers by days of cold storage, and to predict storage days with an error of 0.5 days in cross validation.

- On the same way, was also possible to correctly predict days of storage of eggplant fruits, by both VIS-NIR hyperspectral imaging and FT-NIR spectroscopy.

-Finally the last experiment allowed studying the changes induced on FT-NIR reflectance spectra of eggplants induced by time and temperature of storage using an

ANOVA-simultaneous component analysis (ASCA). With this statistical analysis was confirmed that both temperature and storage time, as also their interaction, significantly affected the spectral profiles. Using this properties, a PLS-DA model was developed for discriminating eggplant fruit based on the temperature. The PLS-DA model could classify fruit by 93% and 87.5% accuracy in calibration and cross-validation sets, respectively.

It is worth stressing that the proposed approaches consent classification among chilled and healthy fruit by a rapid, comparatively cheap and non-invasive technique that thanks to the reduction of used wavelengths can be easily transferred for a multi-spectra prototype. The choice of using images or just FT-NIR spectra may depend from the final use of this prototype. If the device is intended to complement an online vision system for sorting, then hyperspectral imaging system, may be the first option, whereas for other steps of the food chain, as transport or distribution, or also for a new-sensor to be added on the processing or sorting line, a portable or a micro FT-NIR instrument, may be a convenient choice.