



UNIVERSITÀ DEGLI STUDI DI MILANO
Department of Agricultural and Environmental Sciences

*Doctoral School in
“Innovazione tecnologiche per le scienze agro-alimentari e
ambientali” XXV ciclo*

Application of vis/NIR spectroscopy
for ripeness evaluation
and postharvest quality analysis
of agro-food products

PhD Thesis

Valentina Giovenzana
(Matr. Number R08648)

Tutor: Prof. Luigi Bodria

Doctoral School Coordinator: prof. Roberto Pretolani

Academic year 2011/2012

Ai miei genitori

Tables of contents

Preface	5
----------------	---

Part 1

Literature review	13
--------------------------	----

NIR and vis/NIR spectroscopy in food sector	14
--	----

Electromagnetic radiation	14
---------------------------	----

Transitions in the Near Infrared Region	15
---	----

Instruments	16
-------------	----

Chemometrics	23
--------------	----

NIRs Applications	25
-------------------	----

Image analysis (traditional, multispectral and hyperspectral) in food sector	32
---	----

The vision system	34
-------------------	----

Multispectral and hyperspectral images	36
--	----

Image analysis applications	40
-----------------------------	----

References	48
-------------------	----

Part 2

Objectives	61
-------------------	----

Experimental studies	65
-----------------------------	----

Section 1 _Testing of a vis/NIR system for a better management of apple storage	67
---	----

Section 2 _Vis/NIR spectroscopy for quick evaluation of olive fruit ripeness	95
--	----

Section 3 _Application of vis/NIR spectroscopy for a quick quality evaluation of craft beers	113
Section 4 _Quick Quality Evaluation of Chilean Grapes by a Portable vis/NIR Device	135
Section 5 _Application of non-destructive techniques for the evaluation of fresh-cut Lamb's lettuce (<i>Valerianella locusta</i> Laterr.) shelf life: a preliminary study	151
Section 6 _Wavelengths selection with a view to a simplified handheld optical system to estimate grape ripeness	163
Section 7 _Feasibility of a simplified handheld optical system to evaluate fresh-cut <i>Valerianella locusta</i> Laterr.	193
Section 8 _ Derivation of a blueberry ripeness index with a view to a low-cost, handheld optical sensing device for supporting harvest decisions	209
Section 9 _Design of a simplified LED based system for a quick monitoring of grape ripening	235

Part 3

General conclusions and future works	263
Acknowledgments	269

Preface

The food sector is one of the most important item of the world economic system as it fulfills one of the main needs of man.

In the food industry, some quality evaluation is still performed manually by qualified staff, but this is often tedious, laborious, expensive and unreliable due to its subjective nature. Increased demands for objectivity, consistency and efficiency moved the focus on optical non-destructive systems.

In recent years optical techniques have been applied increasingly for food quality evaluation.

Lately, computer vision employing image processing techniques has been quickly developed, which can quantitatively characterize complex properties of foods like size, shape, colour and texture.

In order to develop an automated system for food quality evaluation, image processing techniques are often combined with mechanical and instrumental devices to replace, if possible, human manipulation during the process. This techniques give flexibility in application and can be reasonable used in place of human visual decision-making process.

However, image analysis allows only a superficial analysis of the sample. This is sufficient in many cases, but to achieve particular goals, data acquired by imaging analysis technique may lack of useful information.

Among non-destructive techniques, the optical analysis in the region of near infrared (NIR) and visible-near infrared (vis/NIR) has met a significant development in the last 20 years. NIR and vis/NIR analyses are based on the use of spectral information arising from the interaction between food structure and light.

NIR spectroscopy has proved to be one of the most efficient and advanced tools to monitor product quality and for the process controlling of food industry. A lot of works were published on this topics.

NIR spectroscopy is rapid, non-destructive, easy to apply on-line and off-line. With this technology, it is possible to obtain spectroscopic information about the components of the analysed sample. However,

vis/NIR and NIR spectroscopy are punctual analyses and these could be a problem for the evaluation of heterogeneous samples. This technology allows to acquire one measure only in a precisely point of the samples.

In this case problems could be overcome by using hyperspectral imaging (HSI). HSI combines spectroscopy and traditional imaging to form a three-dimensional structure of multivariate data (hypercube). The hyperspectral images consist of many spectral bands acquired in a narrow and contiguous way, allowing to analyze each pixel in the multiple wavelengths simultaneously and, therefore, to obtain a spectrum associated with a single pixel. The data set arising from a hyperspectral image is a kind of data cube, with two spatial directions, ideally remaining on the surface observed, and a spectral dimension. As result, HSI represents a major technological advance in the acquisition of morphological and chemical information from food products.

The advantage of HSI is the large volume of data available in each hypercube (i.e. the information derived from the analysis), but the hypercube contains also redundant information. This abundance of data results both in a need of high computational power and long acquisition times caused by the size of the data matrix. Therefore, it is desirable to reduce this size to manageable levels, especially if the goal is the application of HSI techniques in real time, on-line on production lines.

Chemometrics is the most useful approach for the analysis of these kind of data. With the use of multivariate analysis, the huge dimensionality problems of hyperspectral data can be reduced or eliminated by selecting few spectral data at some important wavelengths. In most cases, not all the spectral bands are required to investigate a particular attribute. Selection of important wavelengths is an optional step based on the speed requirements of the whole process. Generally, the selection of these optimal wavelengths could reduce the size of the data matrix while preserving the most useful information contained in the data space. The selected wavelengths preserve the largest amount of energy among the hyperspectral data maintaining the most important spectral information and valuable details about tested samples. The useful wavelengths should not only maintain any valuable required

details, but also simplify the successive discrimination and classification procedures.

Several essential wavelengths could be sorted from the whole spectral cube through a variety of strategies, such as general visual inspection of the spectral curves and correlation coefficients analysis of spectral differences from the average spectrum, stepwise regression and discriminant analysis, Principal Component Analysis (PCA), Partial Least Square (PLS) and others.

Also NIR and vis/NIR instrumentation must always be coupled with chemometric analysis to extract useful information present in spectra separating it both from not useful one to solve the problem and from spectral noise.

In general, classify or quantify the presence of compounds in a sample is the main purpose of the application of optical analysis in the food sector. Algorithms for classification and regression already exist but their efficiency could be improved, as well as create datasets, identify anomalies or objects with different spectral characteristics, compare optical results with those of data library. These goals can be achieved only if experimental data are processed with chemometric methods.

Chemometrics has been defined as "a chemical discipline that uses statistical and mathematical methods to design or select optimum procedures and experiments, and to provide maximum information by analyzing chemical data". Chemometrics can be considered as a part of the wider field of chemoinformatics, and has close relationships to bioinformatics.

Furthermore if vis/NIR and NIR spectroscopy are compared to image analysis and multispectral/hyperspectral imaging, spectroscopy allows measurements which have the limit of being punctual, but this technology has instead the enormous advantage of analyse not only product surface, but also the inner layers of the sample.

The choice of the most suitable optical technologies requires a good understanding of the nature and limitations of the objectives of the work and the various strategies for data processing and interpretation. For the objectives of this work, i.e. ripeness evaluation and postharvest quality characteristics of agro-food products, vis/NIR spectroscopy was chosen.

This thesis is a collection of 9 chapters, divided into three parts.

In the first part (Literature review/State of the art), a review of recent advances in vis/NIR spectroscopy and image analysis (traditional, multispectral and hyperspectral images) for food quality evaluation is presented.

This chapter essentially analyses the theoretical principles of the most important technologies currently used in the food industry, namely vis/NIR and NIR spectroscopy, image analysis with particular regard to hyperspectral image analysis. This review focuses on the use of NIR spectroscopy and hyperspectral imaging for the analysis of foods such as meat, fruit, grain, dairy products, oil, honey, wine and other areas, and looks at the literature published in the last 10 years.

The second part present, at first, thesis objectives and then the experimental application (sections 1-9) of vis/NIR spectroscopy for ripeness evaluation and postharvest quality analysis of agro-food products are presented.

In particular in this collection of works vis/NIR spectroscopy was used:

- to monitor and manage lots of apples during long storage in cold room, with the aim of classify apples based on their internal features like soluble solids content and firmness (section 1);
- to analyse ripening parameters (soluble sugar content and texture) of olives before to be processed, both for fermentation process of table olives or for olive oil extraction process (section 2);
- to evaluate qualitative parameters (pH and soluble solids content, measured in °Plato) in three types of craft beer (pale, amber and brown) (section 3);
- to evaluate correlation between spectral data and classical destructive ripening parameters of both red and white grapes samples (section 4);
- to investigate the applicability of non-destructive techniques in monitoring freshness decay of fresh-cut lettuce during storage/shelf life (section 5).

Moreover, regarding grapes (section 6), fresh cut salad (section 7) and blueberries (section 8) was studied the selection of the most significant

wavelengths in order to evaluate the feasibility of a simplified, handheld and low-cost optical device.

The last chapter (section 9) presents the realization of the first prototype of a simplified optical device, optimized for use on grapes and characterized by the presence of four LEDs emitting at selected wavelengths.

Each chapter of experimental studies (section 1-9) consists of *Abstract, Keywords, Introduction, Material and Methods, Results and Discussion and Conclusions*.

The third part shows general conclusions highlighting the main achievements and the following proposals for future works.

References used in the thesis are listed at the end of each chapter.

PART 1

Literature review

The food sector is one of the most important voices in the economic field as it fulfills one of the main needs of man. The changes in the society in recent years have radically modified the food industry by combining the concept of globalization with the revaluation of local production. Besides the production needs to be global, in fact, there are always strong forces that tend to re-evaluate the expression of the deep local production like social history and centuries-old tradition.

The increase in productivity, in ever-expanding market, has prompted a reorganization of control systems to maximize product standardization, ensuring a high level of food security, promote greater compliance among all batches produced. The protection of large quantities of production, however, necessarily passes through systems to highlight possible fraud present throughout the production chain: from the raw materials (controlled by the producer) to the finished products (controlled by large sales organizations). The fraud also concern the protection of local productions: the products guaranteed origin must be characterized in such a way to identify specific properties easily and detectable by objective means.

The laboratories employ analytical techniques that are often inadequate because they require many samples, a long time to get the response, staff with high analytical ability. In a context where the speed is an imperative, technology solutions must require fewer samples or, at least no one (non-destructive techniques); they have to provide quick answers, if not immediate, in order to allow the operator to decide quickly about further steps to control or release the product to market; they must be easy to use, to promote their use throughout the production chain where it is not always possible to have analytical laboratories. The technologies must therefore be adapted to this new approach to production: the sensors and the necessary related data modelling, which allows the "measure", are evolving to meet the needs of the agri-food sector. The trial involves, often, Research Institutions on the side of Companies, a sign of a great interest and a high level of expectations. The manufacturers of technologies, often, provide devices that require calibration phases not always easy to perform, but that are

often the subject of actual researches. These are particularly complex when the modelling approach must be based on chemometrics.

NIR and vis/NIR spectroscopy in food sector combined with chemometrics

Among the non-destructive techniques has met a significant development in the last 20 years the optical analysis in the region of near infrared (NIR) and visible-near infrared (vis/NIR), based on the use of information arising from the interaction between the structure of food and light.

Electromagnetic radiation

Spectroscopic analysis is a group of techniques allowing to get information on the structure of matter through its interaction with electromagnetic radiation.

Radiation is characterized by (Fessenden & Fessenden, 1993):

- a wavelength (λ), which is the distance between two adjacent maxima and is measured in nm;
- a frequency (ν), representing the number of oscillations described by the wave per unit of time and is measured in hertz (cycles/s);
- a wave number (n), which represents the number of cycles per centimetre and is measured in cm^{-1} .

The entire electromagnetic spectrum is divided into several regions, each characterized by a range of wavelengths (Figure1)

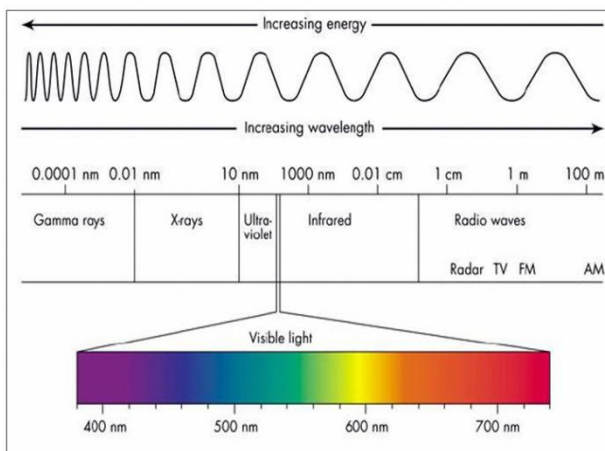


Figure 1. The electromagnetic spectrum (Lunadei, 2008)

Transitions in the near infrared region (NIR)

The radiation from the infrared region is able to promote transitions at vibrational level. The infrared spectroscopy is used to acquire information about the nature of the functional groups present in a molecule. The infrared region is conventionally divided into three sub-regions: near (750-2500 nm), medium (2500-50000 nm) and far infrared (50-1000 μm).

Fundamental vibrational transitions, namely between the ground state and first excited state, take place in the mid-infrared, while in the region of near-infrared absorption bands are due to transitions between the ground state and the second or the third excited state. This type of transitions are called overtones and their absorption bands are generally very weak. The absorption bands associated with overtones can be identified and correlated to the corresponding absorption bands arising from the fundamental vibrational transitions because they fall at multiple wavelengths of these.

Following the process of absorption of photons by molecules the intensity of the radiation undergoes a decrease. The law that governs the absorption process is known as the Beer-Lambert Law:

$$A = \log(I_0/I) = \log(1/T) = \epsilon \cdot l \cdot c \quad (1)$$

where:

A = absorbance [$\log(\text{incident light intensity/transmitted beam intensity})$];

T = transmittance [beam intensity transmitted/incident light intensity];

I_0 = radiation intensity before interacting with the sample;

I = radiation intensity after interaction with the sample;

ϵ = molar extinction coefficient characteristic of each molecule ($\text{l}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$);

l = optical path length crossed by radiation (cm);

c = sample concentration (mol/l).

The spectrum is a graph where in the abscissa is reported a magnitude related to the nature of radiation such as the wavelength (λ) or the wave number (n) and in the Y-axis a quantity related to the change in the intensity of radiation as absorbance (A) or transmittance (T).

Instruments

Since '70s producers developed analysis instruments specifically for NIR analysis trying to simplify them to fit also less skillful users, thanks to integrated statistical software and to partial automation of analysis.

Instruments built in this period can be divided in three groups: desk instruments, compact portable instruments and on-line compatible devices.

Instruments during years devices evolved over the years also for the systems employed to select wavelength. First instruments used filter devices able to select only some wavelength (Figure 2). These devices are efficient when specific wavelength are needed. Since the second half of '80s instruments capable to acquire simultaneously the sample spectrum in a specific interval of wavelength were introduced, recording the average spectrum of a single defined sample area (diode array systems and FT-NIR instruments) (Stark & Luchter, 2003). At the same time, chemometric data analysis growth helped to diffuse NIR analysis.

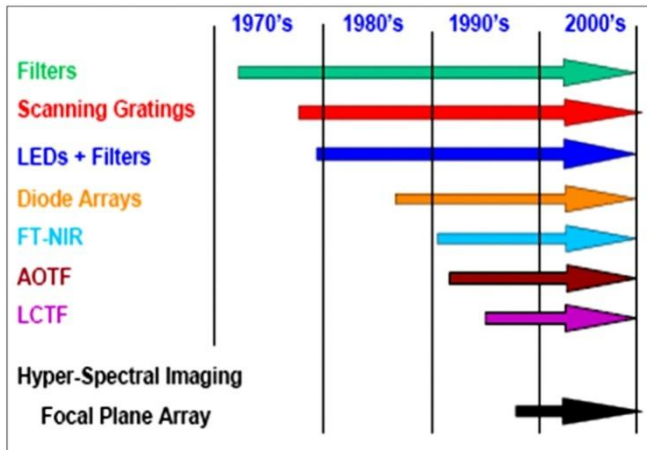


Figure 2. Development of the different analysis technologies scheme (Stark & Luchter, 2003).

Particularly, food sector showed interest towards NIR and vis/NIR instruments, both mobile and on-line. Devices based on diode array spectrophotometers and FT-NIR desk systems proved to be the best for this sector.

Both in the case of portable and stationary instruments, the fundamental components of these systems are common and are four:

- Light source;
- Light radiation transport system;
- Sample compartment and measurement zone;
- Spectrophotometer and Personal Computers.

Light source

Tungsten filament halogen lamps are chosen as the light source by most of the instruments. This is due to a good compromise between good performance and relatively low cost. This type of lamps are particularly suitable for use in low voltage. A little drawback may be represented by sensitivity to vibration of the filament.

Halogen bulbs are filled with halogen gas to extend their lives by using the return of evaporated tungsten to the filament. The life of the lamp depends on the design of the filament and the temperature of use, on average ranges from a minimum of 50 hours and a maximum of 10000

hours at rated voltage. The lamp should be chosen according to the use conditions and the spectral region of interest. An increase in the voltage of the lamp may cause a shift of the peaks of the emission spectrum towards the visible region but can also lead to a reduction of 30% of its useful life. On the contrary, use of lower voltages can increase the lamp life together, however, with an intensity reduction of light radiation, especially in the visible region. Emission spectrum of the tungsten filament changes as a function of temperature and emissivity of the tungsten filament. The spectrum shows high intensity in the VNIR region (NIR region close to the area of the visible).

Even if less common, alternative light sources are available. For example, LED light sources and ad laser sources could be used. LED sources (light emitting diodes) are certainly interesting sources thank to their efficiency and their small size. They meet, however, a limited distribution due to limited availability of LEDs emitting at wavelengths in the NIR region. Technology to produce LEDs to cover most of the NIR region already exists, but demand for this type of light sources is currently too low and the development of commercial product of this type is still in an early stage.

The use of laser sources guarantees very intense emission in a narrow band. But the reduced spectral range covered by each specific laser source can cause problems in some applications. In any case the complexity and high cost of these devices have limited very much their use so far, mostly restricted to the world of research.

Light radiation transport system

Light source must be very close to the sample to light it up with good intensity. This is not always possible, so systems able to convey light on the samples are needed. Thanks to optic fibers this problem was solved, allowing the development of different shapes devices.

The use of fiber optics allows to separate the area of placement of the instrument from the measuring proper area. There are indeed numerous circumstances on products sorting line in which environmental conditions do not fulfill direct installation of measure instruments. For example, high temperature, excessive vibrations or lack of space are restricting factors to the use of on-line NIR devices. In all these situations optic fibers are the solution to the problem of conveying light.

They transmit light from lamp to sample and from sample to spectrophotometer. They allow to have an immediate measure on a localized sample area, thanks to their small dimensions, reaching areas difficult to access. Furthermore, they are made of a dielectric material that protects from electric and electromagnetic interferences. 'Optic fibers' means fibers optically transparent, purposely studied to transmit light thanks to total internal reflection phenomenon. Internal reflection is said to be total because it is highly efficient, in fact more than 99,999% radiation energy is transmitted in every reflection. This means that radiation can be reflected thousands of times during the way without suffer an appreciable attenuation of intensity (Osborne et al., 1993). Optic fiber consists of an inner core, a covering zone and of an external protection cover. Core is usually of pure silica, but plastic materials or particular glasses can be employed. The core is usually made of pure silica, but can also be used plastics or special glasses. The cladding area consists of material with a lower refractive index, while the exterior is only to protect the fiber from mechanical, thermal and chemical stress.

In Figure 3 are shown the inner core and the cladding of an optical fiber with the respective indices of refraction n_1 and n_2 , where $n_1 > n_2$. Each ray of light that penetrates inside the fiber with an angle $\leq \theta_{\max}$ (acceptance angle) is totally reflected with high efficiency within the fiber.

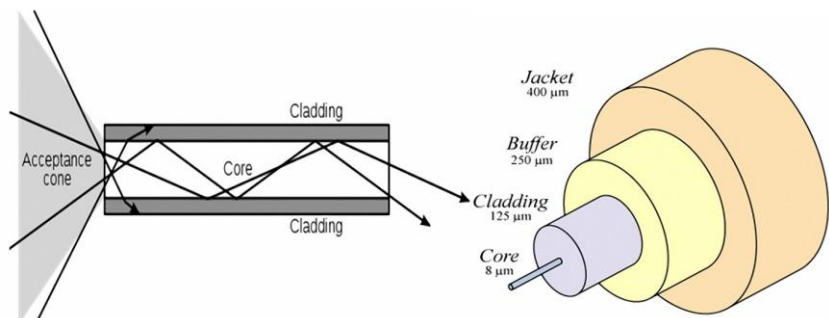


Figure 3. Scheme of an optical fiber, where n_1 and n_2 are respectively the refractive indices of the inner core and cladding. The acceptance cone is determined by the critical angle for incoming light radiation. Buffer absorbs the radiation not reflected by the cladding. Jacket has a protective function.

Sample compartment and measurement zone

Samples compartment and measurement zone are highly influenced by the technique of acquisition of spectra. Different techniques are employed, depending on type of samples, solid or liquid, small or large, to be measured in plan or in line, that influence the geometry of the measurement zone.

The techniques to acquire spectra are four: transmittance, reflectance, transreflectance and interactance. They are different mainly for the different positioning of the light source and of the measurement sensor around the sample (Figure 4).

Transmittance - The transmittance measurements are based on the acquisition of spectral information by measuring the light that goes through the whole sample (Lu & Ariana, 2002). The use of analysis in transmittance can explore much of the internal structure of the product. This showed that is a technique particularly well suited to detect internal defects. To achieve significant results with this technique is required a high intensity light source and a high sensitivity measuring device. This because intensity of light able to cross the product is often very low. The transmittance measurements generally require a particular geometry of the measuring chamber, which can greatly influence the design of the instrument.

Reflectance - This technique measures the component of radiation reflected from the sample. The radiation is not reflected on the surface but penetrates into the sample a few millimeters, radiation is partly absorbed and partly reflected back again. Measuring this component of reflected radiation after interacting with the sample is possible to establish a relationship of proportionality between reflectance and analyte concentration in the sample. The reflectance measurement technique is well suited to the analysis of solid matrices because the levels of intensity of light radiation after the interaction with the sample are high.

This technique also allows to put in a limited space inside a tip the bundle of fibers that illuminate the sample and the fibers leading to the spectrophotometer the radiation after the interaction with the product. Therefore the use of this type of acquisition technique is particularly versatile and is suitable for compact, portable instruments, designed for use in field or on the process line. The major drawback using this

technique is related to the possibility to investigate only the outer area of the sample without having the chance to go deep inside.

Transflectance - This technique is used in case it is preferable to have a single point of measurement, as in the case of acquisitions in reflectance. In this case, however, the incident light passes through the whole sample, is reflected by a special reflective surface, recross the sample and strikes the sensor located near the area of illumination. The incident light so makes a double passage through the sample. Obviously this type of technique can be used only in the case of samples very permeable to light radiation such as partially transparent fluid. It is therefore not applicable to solid samples.

Interactance - This technique is considered a hybrid between transmittance and reflectance, as it uses characteristics of both techniques previously seen. In this case the light source and sensor are located in areas near the sample but between them physically separated. So the radiation reaches the measurement sensor after interacting with part of internal structure of the sample. This technique is mainly used in the analysis of big solid samples, for example, a whole fruit. Interactance is thus a compromise between reflectance and transmittance and has good ability to detect internal defects of the product combined with a good intensity of light radiation. This analysis is widely used on static equipment where, through the use of special holders, is easily obtained the separation between the areas of incidence of light radiation and the area to which the sensor is placed. It is instead difficult to use this configuration on-line because is complicated to place a barrier between incident and returning light to the sensor directly on the process line.

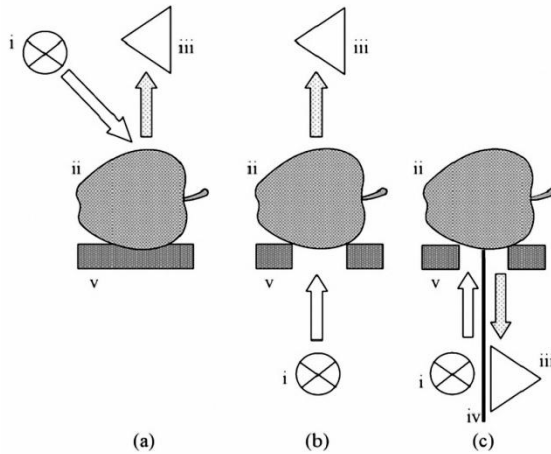


Figure 4. Setup for the acquisition of (a) reflectance, (b) transmittance, and (c) interactance spectra, with (i) the light source, (ii) fruit, (iii) monochromator/detector, (iv) light barrier, and (v) support. In interactance mode, light due to specular reflection is physically prevented from entering the monochromator by means of a light barrier (Nicolai et al., 2007).

Spectrophotometer and personal computers

Spectrophotometer can be considered the heart of an instrument for NIR analysis. The employed technology for the wavelengths selection greatly influences the performance of the instrument. For example, the use of filters allows instruments to record the signal of a single wavelength at a time. Modern instruments (diode array instruments and interferometers) allow to record the spectrum of the entire wavelengths range.

Instruments equipped with a diode array spectrophotometer are those who have met the increased use for portable and online applications in food sector. This is due to their compact size, versatility and robustness, thanks to the lack of moving parts during operation and also thanks to a relatively low cost.

As seen before, fiber optic sensor collects the portion of the electromagnetic radiation after interaction with the internal structure of the sample and transfers it to the spectrophotometer. The optical fiber is connected to the optical bench of the instrument. The optical bench allows to decompose the electromagnetic radiation and recording the intensity at different wavelengths.

Optical bench of this type of instrument generally consists of five components:

1. Optical fiber connector: connects the fiber optic with the optical bench of the instrument.
2. First spherical mirror (collimating mirror), has the function of collimating the light and send it to the diffraction grating.
3. Diffraction grating: in this area of the instrument, the light is split into different wavelengths and sent to the second spherical mirror.
4. Second spherical mirror (focussing mirror), collects diffracted radiation from the grating and sends them to the CCD sensor.
5. Matrix CCD sensor (diode array): records the signal intensity at each wavelength.

High sensitivity of the CCD matrix sensor compensate the low intensity of light radiation input due to the reduced diameter of the optical fibers used. Sensors used are generally Si-diode array or InGaAs-diode array. The first ones are certainly the most common and cheap and allow the acquisition of the spectrum in the range between 400 and 1100 nm, so are used for vis/NIR analysis. InGaAs sensors, more expensive, are used in applications requiring the acquisition of spectra at longer wavelengths, their use should range from 900 to 2300 nm.

Recorded signal by the CCD sensor is digitized and acquired by a PC using the software management tool of the instruments. Software records and allows to display graphically the spectrum of the analysed sample. The management software also allows to interface with the spectrophotometer enabling to change some parameters during the acquisition of spectra.

Chemometrics

Chemometrics is an essential part of NIR and vis/NIR spectroscopy in food sector. NIR and vis/NIR instrumentation in fact must always be complemented with chemometric analysis to enable to extract useful information present in the spectra separating it both from not useful information to solve the problem and from spectral noise. Chemometric techniques most used are the principal component analysis (PCA) as a technique of qualitative analysis of the data and PLS regression analysis as a technique to obtain quantitative prediction of the

parameters of interest (Naes et al., 2002; Wold et al., 2001; Nicolai et al., 2007; Cen & He, 2007).

The developed models should be tested using independent samples as validation sets to verify model accuracy and robustness. To evaluate model accuracy, the statistics used were the coefficient of correlation in calibration (r_{cal}), coefficient of correlation in prediction (r_{pred}), root mean square error of calibration (RMSEC), and root mean square error of prediction (RMSEP).

Correlation coefficients (r_{cal} and r_{pred}):

$$r_{\text{cal}} \text{ OR } r_{\text{pred}} = \sqrt{1 - \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2}} \quad (2)$$

where y_i are the reference values, \hat{y}_i are the values predicted by the PLS model, and \bar{y} is the averaged reference value.

Standard errors of calibration and prediction (RMSEC and RMSEP):

$$\text{RMSEC or RMSEP} = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{n}} \quad (3)$$

where n is the number of validated objects, and \hat{y}_i and y_i are the predicted and measured values of the i^{th} observation in the calibration or validation set, respectively. This value gives the average uncertainty that can be expected for predictions of future samples. The optimum calibrations should be selected based on minimizing the RMSEP. Percent errors (RMSEC% and RMSEP%) could be also calculated as: RMSEC (%) = RMSEC/averaged reference values of each parameter.

Prediction capacity of a model can be evaluated with the ratio performance deviation (RPD) (Williams & Sobering, 1996). The RPD is defined as the ratio of the standard deviation of the response variable to the RMSEP. An RPD value > 2.5 means that the model has good prediction accuracy.

NIRs applications

During the last 50 years, there has been a lot of emphasis on the quality and safety of the food products, of the production processes, and the relationship between the two (Burns and Ciurczak, 2001).

Near infrared (NIR) spectroscopy has proved to be one of the most efficient and advanced tools for monitoring and controlling of process and product quality in food industry. A lot of work has been done in this area. This review focuses on the use of NIR spectroscopy for the analysis of foods such as meat, fruit, grain, dairy products, oil, honey, wine and other areas, and looks at the literature published in the last 10 years.

Fruit and vegetables

Water is the most important chemical constituent of fruits and vegetables and water highly absorbs NIR radiation, so the NIR spectrum of such materials is dominated by water. Further, the NIR spectrum is essentially composed of a large set of overtones and combination bands. This, in combination with the complex chemical composition of a typical fruit or vegetable causes the NIR spectrum to be highly convoluted. Multivariate statistical techniques are required to extract the information about quality attributes which is buried in the NIR spectrum. Developments in multivariate statistical techniques such as partial least squares (PLS) regression and principal component analysis (PCA) are then applied to extract the required information from such convoluted spectra (Cozzolino et al., 2006b; McClure, 2003; Naes et al., 2004; Nicolai et al., 2007;).

The availability of low cost miniaturised spectrophotometers has opened up the possibility of portable devices which can be used directly on field for monitoring the maturity of fruit.

Guidetti et al. (2008) tested a portable vis/NIR device (450-980 nm) for the prediction of ripening indexes (soluble solids content and firmness) and presence of compounds with functional properties (total anthocyanins, total flavonoids, total polyphenols and ascorbic acid) of blueberries ('Brigitta' and 'Duke' varieties). Good predictive statistics were obtained with correlation coefficients (r) between 0.80 and 0.92 for the regression models built for fresh berries (Table 3). Similar results were obtained for the regression models for homogenized samples with

$r > 0.8$ for all the indexes. Results showed that vis/NIR spectroscopy is an interesting and rapid tool for assessing blueberry ripeness.

Table 3. Results of PLS models for fresh 'Duke' berry samples (r = coefficient of correlation; RMSEC = root mean square of the standard error in calibration; RMSECV = root mean square of the standard error in cross-validation; LV = latent variables). All data were preprocessed by second derivative of reduced and smoothed data.

Dependent variable	Units	LV	Calibration		Cross validation	
			r_{cal}	RMSEC	r_{cv}	RMSECV
TSS	°Brix	4	0.86	0.78	0.85	0.79
Young's Module	MPa	3	0.87	0.65	0.87	0.66
Total anthocyanins	mg g _{f.w.} ⁻¹	4	0.87	0.31	0.87	0.31
Total flavonoids	mg _{cat} g ⁻¹	4	0.87	0.37	0.86	0.37
Total polyphenols	mg _{cat} g _{f.w.} ⁻¹	11	0.82	0.20	0.81	0.20
Ascorbic acid	(mg/100g _{f.w.} ⁻¹)	4	0.84	1.01	0.83	1.02

Meat

In literature there are numerous applications of NIR spectroscopy for the analysis of meat quality. One of the most important aim is to monitor the freshness of meat products. Sinelli et al. in 2010 investigated the ability of Near Infrared spectroscopy to follow meat freshness decay. PCA was applied by authors to the data and was able to discriminate samples on the basis of storage time and temperature. The modelling of PC scores versus time allowed the setting of the time of initial freshness decay for the samples (6-7 days at 4.3 °C, 2-3 days at 8.1 °C and less than 1 day at 15.5 °C). Authors reported that results showed the feasibility of NIR for estimating quality decay of fresh minced beef during marketing.

Sierra et al. in 2007 conducted a study for the rapid prediction of the fatty acid (FA) profile of ground using near infrared transmittance spectroscopy (NIT). The samples were scanned in transmittance mode from 850 to 1050 nm. NIT spectra were able to accurately predict

saturated $R^2=0.837$, branched $R^2=0.701$ and monounsaturated $R^2=0.852$ FAs. Results were considered interesting because intramuscular fat content and composition influence consumer selection of meat products.

Andrés et al. in 2007 implemented a study to evaluate the potential of visible and near infrared reflectance (NIR) spectroscopy to predict sensory characteristics related to the eating quality of lamb meat samples. A total of 232 muscle samples from Texel and Scottish Blackface lambs was analyzed by chemical procedures and scored by assessors in a taste panel and these parameters were predicted from vis/NIR spectra. The results obtained by authors suggested that the more important regions of the spectra to estimate the sensory characteristics are related to the absorbance of intramuscular fat and water content in meat samples.

Even in the meat industry have been tried online applications of NIR spectroscopy. A study was conducted by Prieto et al. in 2009a to assess the on-line implementation of visible and near infrared reflectance (vis/NIR) spectroscopy as an early predictor of beef quality traits, by direct application of a fibre-optic probe to the muscle immediately after exposing the meat surface in the abattoir. Authors reported good correlation results only for prediction of colour parameters while less good results were achieved for sensory parameters.

NIR spectroscopy could be used for the detection of beef contamination from harmful pathogens and the protection of consumer safety. Amamcharla et al. in 2010 investigated the potential of Fourier transform infrared spectroscopy (FTIR) to discriminate the Salmonella contaminated packed beef. Principal component analysis was performed on the entire spectrum ($4000-500\text{ cm}^{-1}$). Authors obtained encouraging classification results with different techniques and confirmed that NIR could be used for non-destructive discrimination of Salmonella contaminated packed beef samples from uncontaminated ones.

A review published by Prieto et al. in 2009b indicates that NIR showed high potential to predict chemical meat properties and to categorize meat into quality classes. But authors underlined also that NIR showed in different cases limited ability for estimating technological and sensory attributes, which may be mainly due to the heterogeneity of the meat

samples and their preparation, the low precision of the reference methods and the subjectivity of assessors in taste panels.

Grains, bread and pasta

Grains including wheat, rice, and corn are main agricultural products in most countries. Grain quality is an important parameter not only for harvesting, but also for shipping (Burns and Ciurczak, 2001). In many countries, the price of grain is determined by its protein content, starch content, and/or hardness, often with substantial price increments between grades.

Measurement of carotenoid content of maize by vis/NIR spectroscopy was investigated by Brenna and Berardo (2004). They generated calibrations for several individual carotenoids and the total carotenoid content with good results (R^2 about 0.9).

Several applications can be found in literature regarding the use of NIR for the prediction of the main physical and rheological parameters of pasta and bread. De Temmerman et al. in 2007 proposed near-infrared (NIR) reflectance spectroscopy for in-line determination of moisture concentrations in semolina pasta immediately after the extrusion process. Several pasta samples with different moisture concentrations were extruded while the reflectance spectra between 308 and 1704 nm were measured. An adequate prediction model was developed based on the Partial Least Squares (PLS) method using leave-one-out cross-validation. Good results were obtained with $R^2 = 0.956$ and very low level of RMSECV. This creates opportunities for measuring the moisture content with a low-cost sensor.

Zardetto & Dalla Rosa in 2006 studied the evaluation of the chemical and physical characteristics of fresh egg pasta samples obtained by using two different production methodologies: extrusion and lamination. Authors evaluated that it is possible to discriminate the two kinds of products by using FT-NIR spectroscopy. FT-NIR analysis results suggest the presence of a different matrix–water association, a diverse level of starch gelatinization and a distinct starch–gluten interaction in the two kinds of pasteurised samples.

The feasibility of using near infrared spectroscopy for prediction of nutrients in a wide range of bread varieties mainly produced from wheat and rye was investigated by Sørensen in 2009. Very good results were

reported for the prediction of total contents of carbohydrates and energy from NIR data with R^2 values of 0.98 and 0.99 respectively.

Finally, a quick, non-destructive method, based on Fourier transform near-infrared (FT-NIR) spectroscopy for egg content determination of dry pasta was presented by Fodor et al. (2011) with good results.

Wine

Quantification of phenolic compounds in wine and during key stages in wine production is therefore an important quality control goal for the industry and several reports describing the application of NIR spectroscopy to this problem have been published.

Grape composition at harvest is one of the most important factors determining the future quality of wine. Measurement of grape characteristics that impact product quality is a requirement for vineyard improvement and for optimum production of wines (Carrara et al., 2008). Inspection of grapes upon arrival at the winery is a critical point in the wine production chain (Elbatawi & Ebaid, 2006).

An optical, portable, experimental system (vis/NIR spectrophotometer) for non-destructive and quick prediction of ripening parameters of fresh berries and homogenized samples of grapes in the wavelength range 450-980 nm was built and tested by Guidetti et al. (2010) (Figure 9). Calibrations for technological ripening and for anthocyanins had good correlation coefficients ($r_{cv} > 0.90$). These models were extensively validated using independent sample sets. Good statistical parameters were obtained for soluble solids content ($r > 0.8$, $SEP < 1.24$ °Brix) and for titratable acidity ($r > 0.8$, $SEP < 2 \text{ g}_{\text{tartaric acid}} \text{ L}^{-1}$), showing the validity of the vis/NIR spectrometer. Similarly, anthocyanins could be predicted accurately compared with the reference determination (Table 4). Finally, for qualitative analysis, spectral data on grapes were divided into two groups on the basis of grapes' soluble content and acidity in order to apply a classification analysis (PLS-DA). Good results were obtained with the vis/NIR device, with 89% of samples correctly classified for soluble content and 83% of samples correctly classified for acidity. Results indicate that the vis/NIR portable device could be an interesting and rapid tool for assessing grape ripeness directly in the field or upon receiving grapes in the wine industry.



Figure 9. Images of spectral acquisition phases on fresh berries and on homogenized samples.

Table 4. Results of PLS models for homogenized samples.

Parameter	Units	Pretreatment ¹	Calibration Validation				
			r	RMSEC	r	RMSEP	
TSS	(°Brix)	MSC+d2	5	0.93	0.95	0.75	0.95
Titrateable acidity	(g _{tart. acid} dm ⁻³)	MSC+d2	6	0.95	1.16	0.85	1.12
pH		MSC+d2	5	0.85	0.08	0.80	0.13
PA	(mg dm ⁻³)	MSC+d2	5	0.95	80.90	0.78	129.00
EA	(mg dm ⁻³)	MSC+d2	3	0.93	57.70	0.84	77.70
TP	(OD 280 nm)	MSC+d2	4	0.80	3.74	0.70	5.81

¹MSC = multiplicative scatter correction, and d2 = second derivative.

The application of some chemometric techniques directly to NIR spectral data with the aim of following the progress of conventional fermentation and maturation was investigated by Cozzolino et al. (2006b). The application of principal components analysis (PCA) allowed similar spectral changes in all samples to be followed over time. The PCA loading structure could be explained on the basis of absorptions from anthocyanins, tannins, phenolics, sugar and ethanol, the contents of which changed according to different fermentation time points. This study demonstrated the possibility of applying NIR spectroscopy as a process analytical tool for the wine industry.

Urbano-Cuadrado et al. (2004) analysed by vis/NIR spectroscopy different parameters commonly monitored in wineries. Coefficients of determination obtained for the fifteen parameters were higher than 0.80 and in most cases higher than 0.90 while SECV values were close to

those of the reference method. Authors said that these prediction accuracies were sufficient for screening purposes.

Römisch et al. in 2009 presented a study on the characterization and determination of the geographical origin of wines. In this paper, three methods of discrimination and classification of multivariate data were considered and tested: the classification and regression trees (CART), the regularized discriminant analysis (RDA) and the partial least squares discriminant analysis (PLS-DA). PLS-DA analysis showed better classification results with percentage of correct classified samples from 88 to 100%.

Finally, PLS and artificial neural networks (ANN) techniques were compared by Janik et al. in 2007 for the prediction of total anthocyanin content in red grape homogenates.

Other applications

The applications of vis/NIR and NIR spectroscopy and their chemometric techniques are present in many other sectors of the food industry. In literature are reported works relating to the dairy, oil, coffee, honey and chocolate industry. In particular, interesting studies have been conducted by some authors for the application of NIR spectroscopy in detecting the geographical origin of raw materials and finished products, defending the protected designation of origin (PDO).

Olivieri et al. (2011) worked out the exploration of three different class-modelling techniques to evaluate classification abilities based on geographical origin of two PDO food products: olive oil from Liguria and honey from Corsica. Authors developed the best models for both Ligurian olive oil and Corsican honey by a potential function technique (POTFUN) with values of correctly classified around 83%.

González-Martín et al. in 2011 presented a work on the evaluation by near infrared reflectance spectroscopy of different sensorial attributes of different type of cheeses, taking as reference data the evaluation of the sensorial properties obtained by a panel of eight trained experts. NIR spectra were collected with a remote reflectance fibre optic probe applying the probe directly to the cheese samples and the calibration equations was developed by using modified partial least-squares (MPLS) regression for 50 samples of cheese. Authors stated that obtained results can be considered good and acceptable for all the

parameters analyzed (presence of holes, hardness, chewiness, creamy, salty, buttery flavour, rancid flavour, pungency and retronasal sensation).

The quality of coffee is related to the chemical constituents of the roasted beans, whose composition depends on the composition of green beans (i.e., un-roasted). Unroasted coffee beans contain different chemical compounds, which react amongst themselves during coffee roasting influencing the final product. For this reason, monitoring the raw materials and the roasting process is very important. Ribeiro et al. in 2011 elaborated PLS models correlating coffee beverage sensory data and NIR spectra of 51 Arabica roasted coffee samples. Acidity, bitterness, flavour, cleanliness, body and overall quality of coffee beverage were considered. Results were good and authors confirmed that it is possible to estimate the quality of coffee using PLS regression models obtained by using NIR spectra of roasted Arabica coffees.

Da Costa Filho in 2009 elaborated a rapid method to determine sucrose in chocolate mass using near infrared spectroscopy. Data were modelled using partial least squares (PLS) and multiple linear regression (MLR), achieving good results (correlation coefficient of 0.998 and 0.997 respectively for the two chemometric techniques). Results showed that NIR can be used as rapid method to determine sucrose in chocolate mass in chocolate factories.

Image analysis (traditional, multispectral and hyperspectral) in food sector

In the food industry, since some time, there is a growing interest in image analysis techniques, since the appearance of a food contains a variety of information directly related to the quality of the product itself and this characteristics are difficult to measure through use of classical methods of analysis. In addition, image analysis techniques: provide information much more accurate than human vision, are objective and continuous over time and offer the great advantage of being non-destructive. These features, enable vision systems to be used in real time on the process lines, allowing on-line control and automation of sorting and classification within the production cycle (Guanasekaran & Ding, 1994).

The objective of the application of image analysis techniques, in the food sector, is the quantification of geometric and densitometric characteristic of image, acquired in a form that represents meaningful information (at macro and microscopic level) of appearance of an object (Diezak, 1988). The evolution of these techniques and their implementation in the vision machine in form of hardware and specialized software, allows a wide flexibility of applications, a high capacity of calculation and a rigorous statistical approach.

The benefits of image analysis techniques (Brosnan & Sun, 2004) that rely on the use of machine vision systems can be summarized as follows:

- are non-destructive techniques;
- techniques are use-friendly, rapid, precise, accurate and efficient;
- generate objective data that can be recorded for analysis deferred;
- allow a complete analysis of the lots and not just a single sample of the lot;
- reduce the involvement of human personnel in performing tedious tasks and allow the automation of various functions that would require intensive work shifts;
- are reasonably affordable cost.

These suggest the reason that drives scientific research, of the agro-food sector, to devote to the study and analysis of machine vision systems to analyse the internal and external quality characteristics of food, valued according to the optical properties of the products. able light source, it is possible to extract information about colour, shape, size and texture. From these features, it is possible to know many objective aspects of the sample, to be able to correlate, through statistical analysis, the characteristics defined by quality parameters (degree of maturation, the presence of external or internal mechanical defects, class , etc.) (Du & Sun, 2006, Zheng et al., 2006).

The image analysis may have several applications in the food industry: as a descriptor or as gastronomic and technologic parameter. Vision machine can be used to know size, structure, colour in order to quantify the macro and microscopic surface defects of a product or for the characterization and identification of foods or to monitor the shelf life (Riva, 1999). "Image analysis" is a wide designation that include, in addition to classical studies in greyscale and RGB images, the analysis

of images collected by mean multiple spectral channels (multispectral) or, more recently, hyperspectral images, technique exploited for its full extension in the spectral direction.

The hyperspectral image (Chemical and Spectroscopic Imaging) is an emerging technology, non-destructive, which complements the conventional imaging with spectroscopy in order to obtain, from an object, information, both spectral that spatial. The hyperspectral images are digital images in which each element (pixel) is not a single number or a set of three numbers, like the colour pictures (RGB), but a whole spectrum associated with that point. They are three-dimensional blocks of data. Their main advantage is that they provide spatial information necessary for the study of non-homogeneous samples. The advantage of this technique is the ability to detect in a foodstuff even minor constituents, isolated spatially.

To support image analysis, chemometric techniques are necessary to process and to model, data sets, in order to extract the highest possible information content. Methods of classification, modelling, multivariate regression, similarity analysis, principal components analysis, methods of experimental design and optimization, must be applied on the basis of each different condition and needs.

The vision system

The machine vision systems, appeared in the early sixties and then spread over time, in many fields of application, are composed of a lighting system, a data acquisition system connected to a computer, via a capture card, which digitizes (converts the signal into numerical form) and stores the analogic electrical signal, at the output from the camera sensor (Russ et al., 1988). The scanned image is then "converted" into a numerical matrix. From the images captured are read, by appropriate processing software, the information you want. In Figure 5 shows an example of vision machine.

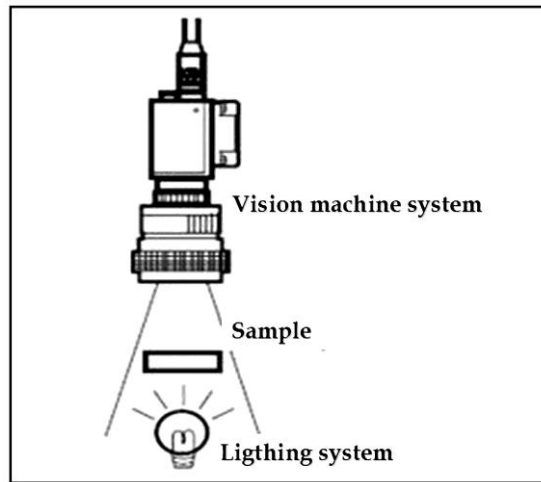


Figure 5. Example of image acquisition of an inspected sample

It is important to choose the localization of light source, but also the type of light source (incandescent, halogen, fluorescent, etc.). Influences the performance analysis. In fact, although the light sources emitting electromagnetic radiation corresponding to the visible (vis, 400-700 nm), ultraviolet (UV 10-400 nm) and near infrared (NIR, 700-1400 nm) are the most widely used, to create a digital image can also be used other types of light sources in order to emit different radiations, depending on the purpose of analysis. For example, to determine the internal structure of objects and / or identify any internal defects, it is possible to use an X-ray source, even if, although this type of source gives good results, its application is much more widespread in the medical field than in the agro-food, this is due to high costs of equipment and low speed of operating.

The digital image

A digital image is generated from the conversion of an analogic video signal produced by a digital camera into an electronic signal (scanning), then stored in the memory of a PC in the form of binary information. Any digital image can be considered as an array of points, the pixels, that make up the smallest element of an image. Each pixel contains a double set of information: its position in space, identified by the values (x, y) and the value of its intensity. Digital images can be represented

using only two colours, typically black and white (binary image), or shades of grey (monochrome image) or a range of colours (multichannel image). The value of light intensity will be different depending on the type of image.

The pixels, in the binary images, can have, as the intensity value, or 0 (equivalent to black) or 1 (white). The value of intensity in monochrome images, will be within a range, defined grey scale, from 0 to L, which usually corresponds to the interval from 0 (or 1) to 255 (or 256), where a value of 0 corresponds to black, a value of 255 corresponds to white, and intermediate values to the various shades of grey.

Finally, in multi-channel images, the colour of each pixel will be identified by three or four values, depending on the reference colour model. For example, in RGB colour space, each pixel will be characterized by a three values, each between 0 and 255, respectively, corresponding to the intensity in the red, green and blue. When all three values are 0, the colour of object is black, and when all three have maximum value, the object will be white, while, when there are equal levels of R, G and B, the grey colour is generated. The images of this type, in fact, may be considered as a three-dimensional matrix, consisting of three overlapping matrices having the same number of rows and columns, where the elements of the first matrix represent the pixel intensity in the red channel, those in the second matrix, the green channel and those of third matrix, in the blue channel.

Multispectral and hyperspectral images

RGB images, represented by three overlapping monochrome images, are the simplest example of multichannel images. In medical applications, in geotechnical, in the analysis of materials and of remote sensing, instead, are often used sensors capable of acquiring multispectral and hyperspectral images, two particular types of multi-channel images. The multispectral images are typically acquired in three/ten spectral bands including in the range of vis, but also in the field of IR, fairly spaced (Alexios et al., 2002). In this way it's possible to extract a larger number of information from the images respect those normally obtained from the analysis of RGB images.

An example of bands normally used in this type of analysis, are the band of blue (430-490 nm), green (491-560 nm), red (620-700 nm), the

NIR (700-1400 nm), the MIR (1400-1750 nm). The spectral combinations can be different depending on the purpose of analysis. The combination of NIR-RG (near infrared, red, green) is often used to identify green areas in satellite images, because the green colour reflects a lot in the NIR wavelength. The combination of NIR-R-B (near infrared, red, blue) is very useful to verify the ripening stage of fruit, this is due to the chlorophyll that shows a peak of adsorption in the wavelength of the red. Finally, the combination of NIR-MIR-blue (NIR, MIR and blue) is useful to observe the sea depth, the green areas in remote sensing images.

Hyperspectral imaging (HSI) combines spectroscopy and the traditional imaging to form a three-dimensional structure of multivariate data (hypercube). The hyperspectral images are consist of many spectral bands acquired in a narrow and contiguous way, allowing to analyze each pixel in the multiple wavelengths simultaneously and, therefore, to obtain a spectrum associated with a single pixels. The set of data constituting an hyperspectral image can be thought as a kind of data cube, with two spatial directions, ideally resting on the surface observed, and a spectral dimension. Extracting a horizontal plane from the cube it is possible to get a monochrome image, while the set of values, corresponding to a fixed position in the plane (x, y), is the spectrum of a pixel of the image (Figure 6).

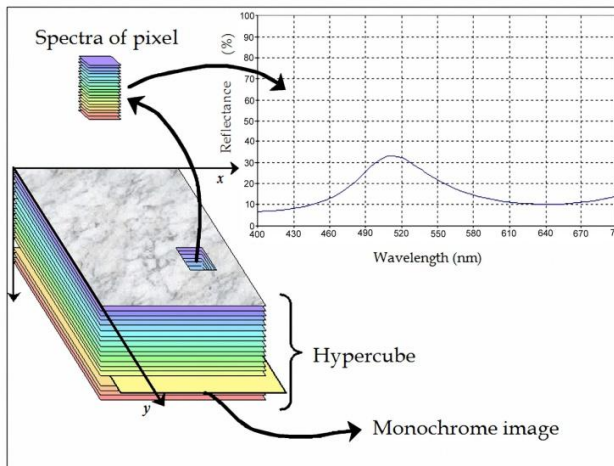


Figure 6. Example of hyperspectral image.

With the hyperspectral imaging, you can acquire the spectra in reflectance, in transmission and fluorescence as a function of the different kind of sample to analysis, even if the most of the scientific works, present in the literature, using spectral images acquired in reflectance, transmission and emission.

The significant time savings that can be made to the industrial production processes, encourage the use of this instrumentation. The hyperspectral image analysis has many advantages, but still has some defects. The advantages of using hyperspectral analysis for what concerns the agro-food sector can be summarized as follows:

- does not necessary to prepare the test sample;
- it is a non-invasive, non-destructive methodology, it avoids the sample loss that can be used for other purposes or analysis;
- can be regarded as an economic tool that it allows a saving of time, labour, reagents, and a strong cost-saving for the waste treatment;
- for each pixel of the analysed sample is possible to have the full spectrum and not a only absorbance value for few wavelength;
- many constituents can be determined simultaneously within a sample, such as colour and morphological characteristics;
- due to its high spectral resolution, it is possible to estimate both qualitative than quantitative information;
- it is also possible to select a single region of interest of the sample, and save it in a spectral library.

As mentioned previously, one of the advantages HSI is the large volume of data available in each hypercube, with which to create the calibration and validation set. But, the information derived from the analysis, contain also redundant information. This abundance of data has two drawback, one due to the high computational load of heavy data size and the second is due to the long acquisition times, given the size of the data being collected (Firtha et al. 2008). Therefore, it is desirable to reduce the load to manageable levels, especially if the goal is the application of HSI techniques in real time, on-line on production lines. In fact, in many cases, the large amount of data acquired from the spectral image, is appropriately reduced (with chemometric processing) so as to select only those wavelengths interesting for the intended purpose.

Once the spectral bands of interest were identified, a multispectral system, with only selected wavelengths, can be engineered a system for industrial application. Another negative aspect is that the spectral image analysis is an indirect method to which it is necessary to apply appropriate chemometric techniques and a procedure of data transfer.

The spectral image is not suitable for liquid and homogeneous samples. In fact, the value of this type of image is evident when applied to heterogeneous samples, and many foods are an excellent heterogeneous matrix. Despite the novelty of applying HSI in the food sector, many jobs are already present in the literature.

The traditional image analysis, based on a computer system, has had a strong development in the food sector with the aim of replacing the human eye on saving costs and improving efficiency, speed and accuracy. But the computer vision technology is not able to select between objects of similar colours, to make complex classifications, to predict quality characteristics (e.g. chemical composition) or detect internal defects. Since the quality of a food is not an individual attribute but it contains a number of inherent characteristics of the food itself, to measure the optical properties of food products has been one of the most studied non-destructive techniques for the simultaneous detection of different quality parameters. In fact, the light reflected from the food contains information about constituents near and at the surface of the foodstuff. Near-infrared spectroscopy technology (NIRS) is rapid, non-destructive, easy to apply on-line and off-line. With this technology, it is possible to obtain spectroscopic information about the components of the sample analysed, but it is not possible to know the position of the component.

The only characteristic of appearance (colour, shape, etc.) however, are easily detectable with conventional image analysis. The combination of image analysis technology and spectroscopy is the chemical imaging spectroscopy that allows to get spatial and spectral information for each pixel of the foodstuff. This technology allowing to know the location of each chemical component in the scanned image. Table 1 summarizes the main differences between the three analytical technologies: imaging, spectroscopy and hyperspectral imaging.

Table 1. Main differences among imaging, spectroscopy and hyperspectral imaging techniques (ElMarsy & Sun, 2010)

Features	Imaging	Spectroscopy	Hyperspectral Imaging
Spatial information	√	x	√
Spectral information	x	√	√
Multi-constituent information	x	√	√
Building chemical images	x	x	√
Flexibility of spectral information extraction	x	x	√

Image analysis applications

The chemical imaging spectroscopy is applied to various fields, from astronomy to agriculture (Baranowski et al., 2008; Monteiro et al., 2007; Smail, 2006.), from the pharmaceutical industry (Lyon et al. 2002; Roggo et al., 2005) to medicine (Ferris et al. 2001; Zheng et al., 2004). But in recent years, has also found use for quality control and safety in food (Gowen et al., 2007).

In general, classify or quantify the presence of compounds in a sample is the main purpose of the application in the food hyperspectral analysis. There already exist algorithms for classification and regression but, improved algorithms efficiency, could be a target, as well as create datasets, identify anomalies or objects with different spectral characteristics, compared hyperspectral image with those of data library. These goals can be achieved only if the experimental data are processed with chemometric methods. K-nearest neighbours and hierarchical clustering are examples of multivariate analysis that allow to get information from spectral and spatial data (Burger & Gowen, 2011). With the use of spectral image, which allows to obtain in a single determination, spectral and spatial information characterizing the sample, it is possible to identify which chemical species are present and how they are distributed in a matrix. Several chemometric techniques are available for the development of regression models (for example partial least squares regression, principal components regression, and linear regression) capable of estimating the concentrations of constituents in a sample, at the pixel level, allowing the spatial

distribution or the mapping of a particular component in the sample analyzed. Moreover the hyperspectral image, combined with chemometric technique, is a powerful method to identify key wavelengths in order to develop of multispectral system, for on-line applications.

Karoui & De Baerdemaeker (2006) wrote a review about the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. Spectroscopic techniques (NIR, MIR, FFFS front face fluorescence spectroscopy, etc.), coupled with chemometric tools have many potential advantages for the evaluation of the identity dairy products (milk, ice cream, yogurt, butter, cheese, etc).

In another review Sankaran et al. (2010), compared the benefits and limitations of advanced techniques and multivariate methods to detect plant diseases in order to assist in monitoring health in plants under field conditions. These technologies include evaluation of volatile profiling (Electronic Nose), spectroscopy (fluorescence, visible and infrared) and imaging (fluorescence and hyperspectral) techniques for disease detection.

In literature it's possible find several examples of applications of spectroscopic image analysis. Hyperspectral imaging could be used as critical control points of food processing to inspect for potential contaminants, defects or lesions. Their absence is essential for ensuring food quality and safety. In some case the application on-line was achieved.

Ariana & Lu (2010), evaluated the internal defect and surface colour of whole pickles, in a commercial pickle processing. They used a prototype of on-line hyperspectral imaging system, operating in the wavelength range of 400–1000 nm. Colour of the pickles was modeled using tristimulus values: there were no differences in chroma and hue angle of good and defective pickles. PCA was applied to the hyperspectral images: transmittance images at 675–1000 nm were much more effective for internal defect detection compared to reflectance images for the visible region of 500–675 nm. A defect classification accuracy was of 86% compared with 70% by the human inspectors.

Mehl et al. (2002), used hyperspectral image analysis and PCA, like chemometrics technique, to reduce the information resulting from HIS and to identify three spectral bands capable of separating normal from contaminated apples. These spectral bands were implemented in a multispectral imaging system. On 153 samples, it's possible to get a good separation between normal and contaminated (scabs, fungal, soil contaminations, and bruises) apples was obtained for Gala (95%) and Golden Delicious (85%), separations were limited for Red Delicious (76%).

HSI application for damage detection on the caps of white mushrooms (*Agaricus bisporus*) was investigated from Gowen et al. (2008). They employed a pushbroom line-scanning HSI instrument (wavelength range: 400–1000 nm). They investigated two data reduction methods. In the first method, PCA was applied to the hypercube of each sample, and the second PC (PC 2) scores image was used for identification of bruise-damaged regions on the mushroom surface. In the second method PCA was applied to a dataset comprising of average spectra from regions normal and bruise-damaged tissue. The second method performed better than the first when applied to a set of independent mushroom samples. Further, they (Gowen et al., 2009) identified mushrooms subjected to freeze damage using hyperspectral imaging. In this case used Standard Normal Variate (SNV) transformation for pretreated the data, then they applied a procedure based on PCA and LDA to classify spectra of mushrooms into undamaged and freeze-damaged groups. The undamaged mushrooms and freeze-damaged mushrooms could be classified with high accuracy (>95% correct classification) after only 45 min thawing (at 23 ± 2 °C) at which time freeze–thaw damage was not visibly evident.

A study on fruits and vegetables (Cubero et al., 2010) used of the ultraviolet or near-infrared spectra to explore defects or features that the human eye is unable to see to apply for automatic inspection. This work present a summary of inspection systems for fruit and vegetables and the latest developments in the application of this technology to the inspection of the internal and external quality of fruits and vegetables.

Li et al. (2011) detected common defects on oranges using hyperspectral (wavelength range: 400-1000) reflectance imaging. The

disadvantage of studied algorithm is that it could not discriminate between different types of defects.

Bhuvaneswari et al. 2011 compared three methods (electronic speck counter, acid hydrolysis and flotation and near-infrared hyperspectral imaging) to investigate the presence of detecting insect fragments (*Tribolium castaneum*_ Coleoptera: Tenebrionidae) in the semolina (ingredient for pasta and couscous). NIR hyperspectral imaging are rapid, non-destructive method, as electronic speck counter, but they showed different correlation between insect fragments in the semolina and detection of specks in the samples: $R^2 = 0.99$ and $0.639-0.767$ respectively. For NIR hyperspectral image technique, the prediction model were developed by PLS regression.

The most important features in meat are tenderness, juiciness and flavour. Jackmana et al., (2011) wrote a review about recent advances in the use of computer vision technology in the quality assessment of fresh meats. The researcher support that the best opportunities for improving computer vision solutions is the application of hyperspectral imaging in combination with statistical modelling. This synergy can provide some additional information on the meat composition and structure. However, in parallel, new image processing algorithms, that developed in other scientific disciplines, should be carefully considered for potential application to meat images.

Others applications concern the possibility of estimating a correlation between characteristics (physical or chemical) of the food and the spectra acquired with spectroscopic image. Moreover these techniques being able to locate and quantify the characteristic of interest within the image.

In most cases the range of wavelength used in applications of hyperspectral images is 400-1000 nm but Maftoonazad et al. (2010) used artificial neural network (ANN) modelling of hyperspectral radiometric (350-2500 nm) data for quality changes associated with avocados during storage. Respiration rate, total colour difference, texture and weight loss of samples were measured as conventional quality parameters during storage. Hyperspectral imaging was used to evaluate spectral properties of avocados. Results indicated ANN models

can predict the quality changes in avocado fruits better than the conventional regression models.

While Mahesh et al. (2011) used near-infrared hyperspectral images (wavelength range: 960–1700 nm), applied to a bulk samples, to classify the moisture levels (12, 14, 16, 18, and 20%) on the wheat. Principal components analysis (PCA) was used to identify the region (1260–1360 nm) with more information. The linear and quadratic discriminant analyses (LDA) and quadratic discriminant analysis (QDA) could classify the sample based on moisture contents than also identifying specific moisture levels with a god levels of accuracy (61- 100% in several case). Spectral features at key wavelengths of 1060, 1090, 1340, and 1450 nm were ranked at top in classifying wheat classes with different moisture contents.

Manley et al. (2011) used near infrared hyperspectral imaging combined with chemometrics techniques for tracking diffusion of conditioning water in single wheat kernels of different hardnesses. NIR analysers is a commonly, non-destructive, non-contact and fast solution for quality control, used tool for detect the moisture-content of carrot samples during storage but Firtha (2009) used hyperspectral system that is able to detect the spatial distribution of reflectance spectrum as well. Statistical analysis of the data has shown the optimal intensity function to describe moisture-content.

The intent of Junkwon et al. (2009), was to develop a technique for weight and ripeness estimation of oil palm (*Elaeis guineensis* Jacq. var. *tenera*) bunches from hyperspectral and RGB colour images. In the hyperspectral images, the total number of pixels in the bunch was also counted from an image composed of three wavelengths (560 nm, 680 nm, and 740 nm), while the total number of pixels of space between fruits was obtained at a wavelength of 910 nm. Weight-estimation equations were determined by linear regression (LR) or multiple linear regression (MLR). As a result, the coefficient of determination (R^2) of actual weight and estimated weight were at a level of 0.989 and 0.992 for colour and hyperspectral images, respectively. About the estimation of oil palm bunch ripeness the bunches was classified in 4 classes of ripeness (overripe, ripe, underripe, and unripe) (Figure 10). Euclidean distances between the test sample and the standard 4 classes of

ripeness were calculated, and the test sample was classified into the ripeness class. In the classification based on colour image, (average RGB values of concealed and not-concealed areas), and by hyperspectral images (average intensity values of fruits pixels from the concealed area), the results of validation experiments with the developed estimation methods indicated acceptable estimation accuracy.



Figure 10. Bunches of oil Palm: a) unripe, b) underripe, c) ripe, and d) overripe (Junkwon et al., 2009)

Nguyen et al. (2011) illustrated the potential of combination of hyperspectral imaging chemometrics and image processing as a process monitoring tool for the potato processing industry. They predicted the optimal cooking time by hyperspectral imaging (wavelength range 400–1000 nm). By partial least squares discriminant analysis (PLS-DA), cooked and raw parts of boiled potatoes, were discriminated successfully. By modelling the evolution of the cooking front over time the optimal cooking time could be predicted with less than 10% relative error.

Yu H. & MacGregor J.F. (2003) applied multivariate image analysis and regression for prediction of coating content and distribution in the production of snack foods. Elaboration tools based on PCA and PLS was used for the extraction of features from RGB colour images and for their use in predicting the average coating concentration and the coating distribution. On-line and off-line imaging were collected from several

different snack food product lines and were used to develop and evaluate the methods. The better methods are now being used in the snack food industry for the on-line monitoring and control of product quality.

Siripatrawan et al., (2011) developed a rapid method to detection of *Escherichia coli* contamination in packaged fresh spinach using hyperspectral imaging (400-1000 nm) and chemometrics techniques. The PCA was implemented to remove redundant information of the hyperspectral data and artificial neural network (ANN) to correlate spectra with number of *E. coli* and to construct a prediction map of all pixel spectra of an image to display the number of *E. coli* in the sample.

In this study (Barbin et al. 2012) a hyperspectral imaging technique (range from 900 to 1700 nm) was developed to achieve fast, accurate, and objective determination of pork quality grades. The sample investigated were 75 pork cuts of *longissimus dorsi* muscle from three quality grades. Six significant wavelengths (960, 1074, 1124, 1147, 1207 and 1341 nm) that explain most of the variation among pork classes were identified from 2nd derivative spectra. PCA was carried out and the results indicated that pork classes could be precisely discriminated with overall accuracy of 96%. Algorithm was developed to produce classification maps of the investigated sample.

Valous et al. (2010) communicated perspectives and aspects, relating to imaging, spectroscopic and colourimetric techniques on the quality evaluation and control of hams. These no-contact and no-destructive techniques, can provide useful information regarding ham quality. Hams are considered a heterogenic solid system: varying colour, irregular shape and spatial distribution of pores. Fat-connective tissue, water, protein contribute to the microstructural complexity. This review paid attention on applications of imaging and spectroscopy techniques, for measuring properties and extracting features that correlate with ham quality.

In literature is present a review (Mathiassena et al., 2011) that focused attention on application of imaging technologies (vis/NIR imaging, vis/NIR imaging spectroscopy, planar and computed tomography X-ray imaging, and magnetic resonance imaging) to inspection of fish and fish products.

Nicolai et al. (2007) wrote a review about the applications of non-destructive measurement of fruit and vegetable quality. Measurement principles are compared, and novel techniques (hyperspectral imaging) are reviewed. Special attention is paid to recent developments in portable systems. The problem of calibration transfer from one spectrophotometer to another is introduced, as well as techniques for calibration transfer. Chemometric is an essential part of spectroscopy and the choice, of corrected techniques, is primary (linear or nonlinear regression, such as kernel-based methods are discussed). The principal objective of spectroscopy system applications in fruit and vegetables sector have focused on the non-destructive measurement of soluble solids content, texture, dry matter, acidity or disorders of fruit and vegetables. (root mean square error of prediction want to be achieved).

References

- Aleixos, N.; Blasco, J.; Navarrón, F. & Moltó, E., 2002. Multispectral inspection of citrus in real-time using machine vision and digital signal processors. *Computers and Electronics in Agriculture*, 33(2), 121-137
- Amamcharla, J.K.; Panigrahi, S.; Logue, C.M.; Marchello, M. & Sherwood, J.S., 2010. Fourier transform infrared spectroscopy (FTIR) as a tool for discriminating *Salmonella typhimurium* contaminated beef. *Sens. & Instrumen. Food Qual.*, 4, 1-12
- Andrés, S.; Murray, I.; Navajas, E.A.; Fisher, A.V.; Lambe, N.R. & Bünger, L., 2007. Prediction of sensory characteristics of lamb meat samples by near infrared reflectance spectroscopy. *Meat Science*, 76, 509-516.
- Ariana, D.P. & Lu, R.A., 2010. Evaluation of internal defect and surface color of whole pickles using hyperspectral imaging. *Journal of Food Engineering*, 96, 583-590
- Baranowski, P.; Lipecki, J.; Mazurek, W. & Walczak, R.T., 2008. Detection of watercore in 'Gloster' apples using thermography. *Postharvest Biology and Technology*, 47, 358-366
- Barbin, D.; Elmasry, G.; Sun, D.W.; Allen, P., 2012. Near-infrared hyperspectral imaging for grading and classification of pork. *Meat Science*. *Meat Science*, 90(1), 259-268
- Basilevsk, A., 1994. Statistical factor analysis and related methods: theory and applications. Wiley-Interscience Publication. ISBN 0-471-57082-6
- Bhuvaneshwari, K.; Fields, P.G.; White, N.D.G.; Sarkar, A.K.; Singh, C.B. & Jayas, D.S., 2011. Image analysis for detecting insect fragments in semolina. *Journal of Stored Products Research* 47, 20-24
- Brenna, O.V. & Berardo, N., 2004. Application of near-infrared reflectance spectroscopy (NIRS) to the evaluation of carotenoids content in maize. *J. Agric. Food Chem.*, 52(18), 5577-5582

- Brosnan, T. & Sun, D.W., 2004. Improving quality inspection of food products by computer vision: a review. *Journal of Food Engineering*, 61, 3-16
- Burger, J. & Gowen, A., 2011. Data handling in hyperspectral image analysis. *Chemometrics and intelligent Laboratory Systems*, 108, 13-22
- Burns, D.A. & Ciurczak, E.W., 2001. Second ed. In: *Handbook of Near-Infrared Analysis.*, Marcel Dekker, New York, 27(28), 729-782
- Carrara, M.; Catania, P.; Vallone, M. & Piraino, S., 2008. Mechanical harvest of grapes: Assessment of the physical-mechanical characteristics of the berry in order to improve the quality of wines. In *Proc. Intl. Conf. on Agricultural Engineering: Agricultural and Biosystems Engineering for a Sustainable World (AgEng 2008)*. CIGR
- Cen, H. & He, Y., 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. *Trends in Food Science & Technology*, 18, 72-83
- Cozzolino, D.; Cynkar, W.; Janik, L.; Damberg, R.G. & Gishen, M., 2006a. Analysis of grape and wine by near infrared spectroscopy — A review. *J Near Infrared Spectros*, 14, 279-289
- Cozzolino, D.; Parker, M.; Damberg, R.G.; Herderich, M. & Gishen, M., 2006b. Chemometrics and visible-near infrared spectroscopic monitoring of red wine fermentation in a pilot scale. *Biotechnol. Bioeng.*, 95, 1101-1107
- Cubero, S.; Aleixos, N.; Moltó, E.; Gómez-Sanchis, J. & Blasco, J., 2010. Advances in Machine Vision Applications for Automatic Inspection and Quality Evaluation of Fruits and Vegetables. *Food Bioprocess Technol.*, 4, 487-504
- Da Costa Filho, P.A., 2009. Rapid determination of sucrose in chocolate mass using near infrared spectroscopy. *Analytica Chimica Acta*, 631, 206-211
- De Temmerman, J.; Saeys, W.; Nicolai, B. & Ramon, H., 2007. Near infrared reflectance spectroscopy as a tool for the in-line

determination of the moisture concentration in extruded semolina pasta. *Biosystems Engineering*, 97, 313-321

Diezak, J.D., 1988. Microscopy and image analysis for R&D, Special report. *Food Technol.*, 110-124

Du, C.J. & Sun, D.W., 2006. Learning techniques used in computer vision for food quality evaluation: a review. *Journal of food engineering*, 72(1), 39-55

Elbatawi, I.E. & Ebaid, M.T., 2006. A new technique for grape inspection and sorting classification. *Arab Universities J. Agric. Sci.*, 14(2), 555-573

ElMarsy, G. & Sun, D.W., 2010. Hyperspectral imaging for food quality, analysis and control. Book, Chapter 1, 3-43. ISBN: 978-0-12-374753-2.
(<http://elsevier.insidethecover.com/searchbook.jsp?isbn=9780123740854>)

Ferris, D.; Lawhead, R.; Dickman, E.; Holtzapple, N.; Miller, J.; Grogan, S.; et al., 2001. Multimodal hyperspectral imaging for the noninvasive diagnosis of cervical neoplasia. *Journal of Lower Genital Tract Disease*, 5(2), 65-72

Fessenden, R.J. & Fessenden, J.S., 1993. *Chimica organica. Cap. 9: Spettroscopia I: Spettri infrarossi, Risonanza Magnetica Nucleare.* Piccin Padova, Italy

Firtha, F., 2009. Detecting moisture loss of carrot samples during storage by hyperspectral imaging system. *Acta Alimentaria*, 38(1), 55-66

Firtha, F.; Fekete, A.; Kaszab, T.; Gillay, B.; Nogula-Nagy, M.; Kovács, Z. & Kantor, D.B., 2008. Methods for improving image quality and reducing data load of nir hyperspectral images. *Sensors*, 8, 3287-3298

Fodor, M.; Woller, A.; Turza, S. & Szigedi, T., 2011. Development of a rapid, non-destructive method for egg content determination in dry pasta using FT-NIR technique. *Journal of Food Engineering*, 107, 195-199

- Frank, I.E. & Todeschini, R., 1994. The Data Analysis Handbook. Elsevier. ISBN 0-444-81659-3, included in series: Data Handling in Science and Technology
- González-Martín, M.I.; Severiano-Pérez, P.; Revilla, I.; Vivar-Quintana, A.M.; Hernández-Hierro, J.M.; González-Pérez, C. & Lobos-Ortega, I.A., 2011. Prediction of sensory attributes of cheese by near-infrared spectroscopy. *Food Chemistry*, 127, 256-263
- Gowen, A.A.; O'Donnell, C.P.; Cullen, P.J.; Downey, G. & Frias, J.M., 2007. Hyperspectral imaging - an emerging process analytical tool for food quality and safety control. *Trends in Food Science & Technology*, 18, 590-598
- Gowen, A.A.; O'Donnell, C.P.; Taghizadeh, M.; Cullen, P.J.; Frias, J.M. & Downey, G., 2008. Hyperspectral imaging combined with principal component analysis for bruise damage detection on white mushrooms (*Agaricus bisporus*). *J. of chemometric*, 22(3-4), 259-267
- Gowen, A.A.; Taghizadeh, M. & O'Donnell, C.P., 2009. Identification of mushrooms subjected to freeze damage using hyperspectral imaging. *Journal of Food Engineering*, 93, 7–12
- Guanasekaran, S. & Ding, K., 1994. Using computer vision for food quality evaluation. *Food Technol.*, 15, 1-54;
- Guidetti, R.; Beghi, R. & Bodria, L., 2010. Evaluation of Grape Quality Parameters by a Simple Vis/NIR System. *Transaction of the ASABE*, 53 N.2, 477-484, ISSN: 2151-0032
- Guidetti, R.; Beghi, R. & Giovenzana, V., 2012. Chemometrics in Food Technology. In *Chemometrics*. InTech: E-Publishing, ISBN: 978-953-51-0438-4. Chapter 10, 217-252.
- Guidetti, R.; Beghi, R.; Bodria, L.; Spinardi, A.; Mignani, I. & Folini, L., 2008. Prediction of blueberry (*Vaccinium corymbosum*) ripeness by a portable Vis-NIR device. *Acta Horticulturae*, n° 310, ISBN 978-90-66057-41-8, 877-885
- Jackmana, P.; Sun D.W. & Allen P., 2011. Recent advances in the use of computer vision technology in the quality assessment of fresh meats. *Trends in Food Science & Technology*, 22, 185-197

- Jackson, J.E., 1991. A user's guide to principal components. Wiley-Interscience Publication. ISBN 0-471-62267-2
- Janik, L.J.; Cozzolino, D.; Damberg, R.; Cynkar W. & Gishen, M., 2007. The prediction of total anthocyanin concentration in red-grape homogenates using visible-near-infrared spectroscopy and artificial neural networks. *Anal. Chim. Acta*, 594(1), 107-118
- Junkwon, P.; Takigawa, T.; Okamoto, H.; Hasegawa, H.; Koike, M.; Sakai, K.; Siruntawinetti, J.; Chaeychomsri, W.; Sanevas, N.; Tittinuchanon, P. & Bahalayodhin, B., 2009. Potential application of color and hyperspectral images for estimation of weight and ripeness of oil palm (*elaeis guineensis jacq. var. tenera*). *Agricultural Information Research*, 18(2), 72-81
- Karoui, R. & De Baerdemaeker, J., 2006. A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. *Food Chemistry*, 102, 621-640
- Li, J.; Rao, X. & Ying, Y., 2011. Detection of common defects on oranges using hyperspectral reflectance imaging. *Computers and Electronics in Agriculture*, 78, 38-48
- Lu, R. & Ariana, D., 2002. A Near-Infrared Sensing Technique for Measuring Internal Quality of Apple Fruit. *Applied Engineering in Agriculture*, 18(5), 585-590
- Lunadei, L., 2008. L'analisi dell'immagine come metodologia per migliorare la fase di selezione dei prodotti alimentari. PhD thesis Università degli Studi di Milano, Italy
- Lyon, R.C.; Lester, D.S.; Lewis, E.N.; Lee, E.; Yu, L.X.; Jefferson, E.H.; et al., 2002. Near-infrared spectral imaging for quality assurance of pharmaceutical products: analysis of tablets to assess powder blend homogeneity. *AAPS PharmSciTech.*, 3(3), 1-15
- Maftoonazad, N.; Karimi, Y.; Ramaswamy, H.S. & Prasher, S.O., 2010. Artificial neural network modelling of hyperspectral radiometric data for quality changes associated with avocados during storage. *Journal of Food Processing and Preservation* ISSN 1745-4549

- Mahesh, S.; Jayas, D.S.; Paliwal, J. & White, N.D.G., 2011. Identification of wheat classes at different moisture levels using near-infrared hyperspectral images of bulk samples. *Sens. & Instrumen. Food Qual.*, 5, 1-9
- Manley, M.; Du Toit, G. & Geladi, P., 2011. Tracking diffusion of conditioning water in single wheat kernels of different hardnesses by near infrared hyperspectral imaging. *Analytica Chimica Acta*, 686, 64-75
- Massart, D.L.; Buydens, L.M.C.; De Jong, S.; Lewi P.J. & Smeyers-Verbek, J., 1998. Handbook of Chemometrics and Qualimetrics: Part B. Edited by B.G.M. ISBN: 978-0-444-82853-8, included in series: Data Handling in Science and Technology
- Massart, D.L.; Vandeginste, B.G.M.; Buydens, L.M.C.; De Jong, S.; Lewi P.J. & Smeyers-Verbek, J., 1997. Handbook of Chemometrics and Qualimetrics: Part A. Elsevier, ISBN: 0-444-89724-0, included in series: Data Handling in Science and Technology
- Mathiassena, J.R.; Misimib, E.; Bondøb, M.; Veliyulinb, E. & Ove Østvik, S., 2011. Trends in application of imaging technologies to inspection of fish and fish products. *Trends in Food Science & Technology* 22, 257-275
- McClure, W.F., 2003. 204 years of near infrared technology: 1800 - 2003. *Journal of Near Infrared Spectroscopy*, 11, 487-518
- Mehl, P. M.; Chao, K.; Kim, M.; Chen, Y. R., 2002. Detection of defects on selected apple cultivars using hyperspectral and multispectral image analysis. *Applied Engineering in Agriculture*, 18(2), 219-226
- Monteiro, S.; Minekawa, Y.; Kosugi, Y.; Akazawa, T. & Oda, K., 2007. Prediction of sweetness and amino acid content in soybean crops from hyperspectral imagery. *ISPRS Journal of Photogrammetry and Remote Sensing*, 62(1), 2-12
- Naes, T.; Isaksson, T.; Fearn, T. & Davies, T., 2002. A user-friendly guide to multivariate calibration and classification. Chichester, UK: NIR Publications ISBN 0-9528666-2-5

- Nguyen, D.; Trong, N.; Tsuta, M.; Nicolaï, B.M.; De Baerdemaeker, J. & Saeys, W., 2011. Prediction of optimal cooking time for boiled potatoes by hyperspectral imaging. *Journal of Food Engineering*, 105, 617-624
- Nicolai, B. M.; Beullens, K.; Bobelyn, E.; Peirs, A.; Saeys, W.; Theron & K. I., Lammertyna J., 2007. Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. *Postharvest Biology and Technology*, 46, 99-118
- Osborne, B.G.; Fearn, T. & Hindle, P.H., 1993. *Practical NIR Spectroscopy with Applications in Food and Beverage Analysis*. Cap. 4: Fundamentals of near infrared instrumentation, 73-76. Longman Scientific & Technical
- Prieto, N.; Roehe, R.; Lavín, P.; Batten, G. & Andrés, S., 2009b. Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. *Meat Science*, 83, 175–186
- Prieto, N.; Ross, D.W.; Navajas, E.A.; Nute, G.R.; Richardson, R.I.; Hyslop, J.J.; Simm, G. & Roehe, R., 2009a. On-line application of visible and near infrared reflectance spectroscopy to predict chemical–physical and sensory characteristics of beef quality. *Meat Science*, 83, 96–103
- Ribeiro, J.S.; Ferreira, M.M.C. & Salva, T.J.G., 2011. Chemometric models for the quantitative descriptive sensory analysis of Arabica coffee beverages using near infrared spectroscopy. *Talanta* 83, pp.1352–1358
- Riva M., 1999. *Introduzione alle tecniche di Image Analysis*. http://www.distam.unimi.it/image_analysis/image0.htm
- Roggo, Y.; Edmond, A.; Chalus, P. & Ulmschneider, M., 2005. Infrared hyperspectral imaging for qualitative analysis of pharmaceutical solid forms. *Analytica Chimica Acta*, 535(1-2), 79-87
- Römisch, U.; Jäger, H.; Capron, X.; Lanteri, S.; Forina, M. & Smeyers-Verbeke, J., 2009. Characterization and determination of the geographical origin of wines. Part III: multivariate discrimination and classification methods. *Eur. Food Res. Technol.*, 230, 31-45

- Russ, J.C.; Stewart, W. & Russ, J.C., 1988. The measurement of macroscopic image. *Food Technol.*, 42, 94-102
- Sankaran, S., Mishra, A., Ehsani, R., Davis, C., 2010. A review of advanced techniques for detecting plant diseases. *Computers and Electronics in Agriculture*, 72, 1–13
- Sierra, V.; Aldai, N.; Castro, P.; Osoro, K.; Coto-Montes, A. & Oliva, M., 2007. Prediction of the fatty acid composition of beef by near infrared transmittance spectroscopy. *Meat Science*, 78, 248-255
- Sinelli, N.; Limbo, S.; Torri, L.; Di Egidio, V. & Casiraghi, E., 2010. Evaluation of freshness decay of minced beef stored in high-oxygen modified atmosphere packaged at different temperatures using NIR and MIR spectroscopy. *Meat Science*, 86, 748-752
- Siripatrawan, U.; Makino, Y.; Kawagoe, Y. & Oshita, S., 2011. Rapid detection of *Escherichia coli* contamination in packaged fresh spinach using hyperspectral imaging. *Talanta* 85, 276-281
- Smail, V.; Fritz, A. & Wetzal, D., 2006. Chemical imaging of intact seeds with NIR focal plane array assists plant breeding. *Vibrational Spectroscopy*, 42(2), 215-221
- Sørensen, L.K., 2009. Application of reflectance near infrared spectroscopy for bread analyses. *Food Chemistry* 113, 1318-1322
- Stark, E.K. & Luchter, K., 2003. Diversity in NIR Instrumentation, in *Near Infrared Spectroscopy: Proceeding on the 11th International Conference*. NIR Publication, Chichester, UK, 55-66
- Sun, D.W., 2010. *Hyperspectral imaging for food quality analysis and control*. Edited by Da-Wen Sun. Elsevier. ISBN 978-0-12-374753-2
- Torri, L.; Sinelli, N. & Limbo S., 2010. Shelf life evaluation of fresh-cut pineapple by using an electronic nose. *Postharvest Biology and Technology*, 56, 239-245
- Urbano-Cuadrado, M.; de Castro, M.D.L.; Perez-Juan, P.M.; Garcia-Olmo, J. & Gomez-Nieto, M.A., 2004. Near infrared reflectance, spectroscopy and multivariate analysis in enology—Determination or screening of fifteen parameters in different types of wines. *Anal. Chim. Acta*, 527, 81-88

- Valous, N.A.; F. Mendoza & D.W. Sun, 2010. Emerging non-contact imaging, spectroscopic and colorimetric technologies for quality evaluation and control of hams: a review. *Trends in Food Science & Technology*, 21, 26-43
- Williams, P.C., & Sobering, D., 1996. How do we do it: A brief summary of the methods we use in developing near infrared calibrations. In A. M. C. Davies & P. C. Williams (Eds.), *Near infrared spectroscopy: the future waves*, 185-188. Chichester: NIR Publications
- Wold, S.; Sjöström, M. & Eriksson, L., 2001. PLS-regression: a basic tool of chemometrics. *Chemom. Intell. Lab. Syst.*, 58, 109-130
- Yu, H. & MacGregor, J.F., 2003. Multivariate image analysis and regression for prediction of coating content and distribution in the production of snack foods. *Chemometrics and Intelligent Laboratory Systems*, 67, 125-144
- Zardetto, S. & Dalla Rosa, M., 2006. Study of the effect of lamination process on pasta by physical chemical determination and near infrared spectroscopy analysis. *Journal of Food Engineering*, 74, 402-409
- Zheng, C.; Sun, D.W. & Zheng, L., 2006. Recent developments and applications of image features for food quality evaluation and inspection: a review. *Trends in food Science & Technology*, 17, 642-655
- Zheng, G.; Chen, Y.; Intes, X.; Chance, B. & Glickson, J.D., 2004. Contrast-enhanced near-infrared (NIR) optical imaging for subsurface cancer detection. *Journal of Porphyrins and Phthalocyanines*, 8(9), 1106-1117

PART 2

Objectives

Agro-food composition at harvest time is one of the most important factors determining the future quality of final products (e.g. wine from grapes and oil from olives). Quality parameters change in function of different product matrix. Measurement of fruits characteristics that impacts on product quality is a requirement for production improvement. Inspection of fruits during ripening is a critical point in all agri-food production chains. This control is usually performed only on small samples that are not always representative of the whole lot. The importance of this monitoring operation is easy to understand, as it determines the economic value of the entire stock. Traditionally, fruits quality evaluation is achieved by a visual and taste assessment of them and evaluation of the traditional quality parameters such as total soluble solids, acidity and texture.

The conventional methods to determine fruits quality parameters are time consuming, require preparation of samples, are often expensive, and generally highlight only one or a few aspects of fruits quality.

Therefore, there is a strong need in the modern food industry for a simple, rapid, and easy-to-use method for objectively evaluating the quality of fruits. This kind of tool would enable real-time analyses at the receiving station and would allow the preliminary decision-making about grapes during consignment thank to the rapid analysis of various parameters simultaneously.

Since food quality is not an individual attribute but it contains a number of inherent characteristics of the food itself, to measure the optical properties of food products has been one of the most studied non-destructive techniques for the simultaneous detection of different quality parameters. In fact, the light reflected from food contains information about constituents in the inner layers of sample and at foodstuff surface also.

To achieve objectives of this work, e.g. ripeness evaluation and postharvest quality characteristics of agro-food products, visible near-infrared (vis/NIR) spectroscopy was chosen.

In particular, vis/NIR spectroscopy is a rapid and non-destructive technique requiring minimal sample processing before analysis; coupled

with chemometric methods, appears to be one of the most powerful analytical tools for studying food products.

Chemometrics is an essential part of vis/NIR spectroscopy in food sector. To extract useful information present in the spectra multivariate analysis was carried out. Principal component analysis (PCA) was used for a qualitative analysis of the data and PLS regression analysis as a technique to obtain quantitative prediction of the parameters of interest.

The general aim of this work is to study the application of vis/NIR spectroscopy for ripeness evaluation and postharvest quality analysis of agro-food products. In particular this technology was tested to analyse ripening parameters of olives and grapes before to be processed, or to monitor freshness decay of fresh-cut lettuce and apples during long cold storage in controlled atmosphere.

Usually, with the aid of multivariate analysis, the huge dimensionality problems of spectral data can be reduced or eliminated by selecting spectral data at some importance wavelengths. In most cases, not all the spectral bands are required to address a particular attribute. Selection of important wavelengths is an optional step based on speed requirements of the whole process. Generally, selection of optimal wavelengths reduce the size of the required measurement data while preserving the most important information contained in the data. This approach was used in the presented works to select useful spectral bands present in the spectral data acquired by vis/NIR spectroscopy, for ripeness evaluation and postharvest quality analysis of agro-food products.

Starting from this studies of variable selection, in fact, the feasibility of a simplified handheld and low-cost optical device, based on measurement and processing of diffuse spectral reflectance at a few appropriately selected wavelengths was proposed.

Few essential wavelengths have been sorted among the full vis/NIR spectral variables, through visual inspection of the spectral curves (identification of wavelengths reflecting major spectral differences among spectra) and through the study of loadings plot arising from principal component analysis (PCA) and in particular through the

evaluation of regression coefficients deriving from PLS regression analysis.

This study was focused on identifying the most significant wavelengths able to discriminate in a quick and simple way (i) directly in the field, the blueberries, the grapes, the olives ready to be harvested, (ii) on-line, for the real time monitoring of trend of craft beer fermentation and to estimate qualitative and quantitative parameters or (iii) during shelf life, freshness levels of fresh-cut Lamb's lettuce (*Valerianella locusta* Laterr.).

The final aim of this work is to realize a simplified modular optical device (with few selected wavelengths) for single sample, non-destructive, and quick prediction of fruit ripeness degree and quality parameters evaluation.

The first prototype of simplified optical device was realized for red grapes study and is characterized by the presence of four LEDs emitting at the wavelengths of interest.

LED technology was chosen as illumination source of the sample, and allows obvious advantages in term of simplification and cost reduction.

The design of the prototype of the simplified optical device was realised with particular attention to versatility and modularity. The possibility to adjust the light source with a specific choice of wavelengths for LEDs, makes it possible to use the same simplified optical device for many different application. This modular design allows an easy adjustment for different objective (i.e. ripeness evaluation, chemicals and physical properties prediction or shelf life analysis), and for different kind of sample matrix.

EXPERIMENTAL STUDIES

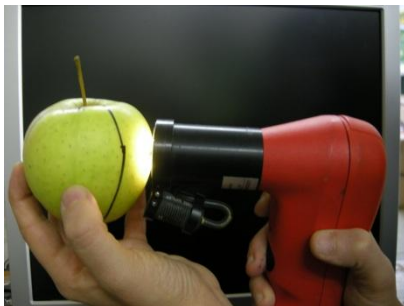
Section 1

Testing of a vis/NIR system for a better management of apple storage

Roberto Beghi¹, Gabriella Giovanelli², Cristina Malegori², Valentina Giovenzana¹, Riccardo Guidetti¹

¹Department of Agricultural and Environmental Sciences – Production, Landscape, Agroenergy, Università degli Studi di Milano, via Celoria 2, 20133, Milano

²Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Via Celoria 2, 20133 Milano



Abstract

The development of diseases during long term storage of apples is a well known issue causing loss of product for warehouses. Non-destructive characterization of fruit can be of help in order to reduce wastes and maximize apples quality. The aim of this study was to evaluate the applicability of the vis/NIR spectroscopy to monitor and manage lots of apples during long term storage in cold room. A bench-top vis/NIR (600-1200 nm) was used to classify apples from two different cultivars, Golden Delicious and Red Delicious, based on their soluble solids content (TSS). The evolution of classes originally created was analyzed during 7 months storage, monitoring TSS and firmness (peak force, penetration energy), and the estimation skills of the vis/NIR device was evaluated.

Results obtained indicate that the spectroscopic technique allows an accurate estimation of chemical-physical parameters for non-destructive classification of apples in homogeneous lots. Regarding the estimation ability of the compact vis/NIR spectrophotometer, results show good prediction skills both for soluble solid content and firmness indices. The use of the instrument for on-line selection and classification of fruits is therefore feasible. This can lead to a better management of postharvest storage and destination of the lots, with a consequent reduction of fruits wastage. This approach is important to plan the opening sequence of storage rooms during the winter season, allowing to enter the market with the best available products all year round.

Keywords Apple, vis/NIR spectroscopy, post-harvest, storage, classification

Introduction

The technologies developed for the preservation of apples (low temperature, controlled humidity and controlled atmosphere) allow long term storage of fruits, fully maintaining their physical, chemical and sensorial characteristics (Beni et al., 2001). External quality is considered of primary importance in the marketing and sale of fruits. The appearance, i.e., size, shape, color and presence of blemishes influences consumer perceptions and therefore determines the level of acceptability prior to purchase. The consumer also associates desirable internal quality characteristics with a certain external appearance. This learned association of internal quality to external quality affects future purchases (Wills et al. 1998; Brosnan & Sun, 2004). Disorders generally develops in more mature fruit and the main contributing factors are high or low air temperatures in the pre-harvest period, poor calcium nutrition, maturity status at harvest and cropping level (Ferguson et al., 1999; Woolf & Ferguson, 2000).

Moreover, some authors suggested that watercore, a typical apple defect, is caused by changes in membrane integrity during maturation and ripening (Bowen & Watkins, 1997; Kumpoun et al., 2003). It has been found that flesh tissue of apples with watercore has higher sorbitol and sucrose concentrations and lower glucose concentrations than tissue without watercore (Watkins et al., 1993; Harker et al., 1999; Yamada & Kobayashi, 1999). While watercore can dissipate during cold storage, fruit with moderate to severe watercore can develop flesh browning (Argenta et al., 2002).

Studies concerning the development of diseases during cold storage are numerous in literature (Snowdon 2010; Johnston et al. 2002). In particular, Volz et al. (1998) assessed fruit maturity indices, respiration rates and CO₂ permeances as predictors of flesh browning which developed in apple during long-term controlled atmosphere storage. Lau (1998) showed that fruits held in controlled atmosphere storage were firmer, higher in titratable acidity and had significantly less core browning and superficial scald than fruit held in air for the same period; however, fruits stored in controlled atmosphere were highly susceptible to Braeburn browning disorder and internal cavities after cool growing seasons.

Today, an important problem in postharvest technology is non-destructive characterization of fruit in order to maximize the quality and reduce wastes. The availability on the market of innovative technologies based on NIR and vis/NIR spectroscopy opened up new ways for fruit testing, which are not encumbered by any of the inconveniences of a traditional analysis (Peirs et al., 2001; Cen & He, 2007).

Novel non-destructive and rapid tools are used both for predicting the optimum harvest time and for a quick monitoring of fruit quality during the postharvest period. The NIR region of the electromagnetic spectrum lies between the visible and infrared regions and spans the wavelength range between 750 and 2500 nm. This region contains information concerning the relative proportions of C-H, N-H and O-H bonds which are the primary structural components of organic molecules (Williams & Norris, 2002).

The use of spectrometers for the evaluation of product quality is simple, fast, non-destructive and therefore applicable to a great number of samples. Interesting applications can be found since the beginning of the 90's (Frank & Todeschini, 1994; Massart et al., 1997; Massart et al., 1998; Basilevsk, 1994; Jackson, 1991).

Food sector showed interest towards NIR and vis/NIR instruments, both mobile and on-line; devices based on diode array spectrophotometers and FT-NIR desk systems have been developed and successfully applied in this field (Guidetti et al., 2012). These applications are based on the measurement of the optical properties of the product and on the correlation between the spectral data and the content of typical chemical components of the fruit. Apple has been widely involved in the experimentation of non-destructive testing methods, in order to determine a number of sensory and nutritional parameters. Recently, vis/NIR has been successfully used to measure a range of apple quality attributes such as soluble solid content (Quilitzsch & Hoberg, 2003; Zude et al., 2006), titratable acidity (Peirs et al., 2002), starch index (Menesatti et al., 2009), chlorophyll content (Zude-Sasse et al., 2002), volatile compounds with the combined use of electronic nose (Saevels et al., 2004). The results achieved have encouraged the inclusion of other specific applications related to the prediction of fruit firmness (Harker et al., 2002; Mehinagic et al., 2003; McClure, 2003; Nicolai et

al., 2007; Lu et al., 2000) and non-destructive analysis of nutraceutical properties (Beghi et al., 2012).

The aim of this work was to study the ability of a commercial vis/NIR instrumentation to classify Golden Delicious and Red Delicious apples in homogeneous lots. An apple storage life analysis was carried out splitting fruits into two classes based on total soluble content (parameter traditionally used for the characterization of fruit). The evolution of classes originally created was analyzed during long term storage, monitoring TSS and firmness (peak force, penetration energy), and the estimation skills of the vis/NIR device was evaluated.

For the correct management of the lots is necessary to consider the fruits heterogeneity before assessing their prolonged storage. Perform a fruits classification during the conferment can allow an optimization of the cells opening sequence to reduce scrap caused by over-ripening during long storage. Disorders, browning and loss of texture in fact, make the fruit unmarketable, especially in the last phases on the conservation period, with a consequent economic loss for warehouses. An optimization in the management of storage cells can lead to a reduction of waste, and to enter the market with the best available product all year round. Storage centers will achieve the full potential of a preservation technique already consolidated, such as modified atmosphere, by using this way of working.

Materials and methods

Sampling

The research was carried out in Ponte in Valtellina (Sondrio, Northern Italy) on the two most representative local apple varieties: Golden Delicious and Red Delicious that received in 2001 the PGI (Protected Geographical Indication). Data collected in the vintage year 2009 – 2010 were used to obtain prediction models for TSS, penetration energy and peak force evaluation (Giovannelli et al. 2011). Based on TSS model (the one with the best prediction skill), a total of 280 apples, were divided in the two ripening classes: 'lower TSS' and 'higher TSS'. TSS values taken as threshold were obtained in a destructive way by calculating the average of refractometric data collected on 40 Golden and 40 Red apples. Moreover these data were used for the vis/NIR

calibration (bias correction) in order to improve the precision of the model.

Fruits were stored for 7 months, from December 2010 to June 2011, and their evolution during storage was followed. Conditions generally used at the warehouse are different for the two cultivars; in particular, the values of O₂, CO₂, relative humidity and temperature are 1%, 2.5-3%, 94-99% and 1-1.5°C for Golden Delicious and 1%, 2%, 89-90% and 0.5-1°C for Red Delicious apples, respectively. For both varieties, storage conditions include the use of SmartFresh technology with 1-methylcyclopropene (Watkins , 2006).

For the cold storage life monitoring, a portion of the classified apples belonging to the different classes was analyzed monthly using the vis/NIR device and by traditional analytical methods for the evaluation of TSS and firmness indices. 140 fruits, both for Golden and Red variety, were analyzed with the bench top vis/NIR device at 7 sampling dates, acquiring each time the spectrum of 10 lower TSS and 10 higher TSS apples for each cultivar. Spectra were acquired by the vis/NIR spectrometer on each whole apples in two opposite points along the equator region and subsequently destructive determinations were carried out.

Each half apple was analyzed separately, both by spectroscopic and destructive analysis, in order to limit the influence of the variability of different parts of the same fruit. The analytical and spectral data were processed to obtain classification models able to follow the evolution of apple during cold storage.

Automatic bench top vis/NIR system

An automatic bench-top vis/NIR system (QS_200) produced by Unitec SpA (Lugo, Ravenna, Italy) was used in the wavelength range 600-1200 nm. The system consists of four elements: a lighting system, a fiber optic probe, a portable spectrophotometer, a PC for data acquisition and instrument control. In this system, samples were hit by radiation produced by the lighting system, and the reflected component was measured by the spectrophotometer. Light radiation was shone onto the fruit sample through a bidirectional fiber optic probe. The choice of this optical fiber was based on the need to acquire spectra in diffuse

reflectance (Haibo et al., 2008; Guidetti et al., 2008 and 2010; McGlone et al., 2002). Measurements were acquired by a dedicated software (Spectra software package, UNITEC SpA, Italy).



Figure 1. Automatic bench-top vis/NIR detail

Chemical analysis

Total soluble solids (TSS) determination

TSS were measured using a portable digital refractometer (model DBX-55, Atago, Tokyo, Japan). The determination was made after spectrophotometer readings and destructive determination of firmness: the two halves previously analyzed, separated from the rest of the cored apple, were reduced into a puree. A few drops of the latter were placed on the refractometer sensor, previously calibrated with distilled water. Result was directly expressed in Brix degrees ($^{\circ}$ Brix).

Firmness determination

Firmness of fruits was determined using a TA.HDplus Texture Analyser dynamometer (TXT, Stable Micro System), supported by the software "Exponent". The force-distance curve was acquired for each sample. Measurements were carried out on each whole apple in two opposite points along the equator region, corresponding to the spectra acquisition. Performance conditions used during the test were:

- Load cell: full scale 2.5 kN
- Probe: punch with a negligible diameter, if compared to sample size;
- Pre-test speed of 0.17 mm s^{-1} ;
- Threshold load value of 0.2 N;
- Test-speed of 3.33 mm s^{-1} ;
- End test standard: sample penetration for 10 mm;

Firmness parameters obtained from the elaboration of the force-distance curve are:

- *peak force* (N): the maximum force registered during penetration;
- *penetration energy* (N mm): work required to penetrate apple pulp from 5 to 8 mm.

Figure 2 shows an example a track for the analysis of firmness parameters.

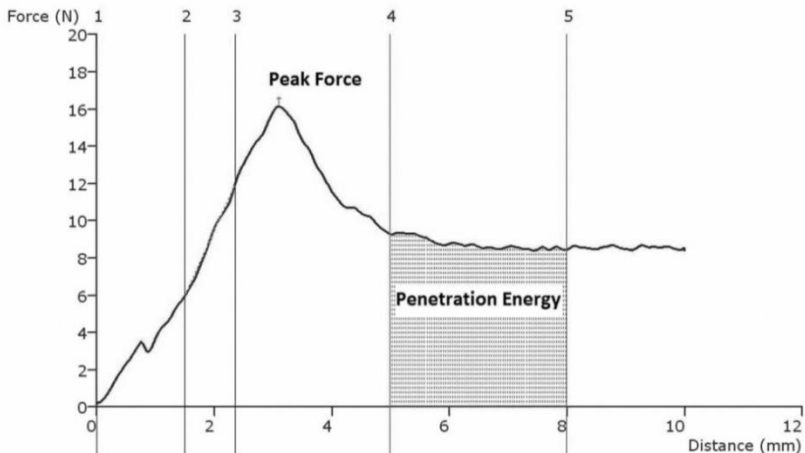


Figure 2. Force-distance curve obtained from the penetration test and identification of the penetration energy (dotted area)

Data processing

Chemometric analysis

Chemometric analyses were performed using The Unscrambler software package (version 9.7, CAMO ASA, Oslo, Norway). Principal component analysis (PCA) was carried out on vis/NIR spectra to

examine sample grouping and to identify outliers (Naes et al., 2002). Different treatments were applied to the vis/NIR spectra, namely scatter correction (Multiplicative Scatter Correction, MSC; Standard Normal Variate, SNV) and derivatives, before building the calibration models. First and second derivatives were performed using Savitzky-Golay transformation and smoothing (15 point and 2nd order filtering).

Regression models

Vis/NIR spectra were correlated with ripening parameters (TSS, firmness) using Partial Least Square (PLS) regression algorithm. The PLS method performs particularly well when the various X-variables express common information, i.e. when there is a large amount of correlation, or even co-linearity, which is the case for spectral data of intact biological material (Nicolaï et al., 2007; Wold et al., 2001).

The following statistical parameters were calculated to evaluate model accuracy: the coefficient of determination in calibration (R^2_{cal}), the root mean standard error of calibration (RMSEC), the coefficient of determination in cross-validation (R^2_{cval}) and the root mean standard error of cross-validation (RMSECV) (Sinelli et al., 2008). Percent errors of RMSECV (RMSECV%) were also calculated as $= RMSECV / \text{averaged reference values of each parameter}$.

Cross-validation is an internal validation method, usually used in the case of a small number of samples available for regression. To perform cross-validation, some samples are kept out of the calibration and used for prediction. This is repeated until all samples have been kept out once. In this case full cross validation was used, so only one sample at a time is kept out of the calibration. The optimum calibrations were selected based on minimizing the RMSECV.

At each sampling date, 10 apples belonging to 'higher TSS' class and 10 belonging to 'lower TSS' one were analyzed by the bench top vis/NIR system, then TSS and firmness were determined on the same samples by refractometer and texture analyser. The aim of this part of the work was to verify prediction skills of the device on an independent sample set. Finally, all available data, 560 measurements (deriving from 280 apples and obtaining 2 measures from each fruit) were used to carry out robust PLS models.

Classification modes

In order to classify the samples by storage time, the vis/NIR spectra were elaborated by Linear discriminant analysis (LDA). The data set was composed of the same 560 samples used for the elaboration of final PLS models. Different pretreatments were applied to vis/NIR spectra (MSC and SNV). The classification technique was carried out after having applied to spectral data the algorithm SELECT as feature selection technique (Casale et al., 2008). Performance of LDA classification was evaluated on the basis of the predictive ability in the cross validation groups (internal prediction rate), which was performed by V-PARVUS package (Forina et al., 2008).

Statistical analysis

In order to highlight statistically significant differences between samples, One-Way Anova and Multiple Range Test were performed using the Statgraphics plus 5.1 package (Graphics Software Systems, Rockville, MD, USA).

Results and Discussion

Traditional shelf life analysis

Considering the 40 Golden Delicious apples used for threshold identification, the average refractometric data was 13 °Brix. Therefore, Golden Delicious apples with a TSS > 13 °Brix were classified as 'higher TSS' while apples with a TSS < 13 °Brix were classified as 'lower TSS'. Similarly, the average refractometric data for the 40 Red Delicious was 12 °Brix: 'higher TSS' for TSS > 12 °Brix and 'lower TSS' for TSS < 12 °Brix.

Regarding the evolution of quality indices analyzed during storage, results showed significant changes of TSS and firmness parameters in some sampling times. Tables 1 and 2 report the average values of quality parameters for Golden Delicious and Red Delicious apples, respectively, for both ripening classes during the 7 months of storage.

Table 1. Average destructive values and standard deviation (n = 10) of the main quality indices of Golden Delicious apples during storage. For each column, values corresponding to different letters are significantly different at p <0.05

Time (day)	Soluble Solids (°Brix)		Texture			
			Penetration Energy (N mm)		Peak Force (N)	
	Lower TSS	Higher TSS	Lower TSS	Higher TSS	Lower TSS	Higher TSS
0	11.1 ± 0.7 ^a	13.3 ± 0.8 ^b	16.9 ± 1.6 ^d	17.1 ± 1.8 ^c	10.2 ± 1.0 ^c	9.7 ± 1.2 ^b
49	11.3 ± 1.0 ^{abc}	13.1 ± 1.1 ^{ab}	14.8 ± 1.8 ^c	15.3 ± 2.2 ^b	9.2 ± 1.0 ^b	8.8 ± 1.5 ^a
76	11.5 ± 0.5 ^{bc}	13.3 ± 0.5 ^{bc}	13.7 ± 1.3 ^b	15.0 ± 1.6 ^b	8.9 ± 0.9 ^b	9.1 ± 1.0 ^{ab}
103	11.7 ± 0.8 ^{bc}	13.5 ± 1.0 ^{bc}	13.9 ± 1.5 ^{bc}	14.5 ± 1.6 ^{ab}	9.0 ± 1.0 ^b	9.0 ± 1.2 ^a
138	11.7 ± 0.6 ^c	13.9 ± 0.5 ^c	13.5 ± 1.4 ^b	14.3 ± 1.2 ^{ab}	8.6 ± 0.7 ^{ab}	8.7 ± 1.1 ^a
168	11.4 ± 0.7 ^{abc}	12.6 ± 0.9 ^a	13.8 ± 1.4 ^b	13.6 ± 1.2 ^a	9.1 ± 0.9 ^b	8.8 ± 1.1 ^a
189	11.2 ± 0.7 ^{ab}	13.1 ± 0.5 ^{ab}	12.3 ± 1.9 ^a	13.8 ± 2.3 ^a	8.1 ± 1.4 ^a	8.6 ± 1.2 ^a
<i>p-value</i>	*	**	***	***	***	*

* p<0.05; ** p<0.01; *** p<0.001

Table 2 - Average destructive values and standard deviation (n = 10) of the main quality indices of Red Delicious apples during storage. For each column, values corresponding to different letters are significantly different at p <0.05

Time (day)	Soluble Solids (°Brix)		Texture			
			Penetration Energy (N mm)		Peak Force (N)	
	Lower TSS	Higher TSS	Lower TSS	Higher TSS	Lower TSS	Higher TSS
0	12.1 ± 0.7 ^{ab}	13.4 ± 1.2 ^a	26.1 ± 3.8 ^e	26.2 ± 5.5 ^d	15.0 ± 1.9 ^d	14.8 ± 2.3 ^d
49	13.0 ± 1.5 ^{cd}	13.9 ± 0.9 ^a	25.4 ± 2.5 ^{de}	22.8 ± 4.2 ^c	14.3 ± 1.6 ^{cd}	13.3 ± 2.2 ^c
76	11.7 ± 0.9 ^a	14.0 ± 1.1 ^a	25.9 ± 3.5 ^{de}	19.2 ± 3.1 ^b	15.2 ± 1.8 ^d	12.3 ± 2.1 ^{bc}
103	12.3 ± 0.9 ^{ab}	13.7 ± 1.0 ^a	23.8 ± 3.0 ^{cd}	20.3 ± 3.4 ^{bc}	13.6 ± 1.8 ^c	12.4 ± 1.6 ^{bc}
138	12.6 ± 0.6 ^{bc}	15.0 ± 1.9 ^b	21.2 ± 5.0 ^b	20.4 ± 5.9 ^{bc}	12.4 ± 2.5 ^b	12.6 ± 3.5 ^c
168	13.4 ± 1.1 ^d	13.6 ± 0.8 ^a	18.3 ± 4.2 ^a	17.9 ± 4.1 ^{ab}	11.1 ± 1.7 ^a	11.0 ± 2.2 ^{ab}
189	12.1 ± 1.1 ^{ab}	13.4 ± 0.6 ^a	22.2 ± 3.7 ^{bc}	15.5 ± 4.2 ^a	13.3 ± 1.5 ^{bc}	9.9 ± 2.4 ^a
<i>p-value</i>	***	***	***	***	***	***

* p<0.05; ** p<0.01; *** p<0.001

Data show that during storage in controlled atmosphere coupled with the use of 1-MCP (Fan et al., 1999), no significant evolution of TSS during the storage period occurred, despite some significant differences of this parameter in some of the sampling dates. TSS ranged between 12 and 15 °Brix and a clear difference between the two ripening classes was maintained all along the storage period (the higher TSS class of apples show a TSS of about 2 °Brix higher than the other one) (Figures 3 and 4). During the last sampling dates non-marketable samples (due to disorders) were eliminated. The number of apples rejected was higher for the higher TSS class respect to the lower TSS (both for Golden and Red samples). This behavior reconfirms the need for a classification tool only to optimize the management of storage and to reduce fruit wastage.

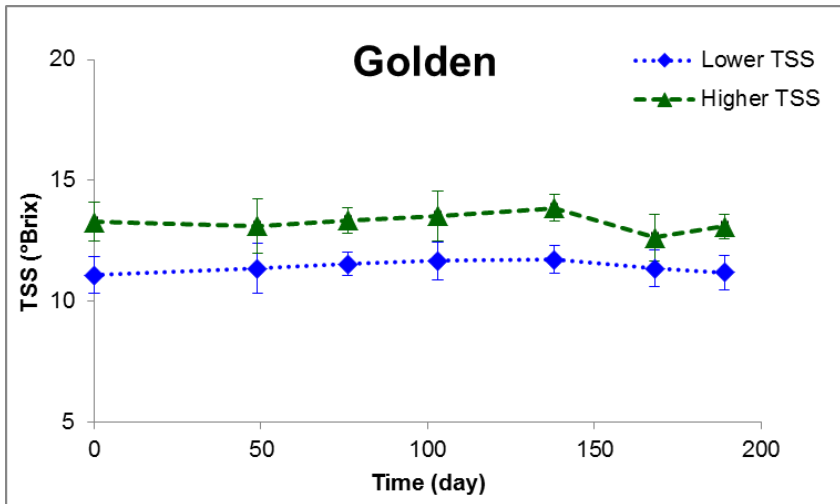


Figure 3. Evolution of TSS during Golden Delicious apple storage life belonging to the two classes of ripeness 'higher TSS' and 'lower TSS'

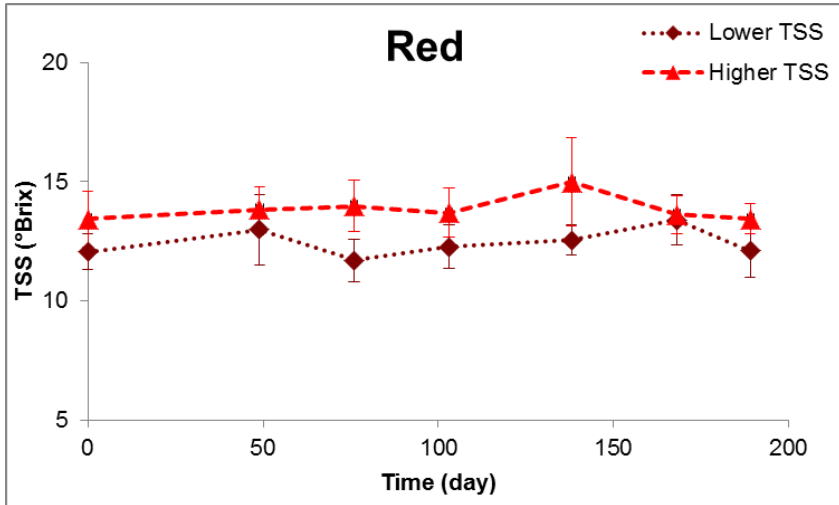


Figure 4. Evolution of TSS during Red Delicious apple storage life belonging to the two classes of ripeness 'higher TSS' and 'lower TSS'

Regarding firmness indices, in response to time progression, the fruits of both classes show a decrease in firmness indexes, especially in the penetration energy. A wider variability for firmness parameters was observed as a result of a significant change in apple texture during storage time. However, there were no differences between the two classes, with clear overlapping patterns particularly for the cultivar Golden Delicious (Figures 5 and 6).

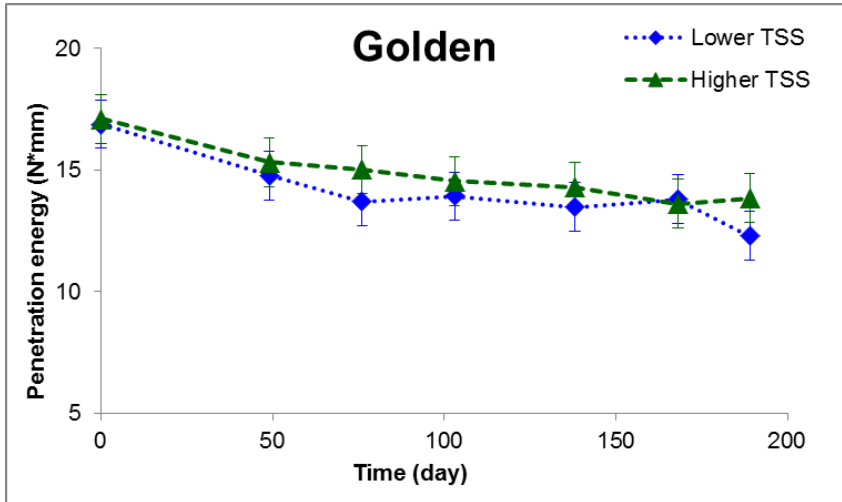


Figure 5. Evolution of penetration energy during Golden Delicious apple storage life belonging to the two classes of ripeness 'higher TSS' and 'lower TSS'

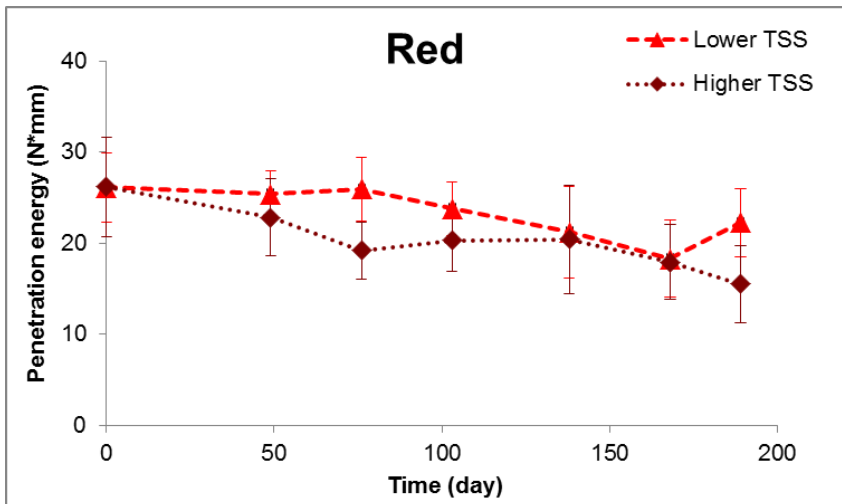


Figure 6. Evolution of penetration energy during Red Delicious apple storage life belonging to the two classes of ripeness 'higher TSS' and 'lower TSS'

Vis/NIR analysis

Figure 7 shows a particular of the vis/NIR bench top instrument and the spectra of some collected samples in the range 600-1200 nm.

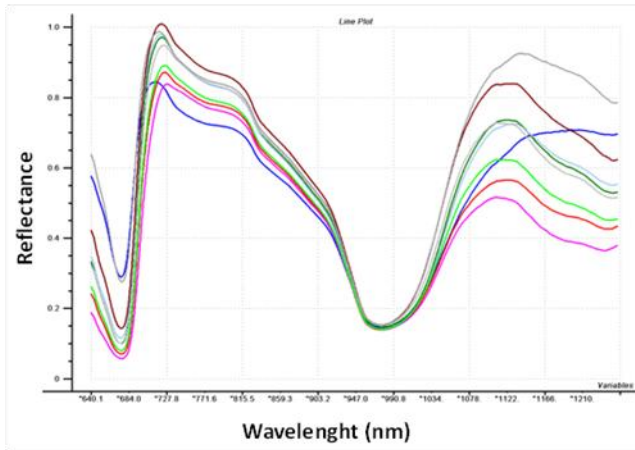


Figure 7. Example of spectra in the 600-1200 nm range

The absorption peak at 675 nm (absorption peak of chlorophyll in the visible region) and the peak at about 970 nm (absorption peak relative to the second overtone of the water O-H bond in the near infrared region) can be clearly detected. The variation of the relative absorption, in particular at the chlorophyll peak, is very important because it is directly related to ripeness and firmness degree of fruits; the in-depth peak at 970 nm is characteristic of the vis/NIR measurements on solid food very rich in water, like apples.

Vis/NIR device prediction skills

At each sampling date, 10 apples belonging to the classes 'higher TSS' and 'lower TSS' were taken from the cells and analyzed both with the bench-top vis/NIR system and in the traditional destructive way by refractometer and texture analyzer. The data set analyzed in this phase consisted of 140 apples for each cultivar (20 fruits for each cultivar every sampling date). For each sample the differences between the estimated data and the analytical data were calculated and the limit of acceptability was defined as a gap equal to 10%. Accordingly, for TSS the gap was fixed at 1 °Brix, while for firmness parameters a gap of 2 N mm for the penetration energy and 1.5 N for the peak force were identified. Data of prediction acceptability are shown in table 3.

Table 3 - Percentage of acceptable and not acceptable prediction of unknown samples for the analyzed parameters. Prediction is acceptable if prediction error < 10%, not acceptable if prediction error > 10%

	Acceptable	Not Acceptable
TSS (°Brix)		
Golden	83%	17%
Red	56%	44%
Penetration Energy (N mm)		
Golden	68%	32%
Red	27%	72%
Peak force (N)		
Golden	52%	48%
Red	63%	37%

Results highlight good percentage of acceptable classification for TSS prediction. In particular, very good results were obtained for Golden Delicious with 83% of samples correctly classified.

Regarding firmness parameters, better results were achieved for Golden Delicious compared with Red Delicious apples, especially for penetration energy. The application of NIR spectroscopy for the analysis of firmness parameters often encountered considerable difficulties, highlighted by some published studies (Nicolaï et al., 2008). Our data also show that it is difficult to obtain high percentages of correct classification in both cultivars for these parameters.

Quantitative vis/NIR analysis

At the end of the sampling period all the available data (vis/NIR spectra and reference destructive analysis) were used for the elaboration of PLS models, based on a large number of samples. Figure 8 shows, as an example, the regression lines of PLS models for penetration energy in Golden Delicious and for TSS in Red Delicious, respectively.

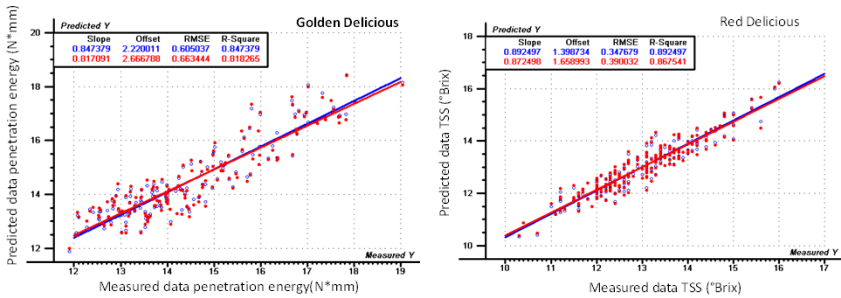


Figure 8. Example of PLS models for Golden Delicious penetration energy and Red Delicious TSS.

Descriptive statistics and statistics of the PLS models for TSS and firmness indices (penetration energy and peak force) are shown in Table 4 for Golden Delicious and Red Delicious respectively. Good results were obtained in both analyzed cultivar. TSS estimation gave high R2 values with a low standard error (RMSECV = 0.3 and 0.4 °Brix for Golden Delicious and Red Delicious, respectively) testifying a high level of prediction performance. In literature, results obtained on various fruits showed values of RMSECV of about 0.6 - 1 °Brix (Nicolaï et al., 2007; Bobelyn et al., 2010; Jha & Ruchi, 2010; Yan-de & Yi-bin, 2004). Results are also similar to those published for similar non destructive applications on other fruits such as apricot (Camps & Christen, 2009) and watermelon (Tian et al., 2007).

Regression models were obtained with good correlation coefficients and low errors in the cross-validation both as regards to the penetration energy (R2cval = 0.81 and 0.77 for Golden Delicious and Red Delicious, respectively) and peak force (R2cval = 0.83 and 0.69 for Golden Delicious and Red Delicious, respectively). Generally, the results obtained for the Golden variety are better than those of the Red variety, both in calibration and in cross-validation.

Table 4 - Descriptive statistics and statistics of the PLS models elaborated on Golden Delicious and Red Delicious apples

Golden Delicious	Units	N° of samples	Mean	SD	Calibration			Cross-validation		
					R ²	RMSEC	LV	R ²	RMSECV	RMSECV%
TSS	°Brix	280 (140)	12.7	1.05	0.94	0.3	7	0.90	0.3	2.4%
Penetration energy	N mm	280 (140)	14.7	2.9	0.85	0.6	6	0.81	0.7	4.8%
Peak force	N	280 (140)	9.1	0.8	0.85	0.4	7	0.83	0.4	4.4%
Red Delicious	Units	N° of samples	Mean	SD	Calibration			Cross-validation		
					R ²	RMSEC	LV	R ²	RMSECV	RMSECV%
TSS	°Brix	280 (140)	13.8	1.5	0.88	0.4	9	0.86	0.4	2.9%
Penetration energy	N mm	280 (140)	23.0	6.0	0.81	1.6	5	0.77	1.7	7.4%
Peak force	N	280 (140)	13.0	1.7	0.72	0.85	7	0.69	1.0	7.7%

R² coefficient of determination; RMSEC root mean square error of calibration; RMSECV root mean square error of cross validation; LV latent variables

Qualitative vis/NIR analysis

Classification techniques (LDA, Linear Discriminant Analysis) were also applied to spectral data, in order to discriminate apples on the basis of storage time (each class corresponds approximately to the months of storage, from time zero to 6 months).

For the classification, classes were defined in accordance to the days of storage: Class 1 is composed of apples at time 0, Classes 2, 3, 4, 5, 6 and 7 are composed of the samples stored for 49, 76, 103, 138, 168 and 189 days, respectively.

Table 5 shows results obtained by LDA classification for Golden Delicious and Red Delicious apples. Vis/NIR spectroscopy gave good classification performances on both apple varieties for both applied pretreatment techniques (SNV and MSC), with average correct classification percentages close to 100%. This result appears very valuable even when compared with other published works where LDA was used as a classification technique, concerning apples (Kavdir & Guyer, 2008) and other products (Craig et al., 2012; Xiangrong et al., 2010; Uddin et al., 2005).

Table 5 - Results obtained using the LDA technique

		% correctly classified samples								
		Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7	Average	
Golden	SNV	Cal	100	100	100	100	100	100	100	100
		Cv	100	100	100	100	100	100	100	100
	MSC	Cal	100	100	100	100	100	100	100	100
		Cv	100	100	100	100	95	100	100	99.3
Red	SNV	Cal	100	100	100	100	100	100	100	100
		Cv	100	100	100	100	100	95	100	99.3
	MSC	Cal	100	100	100	100	100	100	100	100
		Cv	100	100	100	100	100	100	100	100

Cal = calibration, Cv= cross-validation

Conclusions

A compact bench-top spectrophotometer, operating in the range 600-1200 nm, was tested at a refrigerated warehouse for monitoring and optimizing the management of stored apples. Results obtained indicate that the system allows an accurate estimation of chemo-physical parameters (TSS, penetration energy and peak force). Particularly, results show excellent prediction skills of TSS, while acceptable performances in the estimation of firmness parameters were obtained.

The study of quality characteristics during storage demonstrated that the differences between the two ripening classes, initially selected in a non-destructive way, were maintained during storage in controlled atmosphere. The instrument proved to be suitable not only for the evaluation of apple quality parameters, but also for the fruit classification according to the storage time. It can therefore be used as a non-destructive method for apple classification in homogeneous lots with the purpose of better management of the postharvest storage stages and destination of lots for a saving of fruit wastage.

All these advantages are combined with the well known ones regarding the use of this optical technology (saving of time and analysis of a large number of samples).

The use of such tools makes it possible for operators to quickly monitor important characterization parameters attained by the fruit at harvest and during all subsequent storage stages, allowing the management optimization and planning the cell opening sequence according to the fruit characteristics

Acknowledgments

This study received financial support from Regione Lombardia as “VALORVÌ” research project and from Regione Lombardia and European Social Fund for a Post-doctoral Research Fellowship (“Progetto Dote Ricerca”).

References

- Argenta L., Fan X. & Mattheis J., 2002. Impact of watercore on gas permeance and incidence of internal disorders in 'Fuji' apples. *Postharvest Biology and Technology* 24, 113–122
- Basilevsk A., 1994. *Statistical factor analysis and related methods: theory and applications*. Wiley-Interscience Publication. ISBN 0-471-57082-6
- Beghi R., Spinardi A., Bodria L., Mignani I. & Guidetti R., 2012. Apples nutraceutic properties evaluation through a visible and near-infrared portable system. *Food and Bioprocess Technology*, DOI 10.1007/s11947-012-0824-7
- Beni C., Ianicelli V. & Di Dio C., 2001. *Il condizionamento dei prodotti ortofrutticoli*. Edagricole- Edizioni Agricole della Calderini s.r.l., Bologna
- Bobelyn E., Serban A.S., Nicu M., Lammertyn J., Nicolaï, B.M. & Saeys W., 2010. Postharvest quality of apple predicted by NIR-spectroscopy: Study of the effect of biological variability on spectra and model performance. *Postharvest Biology and Technology* 55, 133–143
- Bowen J.H. & Watkins C.B., 1997. Fruit maturity, carbohydrate and mineral content relationships with watercore in 'Fuji' apples. *Postharvest Biology and Technology* 11, 31–38
- Brosnan T. & Sun D.W., 2004. Improving quality inspection of food products by computer vision - a review. *Journal of Food Engineering* 61, 3–16
- Camps C. & Christen D., 2009. Non-destructive assessment of apricot fruit quality by portable visible-near infrared spectroscopy. *Food Science and Technology* 42(6), 1125-1131
- Casale M., Casolino C., Ferrari G. & Forina M., 2008. Near infrared spectroscopy and class modelling techniques for the geographical authentication of Ligurian extra virgin olive oil. *Journal of Near Infrared Spectroscopy* 16 (1), 29-47

- Cen H. & He Y., 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. *Trends in Food Science & Technology* 18, 72-83
- Craig A.P., Franca A.S. & Oliveira L.S., 2012. Discrimination between defective and non-defective roasted coffees by diffuse reflectance infrared Fourier transform spectroscopy. *LWT - Food Science and Technology*. 47(2), 505-511
- Fan X., Blankenship S.M. & Mattheis J.P., 1999. 1-Methylcyclopropene Inhibits Apple Ripening . *Journal of the American Society for Horticultural Science* 124(6), 690-695
- Ferguson I., Volz R. & Woolf A., 1999. Preharvest factors affecting physiological disorders of fruit. *Postharvest Biology and Technology* 15: 255-262
- Forina M., Lanteri S., Armanino C., Casolino C., Casale M. & Oliveri P., 2008. V-PARVUS 2008, Dip. Chimica e Tecnologie Farmaceutiche e Alimentari, University of Genova available (free, with manual and examples) from authors or at [http:// www.parvus.unige.it](http://www.parvus.unige.it).
- Frank I.E. & Todeschini R., 1994. *The Data Analysis Handbook*. Elsevier, ISBN 0-444-81659-3
- Guidetti R., Beghi R., Bodria L., Spinardi A., Mignani I. & Folini L., 2008. Prediction of blueberry (*Vaccinium corymbosum*) ripeness by a portable Vis-NIR device. *Acta Horticulturae*, n° 310, ISBN 978-90-66057-41-8, 877-885
- Guidetti R., Beghi R. & Giovenzana V., 2012. Chemometrics in Food Technology. In: *Chemometrics in Practical Applications*, ISBN: 978-953-51-0438-4, InTech.
- Haibo H., Haiyan Y., Huirong X. & Yibin Y., 2008. Near infrared spectroscopy for on/in-line monitoring of quality in foods and beverages: a review. *Journal of Food Engineering* 87, 303-313
- Harker F.R., Maindonald J., Murray S.H., Gunson F.A., Hallett I.C. & Walker S.B., 2002. Sensory interpretation of instrumental measurements. 1: Texture of apple fruit. *Postharvest Biology & Technology* 24(3), 225-239

- Harker F.R., Watkins C.B., Brookfield P.L., Miller M.J., Reid S., Jackson P.J., Bielecki R.L. & Bartley T., 1999. Maturity and regional influences on watercore development and its postharvest disappearance in 'Fuji' apples. *Journal of the American Society for Horticultural Science* 124, 166-172
- Jackson J.E., 1991. A user's guide to principal components. Wiley-Interscience Publication. ISBN 0-471-62267-2
- Jha S.N. & Ruchi G., 2010. Non-destructive prediction of quality of intact apple using near infrared spectroscopy. *Journal of Food Science and Technology* 47(2), 207-213
- Johnston J.W., Hewett E.W. & Hertog M.L.A.T.M., 2002. Postharvest softening of apple (*Malus domestica*) fruit: A review. *New Zealand Journal of Crop and Horticultural Science* 30(3), 145-160.
- Kavdir I. & Guyer D.E., 2008. Evaluation of different pattern recognition techniques for apple sorting. *Biosystems Engineering*. 99(2), 211-219
- Kumpoun W., Motomura Y. & Harada Y., 2003. Inhibition of *Aspergillus* rot by sorbitol in apple fruit with watercore symptoms. *Postharvest Biology Technology* 29: 121-127
- Lau O.L., 1998. Effect of growing season, harvest maturity, waxing, low O₂ and elevated CO₂ on flesh browning disorders in 'Braeburn' apples. *Postharvest Biology and Technology* 14, 131-141
- Lu R., Guyer D.E. & Beaudry R.M., 2000. Determination of firmness and sugar content of apples using near-infrared diffuse reflectance. *Journal of Texture Studies* 31(6), 615-630
- Massart D.L., Buydens L.M.C., De Jong S., Lewi P.J. & Smeyers-Verbeek J., 1998. *Handbook of Chemometrics and Qualimetrics: Part B*. Edited by B.G.M. ISBN: 978-0-444-82853-8
- Massart D.L., Vandeginste B.G.M., Buydens L.M.C., De Jong S., Lewi P.J. & Smeyers-Verbeek J., 1997. *Handbook of Chemometrics and Qualimetrics: Part A*. Elsevier, ISBN:0-444-89724-0
- McClure W.F., 2003. 204 years of near infrared technology: 1800 - 2003. *Journal of Near Infrared Spectroscopy* 11, 487-518

- McGlone V.A., Jordan R.B. & Martinsen P.J., 2002. Vis/NIR estimation at harvest of pre- and post-storage quality indices for Royal Gala apple. *Postharvest Biology and Technology* 25, 135-144
- Mehinagic E., Royer G., Bertrand D., Symoneaux R., Laurens F. & Jourjon F., 2003. Relationship between sensory analysis, penetrometry and visible-NIR spectroscopy of apples belonging to different cultivars. *Food Quality and Preference* 14, 473-484
- Menesatti P., Zanella A., D'Andrea S., Costa C., Paglia G. & Pallottino F., 2009. Supervised multivariate analysis of hyper-spectral NIR images to evaluate the starch index of apples. *Food And Bioprocess Technology* 2(3), 308-314
- Naes T., Isaksson T., Fearn T. & Davies T., 2002. *A user-friendly guide to multivariate calibration and classification*. Chichester, UK: NIR Publications ISBN 0-9528666-2-5
- Nicolaï B.M., Beullens K., Bobelyn E., Peirs A., Saeys W., Theron K. I. & Lammertyna J., 2007. Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. *Postharvest Biology and Technology* 46, 99–118
- Nicolaï B.M., Verlinden B.E., Desmet M., Saevels S., Saeys W., Theron K., Cubeddu R., Pifferi A. & Torricelli A., 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
- Peirs A., Lammertyn J., Ooms K. & Nicolaï B.M., 2001. Prediction of the optimal picking date of different apple cultivars by means of VIS/NIR-spectroscopy. *Postharvest Biology & Technology* 21(2), 189-1991.
- Peirs A., Scheerlinck N., Touchant K. & Nicolaï B.M., 2002. Comparison of Fourier transform and dispersive near-infrared reflectancespectroscopy for apple measurements. *Biosystems Engineering* 81(1), 305-311
- Quilitzsch R. & Hoberg E., 2003. Fast determination of apple quality by spectroscopy in the near infrared. *Journal of Applied Botany and Food Quality* 77(5-6), 172-176

- Saevels S., Lammertyna J., Berna A.Z., Veraverbeke E.A., Di Natale C. & Nicolaï B.M., 2004. An electronic nose and a mass spectrometry-based electronic nose for assessing apple quality during shelf life. *Postharvest Biology and Technology* 31, 9-19
- Sinelli N., Casiraghi E., Tura D. & Downey G., 2008. Characterisation and classification of Italian virgin olive oils by near- and mid-infrared spectroscopy. *Journal of Near Infrared Spectroscopy* 16(3), 335-342
- Snowdon A.L., 2010. *Post-Harvest Diseases and Disorders of Fruits and Vegetables*; vol. 1, chapter 4. Manson Publishing Ltd; ISBN 9781840765984
- Tian H., Ying Y., Lu H., Fu X. & Yu H., 2007. Measurement of soluble solids content in watermelon by Vis/NIR diffuse transmittance technique. *Journal of Zhejiang University* 8(2), 105-110
- Uddin M., Okazaki E., Turza S., Yumiko Y., Tanaka M. & Fukuda Y., 2005. Non-destructive visible/NIR spectroscopy for differentiation of fresh and frozen-thawed fish. *Journal of Food Science* 70(8), 506-510
- Volz R.K., Biasi W.V., Grant J.A. & Mitcham E.J., 1998. Prediction of controlled atmosphere-induced flesh browning in 'Fuji' apple. *Postharvest Biology and Technology* 13, 97-10
- Watkins C.B., (2006). The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnology Advances* 24, 389-409
- Watkins C.B., Brookfield P.L. & Harker F.R., 1993. Development of maturity indices for the 'Fuji' apple cultivar in relation to watercore incidence. *Acta Horticultural* 326, 267-275
- Williams P. & Norris K., 2002. *Near-Infrared Technology in the Agricultural and Food Industries*. (2nd ed.). St. Paul: American Association of Cereal Chemist
- Wills R.T., Graham D., Mc Glasson W. B. & Joyce D., 1998. *Postharvest: an introduction to the physiology and handling of fruits, vegetables and ornamentals* (4th ed.). Sydney: UNSW Press Ltd

- Wold S., Sjöström M. & Eriksson L., 2001. PLS-regression: a basic tool of chemometrics. *Chemometrics and Intelligent Laboratory Systems* 58, 109-130
- Woolf A.B. & Ferguson I.B., 2000. Postharvest responses to high fruit temperatures in the field. *Postharvest Biology and Technology* 21, 7-20
- Yamada H. & Kobayashi S., 1999. Relationship between watercore and maturity or sorbitol in apples affected by preharvest fruit temperature. *Scientia Horticulturae* 80, 189-202
- Yan-de L. & Yi-bin Y., 2004. Measurement of sugar content in Fuji apples by FT-NIR spectroscopy. *Journal of Zhejiang University* 5(6), 651-655
- Zude M., Herold B., Roger J.M., Bellon-Maurel V. & Landahl S., 2006. Non-destructive tests on the prediction of apple fruit flesh firmness and soluble solids content on tree and in shelf life. *Journal of Food Engineering* 77(2), 254-260
- Zude-Sasse M., Truppel I. & Herold B., 2002. An approach to non destructive apple fruit chlorophyll determination. *Postharvest Biology and Technology* 25(2), 123-133

Section 2

Vis/NIR spectroscopy for quick evaluation of olive fruit ripeness

Roberto Beghi¹, Valentina Giovenzana¹, Raffaele Civelli¹, Enrico Cini², Riccardo Guidetti¹

¹Department of Agricultural and Environmental Sciences
Università degli Studi di Milano, via Celoria 2, Milano, 20133 Italy

²Department of Economics, Engineering, Science and Technology
Agriculture and Forestry, Università di Firenze, Piazzale delle Cascine
15, Firenze, 50144 Italy



Abstract

Increasing consumption of olive oil and table olives has recently determined an expansion of olive tree cultivation in the world. This trend is being fostered by the recognized nutritional value of the Mediterranean diet.

The aim of this work was to test a portable vis/NIR system for the analysis of ripening parameters (soluble sugar content SSC and texture) of olives before to be processed, both for fermentation process of table olives or for extraction process of olive oil. The final goal is to provide the sector with postharvest methods and sorting systems for a quick evaluation of olive fruits ripeness indices.

In the present study analyses were carried out on a total of 109 olives for oil. Olives spectra registered with the optical device and values obtained with destructive analysis in laboratory were analysed. Specific statistical models were elaborated to study their correlations and to evaluate ripening indices obtained through the analysis of the visible-near infrared range (400-1000 nm). Statistical models were processed thanks to chemometric techniques to extract maximum data information. Principal component analysis (PCA) was performed on vis/NIR spectra to examine sample groupings and identify outliers, while Partial Least Square (PLS) regression algorithm was used to correlate samples spectra and physical-chemical properties.

Results are encouraging: PCA showed a significant sample grouping for the different ripening stages. PLS models gave good predictive skills in validation for SSC ($R^2 = 0.67$ and $RMSECV\% = 7.5\%$) and texture ($R^2 = 0.68$ and $RMSECV\% = 8.2\%$).

Keywords: olives, vis/NIR technology, ripening parameters, chemometrics, post-harvest.

Introduction

Growing consumption of olive oil and table olives has recently determined an expansion of olive tree (*Olea europaea* L) cultivation in the world.

Increasing evidence suggests that monounsaturated fatty acids as a nutrient, olive oil as a food, and mediterranean diet as a food pattern are associated with a decreased risk of cardiovascular disease, obesity, metabolic syndrome, type 2 diabetes and hypertension (López-Miranda, Pérez-Jiménez, Ros, De Caterina, Badimón, Covas et al. 2010).

Ripening process control is essential, in fact, during ripening of olive oil fruits, biochemical processes occur: sugar content decreases with time, while the accumulation of oil increases in opposite trend (Cherubini, et al. 2009; Salas Sanchez Ramli, Manaf, Williams & Harood 2002). Moreover olives with a high sugar content may present oils with defects because of sugars fermentation during the production process. Therefore, sugar concentration may be considered an index able to define an appropriate level of olive ripening for processing (Cherubini, et al. 2009).

During ripening process, phenolic compounds decrease as a result of the activity of hydrolytic enzymes. Anthocyanins, belonging to phenol family, contribute to brown colouring typical of ripe fruits. Furthermore phenolic compound content is considered an important parameter in the evaluation of virgin olive oil quality because of phenols contribute to oil flavour and aroma. Phenols also, protect oil from autoxidation. In addition, olives processed at an overripened stage may result in unstable oil during shelf-life owing to the low phenolic compound content (Cherubini, et al. 2009).

During maturation, fruit weight increased. The flesh texture, related to the dry matter content, is a quality parameter for table olive fruits (Beltra, Del Rio, Sanchez, & Martinez, 2004).

Yousfi, K., Cert, R. M., & Garcia, J. M (2006) studied changes in quality and phenolic compounds of virgin olive oils during fruit maturation. They approved that fruit skin colour and firmness allowed a better discrimination at the initial maturity stages than the other methods tested (harvest date, amount of chlorophylls and carotenoids in the oil).

Studies consistently support the concept that olive ripening level, may affect the quality of the oil. In the same manner table olives quality is evaluated.

Marsilio, Campestre, Lanza, & De Angelis (2001) carried out an experimental investigation on olive fruits cultivars, to assess free sugar and polyol compositions and their changes during ripening and processing; Patumi, M., d'Andria, R., Marsilio, V., Fontanazza, G., Morelli, G. & Lanza, B. (2002) established of olive and olive oil quality, after intensive monocone olive growing in different irrigation regimes; Borzillo, Iannotta & Uccella (2000) evaluated quality of Ointoria tables olives during ripening and processing by biomolecular components.

Diaz, Gil, Serrano, Blasco, Moltò & Blasco (2004) compared three algorithms to classify the table olives in four quality categories by means of computer vision. Classification of table olives, according to their quality, was carried out after fermentation process.

Established methods for olive and oil quality assessment are generally based on either colourimetric or chromatography techniques such as HPLC. However, the difficult preparation of samples for this analysis requires a well equipped laboratory, as well as waiting 8 or 10 hours for results. A limited number of laboratories and lack of quick information mean that oil mills must begin the process of oilmaking without having such data available, reducing their chances to diversify production and achieve high quality products.

Therefore, there is a strong need in the modern oil industry for a simple, rapid, and easy to use method for objectively evaluating the quality of olives. A tool enabling real time analysis at the receiving station would allow preliminary decision making about olives during consignment thanks to the rapid analysis of quality parameters (i.e. SSC and texture) simultaneously.

Near infrared spectroscopy has attested to be one of the most efficient and advanced tools to monitor process and control product quality in food industry. It is widely utilized for rapid quality control of several products (Guidetti, Beghi, & Giovenzana 2012).

During fruit ripening, chlorophyll degradation is responsible for the degreening of the ground colour, which is a well-established ripeness

indicator for several species. In completely red-pigmented cultivars of fruits such as apples and peaches, this process is not visible, being masked by anthocyanins in the skin. Optical systems were developed to non-destructively assess the chlorophyll content in these fruits, to estimate ripeness, and to optimize harvesting and postharvest management (Bodria, Fiala, Guidetti & Oberti, 2004).

In literature some works about quality evaluation of olive oil using optical analysis are shown.

Conte, Brussolo, Pizzale, Carazzolo, Meurens & Pavan (2003) considered the application of NIR reflectance analysis to olive oil production quality control; Mailer in 2004 studied rapid evaluation of olive oil quality by NIR reflectance spectroscopy; Marquez, Díaz & Reguera (2005) used optical NIR sensor for on-line virgin olive oils characterization; Bendini, Cerretani, Di virgilio, Belloni, Bonoli-Carbognin & Lercker, (2007a) showed preliminary evaluation of the application of the FTIR spectroscopy to control the geographic origin and quality of virgin olive oils.

Mailer R.J. (2004) calibrated chemical factors, including free fatty acids, induction time, polyphenol content, and FA profiles, for NIR analysis. The results provide evidence of NIR analysis ability to measure most components rapidly and accurately.

Furthermore, this acquisition technique was proved to be suitable for a direct use to monitor quality parameters, SSC in particular obtaining good correlations (Beghi, Spinardi, Bodria, Mignani & Guidetti, 2012; Bodria, Fiala, Guidetti & Oberti, 2004).

With vis/NIR technique it was possible to estimate changes in the firmness and soluble solids sugar content of stored Red Delicious apples undergoing no detectable hue change in the skin (Bodria, Fiala, Guidetti & Oberti, 2004).

NIR and vis/NIR instrumentation must always be complemented with chemometric analysis to extract useful information present in the spectra (Guidetti, Beghi, & Giovenzana 2012). The most used chemometric techniques are the Principal Component Analysis (PCA) as a qualitative analysis of the data and Partial Last Square (PLS)

regression analysis to obtain quantitative prediction of the desired parameters (Nicolai et al., 2007; Cen & He, 2007).

Considering these aspects, the work investigated the capability of a portable and non-destructive optical system (vis/NIR spectroscopy), in combination with multivariate analysis, to distinguish olives based on colour changes of peel and pulp. In particular vis/NIR spectrophotometer was tested to investigate two of the characteristic parameters of olive fruit ripeness (SSC and texture).

In association with chemometric tools, such as Principal Component Analysis or Partial Least Squares methods, vibrational spectroscopy provides both qualitative and quantitative information (Beghi et al. 2010; Bendini et al. 2007). For both parameter (SSC and texture) dedicated chemometric models were created.

Materials and Methods

Sampling

The experimentation was carried out on 109 olives harvested in November and December 2011 by the experimental farm of Montepaldi in Florence (Tuscany, Italy). Olive fruits used in this study were *Moraiolo* and *Frantoio*, typical of the Tuscan hills, in the province of Florence.

Spectral acquisitions were carried on picked fruits, taken randomly in the bin. Samples of olives were classified in four colour classes. Harvested and classified berries were analysed in laboratory to determine qualitative parameters indicating ripening stage.

According to external colour, olives were divided in (Figure 1):

- Ripening stage 1 (**R1**): green sample with small size berries;
- Ripening stage 2 (**R2**): less than 50% black-pigmented samples with medium size berries;
- Ripening stage 3 (**R3**): 50% to completely black-pigmented;
- Ripening stage 4 (**R4**): berries completely coloured.



Figure 1. Image of the four selected olive classes

Spectra measurements were taken on individual berries along their equator region. Subsequently to the spectral acquisition of each olive, analysis of texture and of Soluble Solid Content (SSC) were done using a portable penetrometer (Durofel DFT 100 by Agro-Technologie, Forges les Eaux, France) and a portable refractometer (Pocket Refractometer PAL-1 by ATAGO, Itabashi-ku, Tokyo, Japan). For SSC analysis, fruits were destoned and the flesh (pulp) was crushed.

Spectral data and destructive reference analysis were used for the elaboration of chemometric predictive models.

Vis/NIR device

Spectral acquisitions were realized on samples using an optical portable system (JAZ vis/NIR spectrophotometer by OceanOptics, USA) operating in the wavelength range 340-1000 nm. The JAZ equipment is composed of five components: a) vis/NIR lighting system; b) fiber optic probe for reflection measurement; c) spectrophotometer; d) hardware for data acquisition and instrument control; e) battery for power supplying.

Spectra were acquired in reflectance: light radiation was guided to the sample through a Y-shaped, bidirectional fiber optic probe (OceanOptics, USA). Y-shaped fiber allowed to guide light from halogen

lamp to illuminate the sample while to collect simultaneously the radiation coming from the fruit and guide it back to the spectrophotometer. The tip of the optical probe was equipped with a soft plastic cap to ensure contact with sample skin during measurements, while minimizing environmental light interference (Figure 2).

The integrated spectrophotometer was equipped with a diffractive grating for spectral measurements optimized in the range 340-1000 nm and a CCD sensor with a 2048 pixel matrix, corresponding to a nominal resolution of 0.3 nm.

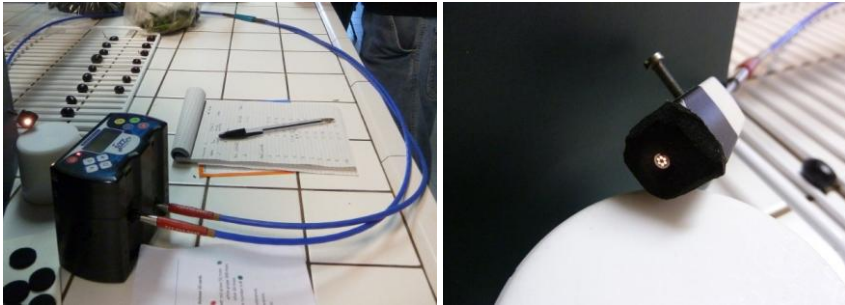


Figure 2. Image of the JAZ vis/NIR system and particular of reflectance probe

Data processing

Chemometric analysis of spectra was based on Unscrambler 9.8 software package (CAMO ASA, Norway). Moving-averaged smoothed spectra were used for Principal Components Analysis (PCA) to explore possible clustering of homogeneous samples spectra belonging to the same shelf-life class (Massart, Vandeginste, Buydens, De Jong, Lewi & Smeyers-Verbek, 1997; Naes, Isaksson, Fearn & Davies, 2002).

Different treatments were applied to the Vis/NIR spectra, namely multiplicative scatter correction (MSC) and derivatives, before building the calibration models. The first and second derivatives were performed using Savitzky-Golay transformation and smoothing (15 point and second order filtering).

All samples available were used for the creation of a chemometric regression model for each parameter considered. The vis/NIR spectra were correlated with ripeness parameters (SSC and texture) using the partial least square (PLS) regression algorithm.

To evaluate model accuracy, the statistics used were the coefficient of determination in calibration (R^2_{cal}), coefficient of determination in cross-validation (R^2_{cv}), root mean square error of calibration (RMSEC), and root mean square error of cross-validation (RMSECV). The best calibrations were selected based on minimizing the RMSECV. Percent errors of cross-validation (RMSECV%) were also calculated as: $RMSECV (\%) = RMSECV / \text{averaged reference values of each parameter}$.

Results and discussion

PCA analysis

Figure 3 shows spectra of the 4 analysed classes. A very different trend could be noticed among classes especially in the area of the visible region (400-700 nm), as expected. The main peak is detectable at 680 nm, corresponding to the absorption peak of chlorophyll.

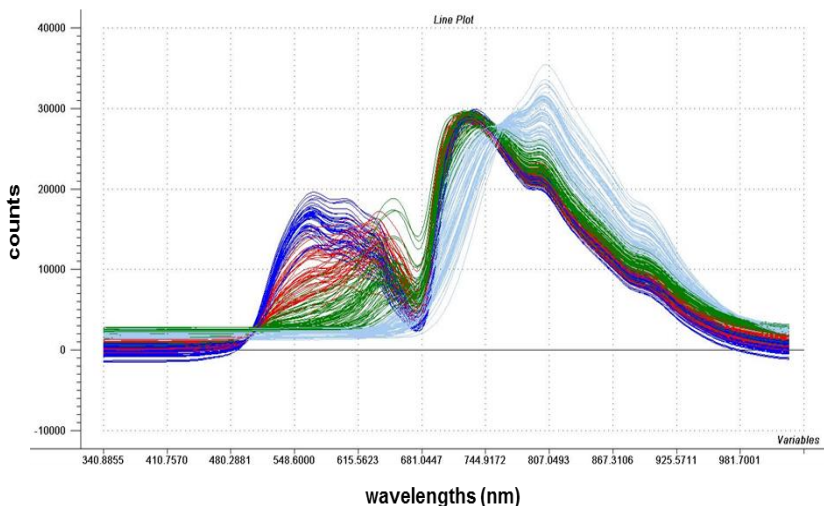


Figure 3. Spectra of the four analysed classes (R1, blue; R2, red; R3, green; R4, azure)

In Figure 4 PCA loadings plot is shown. The loadings plot shows the weight of the different variables (in our case the different wavelengths of the spectrum). The 99% of the total data variance is explained by the

first three principal components (PCs). In particular, PC1 explained 84% of the variability, PC2 slightly more than 11% and 4% for PC3.

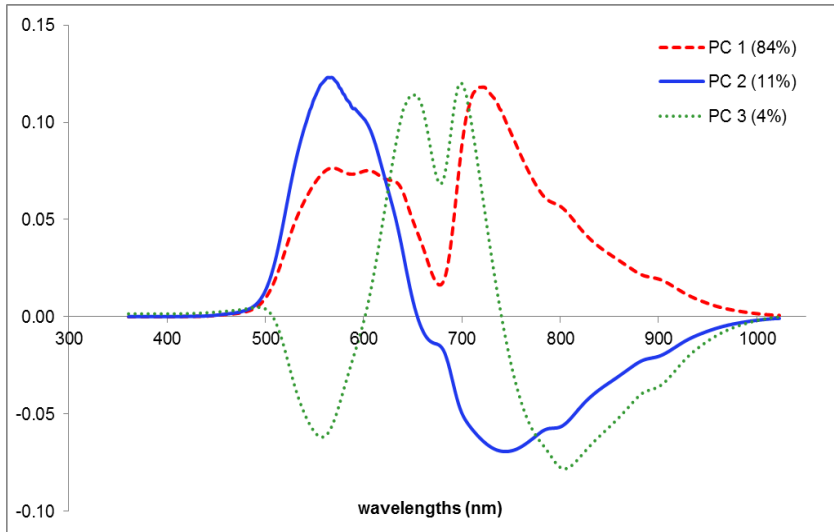


Figure 4. PCA Loadings plot for the first three PCs

Figure 5 and 6 show the PCA scores plot. These graphs show the evolution of the samples in the plane of the PCs, PC1 vs. PC2 (Figure 5) and PC1 vs. PC3 (Figure 6).

An excellent separation among the four analysed classes can be noticed. Some R3 samples appear to be similar to the black olives (R4); this is possible because some R3 olives were border line respect to R4 and not easy to distinguish from them.

Moreover, PC3, despite it explains only 4% of the total variance, associated to PC1 allows an optimum separation between R3 and R4. This behavior is in line with loadings trend which shows high values for the PC3 in the band around 680 nm respect to PC2 (chlorophyll absorption band and classical band used for the monitoring of fruit and vegetables ripening).

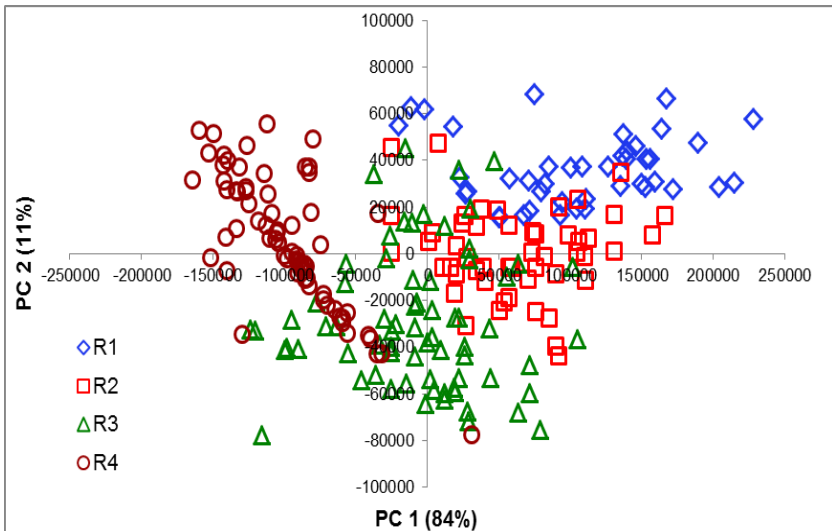


Figure 5. PCA Scores plot PC1 vs PC2

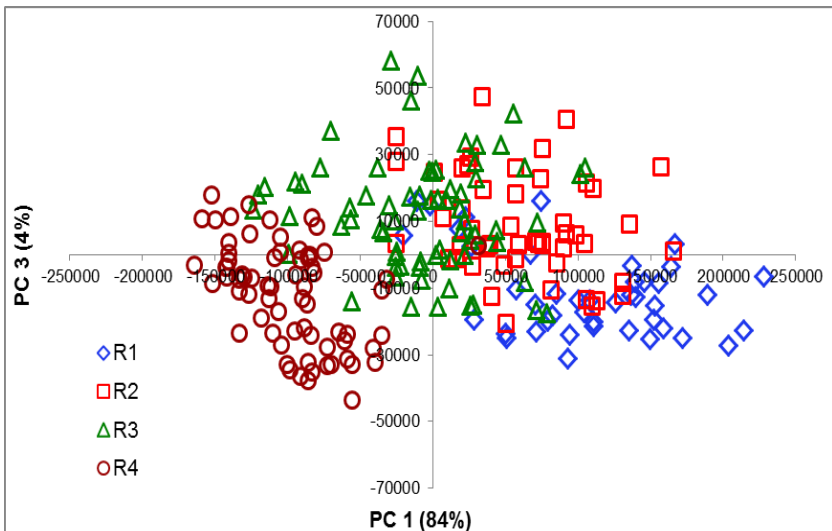


Figure 6. PCA Scores plot PC1 vs PC3

PLS analysis

In Table 1 the results for the PLS regression models for the prediction of SSC and texture are shown.

Table 1. Results of PLS models for the prediction of SSC and texture. R^2 , coefficient of determination; RMSEC, standard error of calibration; RMSECV, standard error of cross validation; LV, latent variables; MSC, multiplicative scatter correction; der2, second derivative of the spectrum.

Qualitative indices	Data pretreatment	N of samples	Calibration			Cross-validation		
			R^2	RMSEC	LV	R^2	RMSECV	RMSECV%
SSC (°Brix)	Smoothing + MSC + der2	80	0.68	1.36	1	0.67	1.40	7.5%
Texture (A.U.)	Smoothing + MSC + der2	88	0.75	4.92	7	0.68	5.62	8.2%

The obtained results are encouraging. In this preliminary study the data set available was not particularly wide, so results could be improved with more samples for the elaboration. Regarding SSC, the performance of the regression model are improvable although model evaluation parameters are not particularly different from results reported in literature for the estimation of this parameter on other fruits (estimation error of 7.5%).

Interesting results were obtained in particular for the prediction of berries texture. The possibility to use the reference data on single berry has allowed to obtain good results for the estimation of a parameter usually difficult to predict, as the texture of a fruit in an optical non-destructive way. This difficulty is usually due to several factors: first, the extreme variability of this parameter among berries, the high instrumental error of penetrometer and, finally, the difficulty of calibrating a model for the estimation of an index not directly associable with a chemical species (and consequently the absorption bands of those chemical bonds).

These reasons usually lead to poor predictive capabilities of the model for this parameter. In this case, however, results are certainly positive, with R^2 about 0.7 in validation and RMSECV% less than 10%.

Conclusions

This work studied the applicability of vis/NIR spectroscopy as a rapid technique for the analysis of the quality of olives directly to the mill just before oil extraction process. In particular a portable vis/NIR device was tested to estimate two quality parameters, SSC and texture of olive berries.

Through the elaboration of prediction models based on multivariate regression techniques, the prediction skills of the system for both parameters were evaluated. The obtained preliminary results were encouraging, especially regarding the texture estimation.

A continuation of the study during the year 2012 is necessary to confirm these early results. In this way the number of samples of the data set available, and consequently the robustness of prediction models will be increased.

For the future a more detailed study of olives spectra could be done with the aim of investigate spectral modifications at particular wavelength bands in order to highlight and select the most informative. In this way a more simple and inexpensive device could be realized to monitor ripeness degree and classify olives entering the mill, for a better management of oil production process.

Acknowledgements

This study received financial support from European Social Fund for a Post-doctoral Research Fellowship (“Progetto Dote Ricerca”).

References

- Beghi, R.; Spinardi, A.; Bodria, L., Mignani, I. & Guidetti, R., 2012. Apples nutraceutic properties evaluation through a visible and near-infrared portable system. *Food and Bioprocess Technology*, DOI 10.1007/s11947-012-0824-7
- Beltra, G.; Del Rio, C.; Sanchez, S. & Martinez, L., 2004. Seasonal changes in olive fruit characteristics and oil accumulation during ripening process. *Journal of the Science of Food and Agriculture*, 84, 1783-1790
- Bendini, A.; Cerretani, L.; Di virgilio, F.; Belloni, P.; Bonoli-Carbognin M. & Lercker, G., 2007a. Preliminary evaluation of the application of the ftir spectroscopy to control the geographic origin and quality of virgin olive oils. *Journal of Food* , 30, 424-437
- Bendini, A.; Cerretani, L.; Di virgilio, F.; Belloni, P.; Bonoli-Carbognin, M.; Lercker, G. & Toschi, T.G., 2007b. In-process monitoring in industrial olive mill by means of FT-NIR. *European Journal of Lipid Science and Technology*, 109, 498-504
- Bodria, L.; Fiala, M.; Guidetti, R. & Oberti, R., 2004. Optical techniques to estimate the ripeness of red-pigmented fruits. *Trans. of ASAE*, 47, 815-820
- Borzillo, A.; Iannotta, N. & Uccella N., 2000. Ointoria tables olives: quality evaluation during ripening and processing by biomolecular components. *European Food Research and Technology*, 212, 113-121
- Cen, H. & He, Y., 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. *Trends in Food Science & Technology*, 18, 72-83.
- Cherubini, C.; Migliorini, M.; Mugelli, M.; Viti, P.; Berti, A.; Cini, E. & Zanoni, B., 2009. Towards a technological ripening index for olive oil fruits. *Journal of the Science of Food and Agriculture*, 89, 671-682
- Conte L. S., Brussolo G., Pizzale L., Carazzolo A., Meurens M. & Pavan O., 2003. Application of near infrared reflectance analysis to olive oil

- production quality control. *Rivista Italiana Sostanze Grasse* 80, 213-217
- Diaz, R., Gil, L., Serrano, C., Blasco, M., Moltò, E. & Blasco J., 2004. Comparison of three algorithms in the classification of table olives by means of computer vision. *Journal of Food Engineering* 61, 101-107
- Guidetti, R., Beghi, R. & Bodria, L., 2010. Evaluation of grape quality parameters by a simple vis/NIR system. *Transactions of the ASABE*, 53(2), 477-484
- Guidetti, R., Beghi, R. & Giovenzana, V., 2012. Chemometrics in Food Technology. In *Chemometrics*. InTech: E-Publishing, ISBN: 978-953-51-0438-4. Chapter 10, 217-252
- López-Miranda, J.; Pérez-Jiménez, F.; Ros, E.; De Caterina, R.; Badimón, L.; Covas, M.I. et al., 2010. Olive oil and health. Summary of the II international conference on olive oil and health consensus report, Jaén and Córdoba (Spain) 2008. *Nutrition, Metabolism & Cardiovascular Diseases*, 20, 284-294
- Mailer R.J., 2004. Rapid evaluation of olive oil quality by NIR reflectance spectroscopy. *Journal of the American Oil Chemists' Society*, 81, 823-827
- Marquez A.J.; Díaz A.M. & Reguera M.I.P., 2005. Using optical NIR sensor for on-line virgin olive oils characterization. *Sensors and Actuators B: Chemical*, 107, 64-68.
- Marsilio, V.; Campestre, C.; Lanza, B. & De Angelis, M., 2001. Sugar and polyol compositions of some European olive fruit varieties (*Olea europaea* L.) suitable for table olive purposes. *Food Chemistry*, 72, 485-490
- Massart, D.L.; Vandeginste, B.G.M.; Buydens, L.M.C.; De Jong, S.; Lewi P.J. & Smeyers-Verbeke, J., 1997. *Handbook of Chemometrics and Qualimetrics: Part A*. Elsevier, ISBN: 0-444-89724-0, included in series: *Data Handling in Science and Technology*
- Naes, T.; Isaksson, T.; Fearn, T. & Davies, T., 2002. *A user-friendly guide to multivariate calibration and classification*. Chichester, UK: NIR Publications ISBN 0-9528666-2-5

- Nicolai, B. M.; Beullens, K.; Bobelyn, E.; Peirs, A.; Saeys, W.; Theron K. I., & Lammertyna J., 2007. Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. *Postharvest Biology and Technology*, 46, 99–118
- Patumi, M.; d'Andria, R.; Marsilio, V.; Fontanazza, G.; Morelli, G. & Lanza, B., 2002. Olive and olive oil quality after intensive monocone olive growing (*Olea europaea* L., cv. Kalamata) in different irrigation regimes. *Food Chemistry*, 77, 27-34
- Salas, J.J.; Sanchez, J.; Ramli, U.S.; Manaf, A.M.; Williams, M. & Harood, J.L., 2002. Biochemistry of lipid metabolism in olive and other oil fruit. *Progress in Lipid Research* 39, 151-180
- Yousfi, K.; Cert, R.M.; & Garcia, J.M., 2006. Changes in quality and phenolic compounds of virgin olive oils during objectively described fruit maturation. *European Food Research and Technology*, 223, 117-124

Section 3

Application of vis/NIR spectroscopy for a quick quality evaluation of craft beers

Valentina Giovenzana, Roberto Beghi, Riccardo Guidetti

Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy. Università degli Studi di Milano. Milano, Italy



Abstract

The recent rediscovery of original and genuine products deeply linked to the territory has allowed an increase in the consumption of craft beer in Italy. In beer production sector quick checks with the use of non-destructive analysis can be widely applied. This is particularly true for craft beer monitoring during fermentation process. Currently microbreweries base qualitative check mainly on Soluble Solids Content (SSC, °Plato) and pH evaluation. These parameters are the criteria used to define trend and the end of fermentation time. Therefore, they are really essential for a successful production of the craft beer.

The present work aims to carry out a preliminary study to verify the possibility of employing an optical, portable and inexpensive non-destructive device, based on visible and near infrared spectroscopy (vis/NIR), directly on the production line of craft beer.

Three types of craft beer were analysed: Montestella (straw, type Helles), Sant'Ambroeus (amber, type Belgian Strong Golden Ale) and Lambrate (copper, type Bock). For each type of craft beer, transmittance spectra were acquired in wavelengths range 450–980 nm and at different stages of fermentation (maximum 12 days of fermentation). Spectral sampling, for each craft beer was conducted in two different condition after the yeast addition: filtered (F) and non-filtered samples (NF). Filtration is necessary to prepare a matrix suitable for laboratory analyses.

The vis/NIR device was tested for the quick evaluation of consolidated parameters (SSC and pH) which were analyzed at each sampling time for each beer sample. Spectra were elaborated in order to perform qualitative analysis (PCA) and to build Partial Least Square (PLS) regression models. PCA results showed how vis/NIR spectroscopy could be effective in discriminating between non-filtered (condition in the process line) and filtered samples. PLS models can be considered good both for the prediction of SSC and pH, with R^2 in cross-validation ranged from 0.61 to 0.82 for SSC and from 0.73 to 0.89 for pH value. Generally, among the different types of craft beer analyzed, better models were elaborated for Lambrate craft beer.

Keywords: vis/NIR spectroscopy, craft beer, quick quality evaluation.

Introduction

A quality beer is produced using malt, hops, water and yeast, which are dosed during an extended production process. Craft beer is a non-filtered and not pasteurized product that maintains unaltered its sensorial characteristics. When compared to industrial beers, craft beer is more subjected to microbial contamination that may be cause of spoilage (turbidity, acidification and production of undesired aromatic compounds). In particular fermentation is one of the most delicate phases of beer production process. In fact, in this step take place, by means of yeast, conversion of sugar into alcohol and development of many secondary fermentation compounds, which are important to determine the flavor profile of product (esters, higher alcohols, sulphur compounds, organic acids, aldehydes and ketones).

Moreover due to absence of pasteurization and microfiltration in craft beer brewing yeast remains in the product: for this reason craft beer remains "alive" and evolves over time. These phenomena justify the need of quality measurements during the whole process and not only on finished beer. Monitoring have to be made on raw materials and in individual process stages.

In industrial process, brewers usually install sensors (hydrometer, oxygen sensor, gas chromatography, hazemeter, spectrophotometer) which enable them to make their measurements automatically, with associated control system responding to the measured values that adjust a relevant parameters for the process control (Bamforth, 2003). For instance, temperature is easily measured remotely during fermentation and, if it rise, may be automatically lowered by triggering the circulation of coolant through the jackets of the fermenters. Small scale breweries have lower investment capacity than industry even if, maintaining, at least, the same requirement of process control. In literature, parameters usually monitored in microbreweries during the fermentation are pH (Lachenmeier, 2007 and Almonacid et al., 2012) and Soluble Solids Content (SSC) or optical density (OD) (Lachenmeier, 2007; Liu et al. 2009; McLeod et al. 2009 and Almonacid et al., 2012). Another parameter often controlled, is the numbers of cells in suspension (dry cell weight, DCW) (McLeod et al. 2009 and Almonacid et al., 2012) that increase while yeast grows, but thereafter, the yeast

count falls, because cells flocculate and leave the body of the beer. During fermentation SSC decreases due to transformation of sugars into ethanol. This phenomena should be monitored also through specific gravity: sugar have much higher specific gravity than water and ethanol has lower specific gravity than water. pH falls during fermentation. Bitterness units, ethanol content, EBC (European Brewing Convention) color and lactic acid are others factors usable to control beer production, considered by Lachenmeier, (2007). Ethanol content was also analysed by McLeod et al. (2009) and Almonacid et al. (2012) to monitoring beer fermentation and by Engelhard et al. (2004) using interpretive Near-Infrared Spectroscopy.

Beers may be divided into ales, lagers and stouts, differentiated according to the type of fermentation that may be high fermentation (using *Saccharomyces cerevisiae*, range temperature 18-22°C) or low fermentation (using *Saccharomyces carlsbergensis*, range temperature 6-15°C) (Bamforth, 2003).

The recent rediscovery of original and genuine products deeply linked to the territory led to an increase in consumption of craft beer in Italy. This reflected in a considerable enhance of the production (from 16000 hl/year in 2005 to 290000 hl/years in 2009) and of the number of small scale breweries. Microbreweries increased by 50% from 2005 to 2010.

Currently, problems related to small quantity available and high price of craft beers result in a difficulty of sale craft beers. Moreover, variability of quality level of craft beer avoid the possibility of replacing industrial beer in consumers uses. However, passion and effectiveness showed in these years by many microbreweries, attract a growing number of persons who became keen on craft beer and this contributes to its spread. Standardize the quality of craft beer at high level is a requirement also for small companies, as microbreweries. To do this, however, a cheap instrumentation and easy to use is desired by producers.

Optical non-destructive analysis and in particular NIR and vis/NIR spectroscopy, therefore, may be considered a valid method for the qualitative and quantitative analysis of many food products (Guidetti et al., 2012) and, among them, also the craft beer. This optical technique was proposed as a viable alternative to classical analytical methods.

Traditional method means destructive analysis performed in laboratory, requires time, professional skills of operators and use of reagents. In addition, vis/NIR spectroscopy is a cheap technology, rapid and effective also in reducing the waste of material for laboratory analysis.

Finally, the optical technology may be applied directly online, providing data in real time, during fermentation process. Applications of vis/NIR spectroscopy are already present in literature concerning evaluation of grapes ripening (Guidetti et al., 2010) and monitoring nutraceutic properties of apples (Beghi et al., 2012). Liu et al. (2009) and Ghasemi-Varnamkhasi et al. (2012) proposed two works about application of optical techniques on beer industry. The first study investigated the screening analysis of beer ageing using near infrared spectroscopy; the second one, carried out correlation modellings between soluble solids content and visible/near infrared spectra. Kirsanov et al. (2010) studied some different beer types like dark, alcohol-free, strong, yeast-containing and filtered white beer. All the samples were analyzed with a number of different spectroscopic techniques (ATR-IR, NIR, Vis and UV). The analyses covered standard characterization of beer quality attributes (extractivity, alcohol content, colour, pH, foam resistance, turbidity, concentrations of bitter-forming components). Madsen and Esbensen (2010) presented results from a comparison study among FT-NIR, FT-MIR and Raman laser spectroscopy applied on the same set of samples obtained from a pilot-scale beer brewing process. Quantitative PLS1 models were elaborated for the most interesting parameters: ethanol, maltose, and total sugar.

This work aims to evaluate the feasibility of using an optical non-destructive, portable and inexpensive device, based on spectroscopy of the visible and near infrared (450 - 980 nm), for quick evaluation of qualitative parameters (pH and soluble solids content, measured in °Plato) in three types of craft beer (pale, amber and brown). The system studied in this work is a more simple and versatile device compared to bench top NIR instruments. These are interesting features especially in small scale breweries with low potential for investment but with a strong need to process control, because they work with highly variable and non-standardized process compared to industrial breweries.

The final objective is to evaluate the possibility of on-line application of similar optical instrumentation for the real time monitoring of trend of craft beer fermentation, qualitative and quantitative analyses. Qualitative analysis has been provided to discriminate craft beer samples according to their optical properties. Instead, quantitative analysis has been developed to predict SSC and pH values, principal parameters used in microbreweries to evaluate the end of fermentation processes.

Materials and methods

Sampling

Sampling of craft beers covered nine months, from November 2010 to July 2011, and was done in collaboration with Birrificio Lambrate, the bigger craft microbrewery in Milan, Italy. Color and features of the three types of craft beer analyzed are shown in Figure 1: Montestella (straw, style Helles), Sant'Ambroeus (amber, style Belgian Strong Golden Ale) and Lambrate (copper, style Bock).



Figure 1: The three types of craft beer analyzed. From left: *Montestella* (straw, style Helles), *Sant'Ambroeus* (amber, style Belgian Strong Golden Ale) and *Lambrate* (copper, style Bock)

TABLE 1 reported the main characteristics of studied craft beers.

TABLE 1: Main characteristics of the three craft beers

Craft beer	Type	Fermentation	Alcoholic strength (% by vol.)	Specific gravity (° Plato)	Color	Style
<i>Montestella</i>	lager	15-25°C	4.9	12	straw gold	Helles
<i>Sant'Ambroeus</i>	ale	high (18-22°C)	7.1	16	amber	Belgian Strong Golden Ale
<i>Lambrate</i>	lager	low (6-10°C)	6.8	16	copper	Bock

For each craft beer, a different number of samples was analyzed. This because fermentation process time changes in function of craft beer type. In particular samples, during fermentation, were taken at different times (18, 24, 48...h, maximum of fermentation process time is 12 days). At the same time samples "as is", non-filtered, named NF, and filtrated ones, before spectral acquisition, called F, were analyzed.

Experimental plan

For each craft beer, three spectra were acquired in transreflectance, in the wavelengths range 450-980 nm, at different fermentation stages. In transreflectance mode, radiation from optical fiber passes through the sample, hits the reference (100%) and returns to the probe. The transreflected component of the light is then read by the spectrophotometer of the vis/NIR system. Analysis were conducted on wort with the addition of yeast (necessary for beer fermentation) then on filtrated and non-filtrated samples (the filtration is necessary for qualitative analysis in laboratory).

Spectra measurements were taken in the laboratory on samples in quartz cuvettes (1 ml) after artificial illumination with a probe tip (Figure 2).

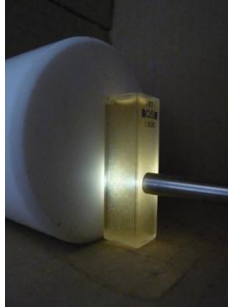


FIGURE 2: Detail of spectral acquisition on a pale craft beer sample. Transreflectance technique was used

Since spectral measurements can be affected by possible influence of environmental conditions especially related to diurnal change of sunlight, spectral acquisitions were done in a darkened zone to standardize as much as possible lighting condition. Tests conducted at different times of the day, confirmed the repeatability of measurements allowed by the artificial lighting which were sufficient to minimize the possible influence of ambient illumination on samples. Possible influence of temperature changes was limited by freezing craft beer samples just taken from line process and thawed at 4°C. In fact, each craft beer has different optimal conditions of brewing, including temperature.

A total of 205 craft beer samples were evaluated (three types of craft beer, samples F and NF), 615 spectra were acquired, three repetitions for each sample (sampling times on fermentation process were variable, due to needs of different kind of craft beer).

For each sampling date, at the microbrewery, chemical analysis of qualitative parameters were carried out. SSC was measured using a portable digital refractometer (model PR-32, Atago, Tokyo, Japan) and reported in °Plato, and pH was measured with a pH-meter (model HI 2211-02, Hanna Instruments, Milan, Italy).

Chemical analyses were performed only on filtered material (as standard protocol), then the value of the chemical parameter used for spectral correlation was the same for samples NF and F (100 samples).

Portable vis/NIR device

For spectral acquisitions, a portable system based on vis/NIR technology in the range (450–980 nm) was used. In this system, samples were hit by radiation produced by a lighting system, and the transflected component was measured by a spectrophotometer and registered through dedicated software (Figure 2). The system was composed of five elements (Guidetti et al. 2010): a lighting system, a fiber optic probe, a portable spectrophotometer, a PC for data acquisition and instrument control, and a battery for power supplying.

Craft beer samples were illuminated by the radiation produced by the lighting system, and the back-scattered component spectrum was measured by a spectrophotometer and saved in a notebook PC by a dedicated software.

The light source was a 50 W halogen lamp (Decostar Coolblue, Osram, Munich, Germany) with a peak in the emission spectrum at 500 nm. The light source was embedded in a metal holder fitting the optical fiber with a SMA connector, specifically designed to have the halogen spotlight focused on the illuminating optical fiber.

Light radiation was shone on the craft beer sample through a Y shaped, bidirectional fiber optic probe (FCR-19IR200-2-ME-S1, Avantes, The Netherlands). Among the fibers available on the market, a model with a large diameter to carry as much light as possible was chosen. It allowed the light radiation produced by the halogen lamp to illuminate the sample while simultaneously collecting the radiation coming from the sample and transferring it to the spectrophotometer. The tip of the optical probe was equipped with a soft plastic cap to ensure the contact with sample during measurements, while avoiding environmental light interference.

The fiber optic probe was connected to a portable spectrophotometer (AvaSpec-2048, Avantes, The Netherlands). The spectrophotometer was equipped with a diffractive grating for spectral measurements optimized in the range 445-970 nm and a CCD sensor with a 2048 pixel matrix, corresponding to a nominal resolution of 0.3 nm.

The system was controlled by a portable PC with dedicated software for data acquisition, pre-processing and logging, as well as for automatic

control of instrument and illumination. In this way, samples were illuminated only for the time necessary for acquisition of the spectrum, thereby avoiding wasting energy, for longer autonomy of system. The system was powered by a 12 V battery, and all components were housed in a backpack.

Data processing

Chemometric analysis was performed using The Unscrambler software package (version 9.8, CAMO ASA, Oslo, Norway). Moving Average smoothing and second derivative treatments were applied to vis/NIR spectra aiming at reducing noise before building the calibration models. Second derivatives were performed using Norris Gap transformation. Principal component analysis (PCA) was performed on vis/NIR spectra to examine samples grouping and to identify outliers.

The vis/NIR spectra were correlated with quality parameters (SSC and pH) using Partial Least Square (PLS) regression algorithm. To evaluate model accuracy, the coefficient of determination in calibration (R^2_{cal}), the root mean standard error of calibration (RMSEC), the coefficient of determination in cross-validation (R^2_{cv}), and the root mean standard error of cross-validation (RMSECV) were applied. Cross-validation is an internal validation method, usually used in case of a small number of samples available for regression. With cross-validation some samples are kept out of the calibration and used for prediction. This is repeated until all samples have been kept out once. In this case leave-one-out cross-validation was used, so only one sample at a time is kept out of the calibration. The optimum calibrations were selected based on minimizing the RMSECV. PLS models were calibrated for each craft beer separately.

Results and Discussion

The three beers, depending on type (Montestella, Sant'Ambroeus and Lambrate), have different fermentation times. Table 2 shows the descriptive statistics results, applied to chemical analysis. For each craft beer analysed, SSC and pH parameters were evaluated, on the basis of at least three repetitions, for each analysis time. Whereas craft beer process is less standardized than industry beer brewing, fermentation time could change (few hours), regarding the same type of craft beer.

Considering the natural variability of craft beer brewing, good repeatability of SSC and pH values, during fermentation, was observed. Sant'Ambroeus is the craft beer most variable both in terms of SSC and pH values.

TABLE 2: Descriptive statistics applied to chemical analysis (SSC and pH) of craft beers analysed at different fermentation times

Kind of beer	Fermentation time (h)	SSC (° Plato)					pH			
		N	Mean	SD	Min	Max	Mean	SD	Min	Max
<i>Montestella</i> (straw, type Helles)	0	6	11.22	0.23	10.9	11.6	5.23	0.04	5.2	5.3
	18	6	10.40	0.28	9.9	10.7	4.98	0.12	4.9	5.2
	48	4	5.78	0.71	5.2	6.9	4.37	0.05	4.3	4.5
	72	3	3.17	0.26	3.0	3.5	4.28	0.01	4.3	4.3
<i>Sant'Ambroeus</i> (amber, type Belgian Strong Golden Ale)	0	9	15.79	0.26	15.3	16.3	5.22	0.03	5.2	5.3
	18	8	13.90	1.80	11.0	15.9	4.83	0.22	4.5	5.1
	48	5	8.56	3.99	4.2	14.3	4.48	0.21	4.3	4.8
	72	4	4.75	1.30	3.2	6.3	4.28	0.02	4.3	4.3
<i>Lambrate</i> (copper, type Bock)	144	3	4.93	2.38	3.2	8.0	4.35	0.06	4.3	4.4
	0	4	15.82	0.25	15.4	16.2	5.23	0.04	5.2	5.3
	18	4	15.23	0.23	14.9	15.5	5.11	0.05	5.1	5.2
	120	3	10.80	1.08	10.1	12.2	4.69	0.03	4.7	4.7
	144	3	9.20	0.47	8.8	9.8	4.58	0.04	4.5	4.6
	288	3	3.45	0.06	3.4	3.5	4.46	0.12	4.4	4.6

Comparing SSC parameter of the three craft beers it is possible to see, already after 18 hours, changes (*Montestella*, 10.4 °Plato; *Sant'Ambroeus* 13.9 °Plato and *Lambrate* 15.23 °Plato). Each craft beer decreases respectively of 7, 12 and 4 % of °Plato from, before fermentation (t_0), and after 18 hours to started process.

Figure 3, 4 and 5 show results of spectra pretreatments. In figure 3 noisy row spectra of craft beer samples are shown, in picture 4 the same spectra after smoothing treatment: peaks are noise-free for a

better instrumental performance. In Figure 5, instead, spectra after smoothing and second derivative treatments are presented.

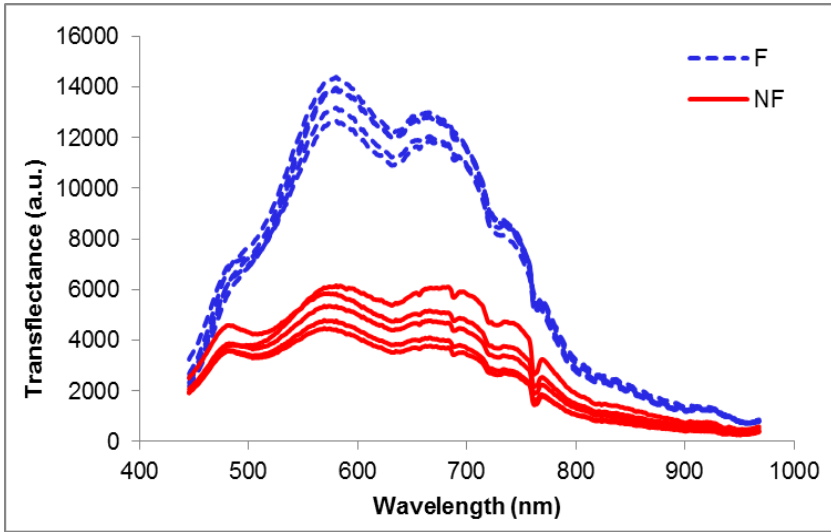


FIGURE 3: Examples of spectra of *Sant'Ambroeus* samples: row spectra. F are filtered samples and NF non-filtered samples

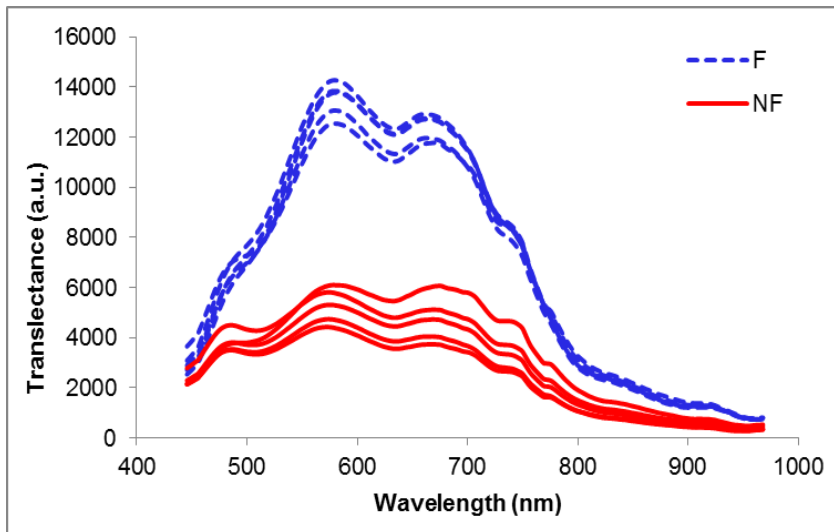


FIGURE 4: Examples of spectra of *Sant'Ambroeus* samples: smoothed spectra. F are filtered samples and NF non-filtered samples

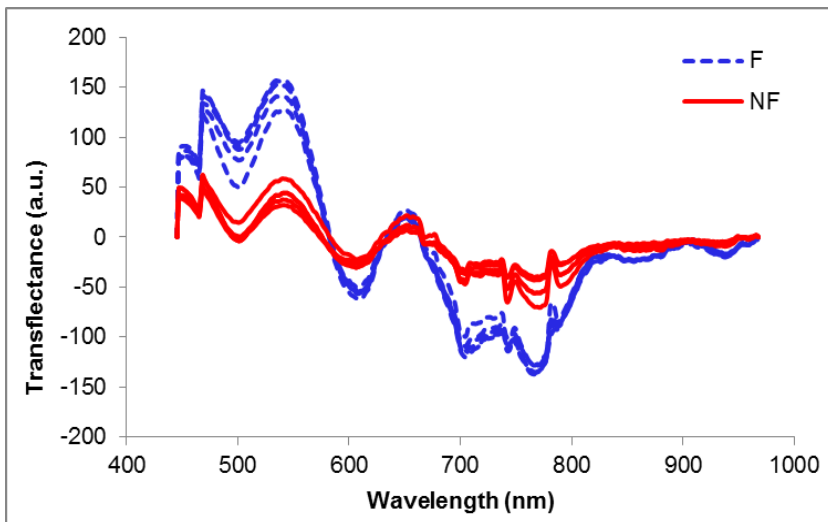


Figure 5: Examples of spectra of *Sant'Ambroeus* samples: spectra after smoothing and second derivative treatments. F are filtered samples and NF non-filtered samples

Evaluating the spectra of the three craft beer collected (Figure 6) changes in the visible region spectra between 500 and 600 nm were observable. This is due to the different pigmentation of each beer. The transfectance pattern of Lambrate craft beer (L) is characterized by a generally lower transfectance throughout the spectrum. This is due to the darkest color, and so, to the greatest capacity for absorption in this part of the visible region compared to the other craft beers.

The water content of beer is around 93% (INRAN, 2012) and is noticeable a small in-depth peak at 760 nm (Figure 6), caused by the third overtone of OH stretching (Clement, et al., 2008; Bertrand, 2000). This absorption band is more evident in figures 4 and 6. In fact, by using the derivative spectra, is possible to remove overlapping peaks and correct the baseline. The derivative brings the overlapping peaks apart and the linear background becomes to a constant level in the first derivative spectrum and zero in the second derivative spectrum (Osborne et al., 1993).

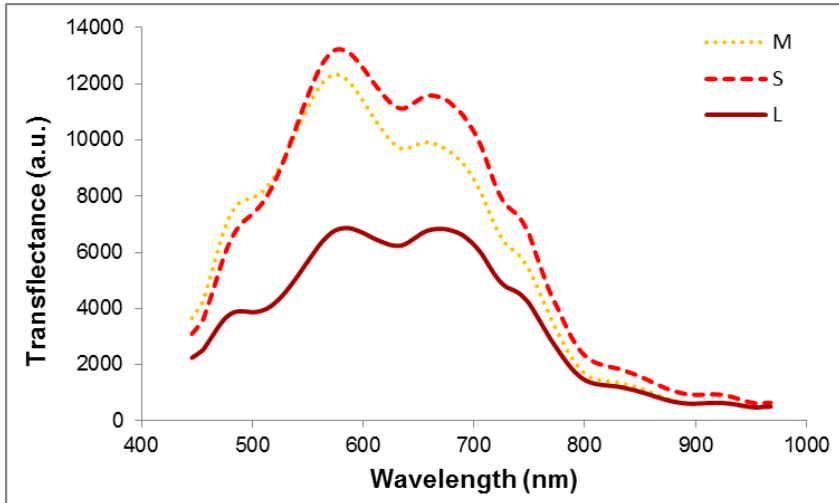


FIGURE 6: Averaged spectra of filtered *Montestella* (M), *Sant'Ambroeus* (S), and *Lambrate* (L) samples. All spectra are referred to fermentation time t_0

PCA discriminates craft beer samples according to their optical properties. In particular, samples spectra of F and NF may be clearly distinguished. Figure 7 shows a good separation along the first component (PC1) between F and NF samples.

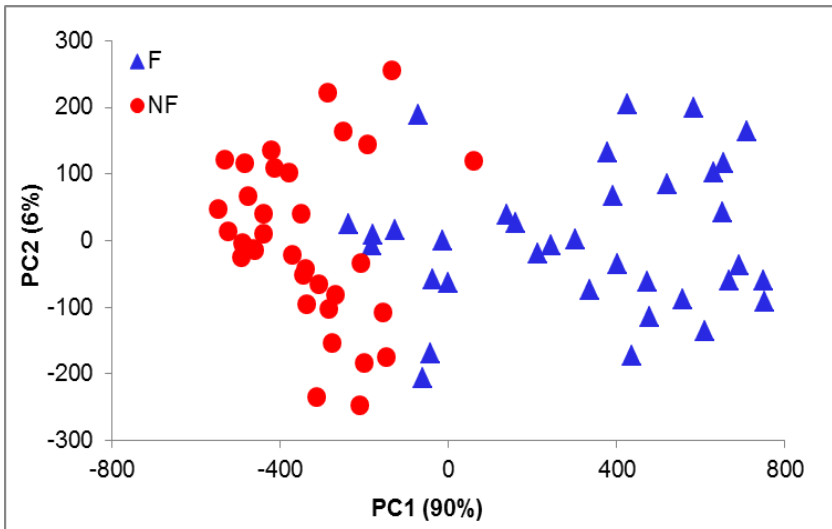


FIGURE 7: PCA of Sant'Ambroeus spectra. F are filtered samples and NF non-filtered samples

The performance of vis/NIR technology to monitor beer fermentation process was evaluated using PLS analysis. Table 3 shows statistics used to assess the regression models accuracy of studied craft beer of filtered and non-filtered samples respectively. Results show more robust PLS models for filtered samples. Regarding SSC models, in validation, R^2_{cv} ranged 0.61-0.82 and RMSECV 1.8-2.8. For pH models R^2_{cv} ranged 0.73-0.89 and RMSECV 0.10-0.19. Only *Sant'Ambroeus* F model gave not adequate results.

TABLE 3: Coefficient of determination in calibration (R^2_{cal}), root mean standard error of calibration (RMSEC), coefficient of determination in cross-validation (R^2_{cv}), root mean standard error of cross-validation (RMSECV) and latent variables (LVs) for the three kind of craft beer and for the two samples F and NF. Spectral pretreatments were smoothing and second derivative for all samples

Sample	n	LVs	Calibration		Cross-Validation	
			R^2_{cal}	RMSEC	R^2_{cv}	RMSECV
°Plato						
<i>Montestella</i> F	26	6	0.88	1.1	0.68	1.8
<i>Montestella</i> NF	23	10	0.96	0.6	0.61	2.1
<i>Sant'Ambroeus</i> F	32	7	0.87	1.8	0.78	2.5
<i>Sant'Ambroeus</i> NF	27	4	0.77	2.3	0.69	2.8
<i>Lambrate</i> F	23	4	0.87	1.6	0.82	1.9
<i>Lambrate</i> NF	21	5	0.78	1.8	0.68	2.4
pH						
<i>Montestella</i> F	21	6	0.91	0.10	0.84	0.15
<i>Montestella</i> NF	20	3	0.76	0.20	0.72	0.19
<i>Sant'Ambroeus</i> F	29	5	0.69	0.20	0.38	0.29
<i>Sant'Ambroeus</i> NF	26	9	0.97	0.06	0.87	0.13
<i>Lambrate</i> F	21	5	0.92	0.10	0.89	0.10
<i>Lambrate</i> NF	19	3	0.80	0.10	0.73	0.14

Concerning SSC, the best model was elaborated for *Lambrate* F with $R^2_{cv}=0.82$ and RMSECV=1.9. Liu et al. in 2009 used a handheld device (325-1075 nm) to calculate PLS model for SSC evaluation. The full-spectrum was used for data processing. The optimal performance of PLS model was $r=0.98$ in validation. The model less robust proposed gives $r=0.92$ in validation.

Regarding regression models for the prediction of pH value, *Lambrate* type provide the most satisfactory results. Good coefficients of determination and low prediction errors were obtained (R^2_{cv} : 0.73-0.89 RMSECV: 0.10-0.14). Lachenmeier (2007) used PLS elaboration

method to correlate Fourier transform infrared (FT-IR) spectra and pH. Accuracy of FT-IR-PLS model was in validation R^2 0.97 if carried out using selected wavelength ranges, while 0.71 (test-set validation) using full spectrum. Guidetti et al. (2010) tested with good results the same optical, portable vis/NIR experimental system used in this work on homogenized samples of grapes with a similar experimental setting. In this study, the best models were calculated for filtered samples. However, also statistics regarding models calculated for NF samples (beer matrix usable in case of application on-line) show good. In fact, in validation, for pH parameter of *Sant'Ambroeus* craft beer the following values were obtained: $R^2_{\text{cal}}=0.97$, $\text{RMSEC}=0.06$, $R^2_{\text{cv}}=0.87$ and $\text{RMSECV}=0.13$. These results encourage the use of an on-line application of vis/NIR technology to control craft beer fermentation process. About this topic, McLeod et al. (2009) evaluated the performance in prediction beer biomass and composition using an online probe in fermentation with a desktop FT-NIR system (1000-2500 nm). The best results achieved by PLS-FT-NIR model, in validation, were $r=0.99$, 0.99, 0.98 and 0.98 for ethanol concentration, specific gravity, optical density and dry cell weight respectively.

In general, more accurate PLS models were obtained for filtered samples. The reason for minor prediction performance using NF spectra might be due to the presence of more noise in data arising from a variable matrix like non-filtered beer during fermentation (caused by little particles in suspension). In fact, filtration process makes samples more homogeneous and less subject to noise during vis/NIR acquisition. Moreover, models could be further improved by selecting only some variables to reduce the spectral noise (Liu et al., 2009; Ghasemi-Varnamkhashtia et al., 2012).

Figure 8 and 9 show, as an example, regression lines of PLS models for the prediction of SSC of *Sant'Ambroeus* and pH value of *Lambrate* respectively. Both F and NF samples are presented.

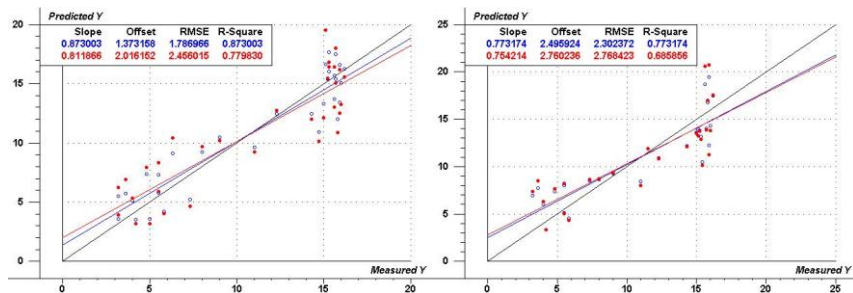


FIGURE 8: regression lines of PLS models for SSC of *Sant'Ambroeus* craft beer, F (left) and NF (right) samples

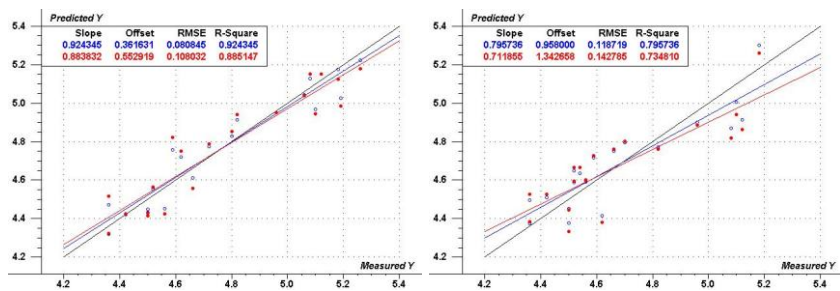


FIGURE 9: regression lines of PLS models for pH of *Lambrate* craft beer, F (left) and NF (right) samples

Conclusions

An optical, portable and inexpensive non-destructive device, based on visible and near infrared spectroscopy (vis/NIR), operating in the range 450-980 nm, was tested for a quick quality evaluation of craft beers and to verify the possibility of employing the device directly on the production line.

The application of PCA on craft beer spectra appropriately treated, allowed a qualitative assessment of the predictive skills of the device. A good separation of filtered craft beer samples was obtained.

Elaboration of regression models using PLS technique was done correlating vis/NIR spectra with the classical chemical data required for the control of fermentation process.

Results obtained show an accurate estimation of chemical parameters related to craft beer quality (SSC and pH). Particularly, for both qualitative indices, good prediction skills were obtained of filtered craft

beers, while fairly good performances in the estimation of non-filtered samples were shown. These results were similar for each type of the three craft beer analyzed.

The system proved to be suitable not only for the evaluation of craft beer quality parameters, but also to identify the perfect time for the end of the fermentation of craft beer. In fact, the possibility of a real time estimation of qualitative characteristics of the wort, and beer arising from it, would be of fundamental importance in order to obtain beers which fully meet the specifications required but with a certain variability typical of craft productions.

In conclusion, the encouraging results obtained show the possibility to use the tested system directly on the production line. Replacement of laboratory traditional analysis with vis/NIR spectroscopy would give some advantages: saving of specialized workforce, saving of time and beer. Furthermore, the instrument is easy to use and inexpensive. These are interesting features especially for small scale breweries with low potential of investment but with a strong need for process control, because they work with highly variable and non-standardized process compared with industrial breweries.

Acknowledgements

This study received financial support from Regione Lombardia and European Social Fund for a Post-doctoral Research Fellowship (“Progetto Dote Ricerca”).

References

- Almonacid, S.F., Nájera, A.L., Young, M.E., Simpson, R.J & Acevedo, C.A., 2012. Comparative study of stout beer batch fermentation using free and microencapsulated yeasts. *Food Bioprocess Technol* 5, 750-758
- Bamforth, C., 2003. Beer tap into the art and science of brewing. Oxford University Press (second ed.), 198 Madison Avenue, New York. ISBN: 13 978-0-19-515479-5
- Beghi, R., Spinardi, A. Bodria, L., Mignani, I. & Guidetti, R., 2012. Apples Nutraceutic Properties Evaluation Through a Visible and Near-Infrared Portable System. *Food Bioprocess Technol*, DOI 10.1007/s11947-012-0824-7
- Bertrand, D., 2000. Spectroscopie de l'eau. In *La Spectroscopie Infrarouge et ses Applications Analytiques*; Bertrand, D., Dufour, E., Eds.; TEC & DOC: Paris, France, 93-105
- Clement, A., Dorais, M., Vernon, M., 2008. Nondestructive Measurement of Fresh Tomato Lycopene Content and Other Physicochemical Characteristics Using Visible-NIR Spectroscopy. *J. Agric. Food Chem.*, 56, 9813-9818
- Engelhard, S., Löhmannsröben, H.G., and Schael, F., 2004. Quantifying Ethanol Content of Beer Using Interpretive Near-Infrared Spectroscopy. *Applied Spectroscopy*, 58(10), 1205-1209
- Ghasemi-Varnamkhasti, M., Mohtasebic, S.S, Rodriguez-Mendez, M. L., Gomesd, A.A., Araújo, M. C. U. & Galvão, R. K.H., 2012. Screening analysis of beer ageing using near infrared spectroscopy and the Successive Projections Algorithm for variable selection. *Talanta*, 89, 286-291
- Guidetti, R., Beghi, R. & Giovenzana, V., 2012. Chemometrics in Food Technology, *Chemometrics in Practical Applications*, Dr. Kurt Varmuza (Ed.), ISBN: 978-953-51-0438-4
- Guidetti, R., Beghi, R., & Bodria, L., 2010. Evaluation of grape quality parameters by a simple Vis/NIR system. *Transactions of the ASABE*, 53, 2, 477-484

INRAN, National Research Institute for Food and Nutrition, 2012.

Kirsanov, D., Legin, A., Papiieva, I., Rudnitskaya, A., Kartsova, A., Bocharnikov, A., Artyushenko, V. & Bogomolov, A., 2010. Beer quality assessment by hybrid spectroscopicelectrochemical technique. Modern Methods of Data Analysis, Seventh Winter Symposium on Chemometrics Russia, Saint Petersburg, February 15-19

Lachenmeier D.W., 2007. Rapid quality control of spirit drinks and beer using multivariate data analysis of Fourier transform infrared spectra. Food Chemistry, 101, 825-832

Liu, F., Jiang, Y. & He, Y., 2009. Variable selection in visible/near infrared spectra for linear and nonlinear calibrations: A case study to determine soluble solids content of beer. Analytica Chimica Acta, 635, 45-52

Madsen, M. & Esbensen, K.H., 2010. PLS2 regression as a tool for selection of optimal analytical modality — a closer look at beer brewing process analysis with alternative spectroscopic data types. Seventh Winter Symposium on Chemometrics Russia, Saint Petersburg, February 15-19, 2010

McLeod, G., Clelland, K., Tapp, H., Kemsley, E.K., Wilson, R.H., Poulter G., Coombs, D., & Hewitt, C.J., 2009. A comparison of variate pre-selection methods for use in partial least squares regression: A case study on NIR spectroscopy applied to monitoring beer fermentation. Journal of Food Engineering, 90, 300–307

Osborne B. G., Fearn T., Hindle P. H., 1993. Practical NIR Spectroscopy with Applications in Food and Beverage Analysis. Cap. 3: Physics of the interaction of radiation with matter, 36-48. Longman Scientific & Technical

Section 4

Quick Quality Evaluation of Chilean Grapes by a Portable vis/NIR Device

Valentina Giovenzana¹, Roberto Beghi¹, Aira Mena¹, Raffaele Civelli¹, Lorenzo F. Leòn G², Stanley Best², Riccardo Guidetti¹

¹Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy. Università degli Studi di Milano. Milano, Italy

²Instituto de Investigaciones Agropecuarias (INIA) Programa de Agricultura de Precisión (Progap-INIA). Chillan, Chile



Abstract

Grape composition at harvest is a key factor determining the quality of future wine. For this reason, measurement of grape characteristics in field during the ripening is a requirement to evaluate the right moment for the harvest. Classical chemical analyses, common at the moment in wine industry, have several limits: these take time, are wasteful, destructive, and ultimately inadequate to test fruit in field. Wineries need new practical and quick instruments, non-destructive, and able to quantitatively estimate, in field and on a large scale, the interesting parameters. The goal of the present study was to test an optical portable system for prediction of ripening parameters of fresh berries of grape (*Vitis vinifera* L. 'Cabernet Sauvignon' and 'Sauvignon Blanc'). In particular, the evaluation of the correlation between vis/NIR spectra and classical destructive ripening parameters was targeted, for samples of both red and white grapes. Each sample was obtained in a different date during the last period of ripening, by averaging the spectral acquisitions of several individual berries. A chemometric regression model was created for each parameter considered. Vis/NIR spectra were correlated with ripening parameters (total soluble solids content TSS, titratable acidity, pH, weight of 200 berries, potential alcoholic degree PAD and sugar/acidity ratio) and with phenolic ripening parameters (extractable anthocyanins EA, total anthocyanins TA and tannins) using the partial least square (PLS) regression algorithm. Principal component analysis (PCA) was performed on spectra too. For both red and white grapes, PCA showed a significant sample grouping for the different acquisition times. PLS models on red grape gave good predictive skills for TSS, acidity and PAD ($0.7 < R^2 < 0.8$, low values of RMSE), and for EA ($R^2_{cv} = 0.74$), while less accurate was the model elaborated for TA. Similar results were obtained for white grape (RMSE < 0.9 °Brix for TSS).

Keywords: spectroscopy, optical transportable system, fruit quality, non-destructive measurement, ripening parameters prediction

Introduction

Measurement of grape characteristics is a requirement for vineyard improvement and for optimum production of wines, since the quality of the final product is significantly influenced by grape composition (Carrara et al., 2008). Inspection of grapes upon arrival at the winery is a critical point in the wine production chain (Elbatawi and Ebaid, 2006). In literature, the application of some chemometric techniques directly to NIR spectral data with the aim of following the progress of conventional fermentation and maturation was investigated (Cozzolino et al., 2006). Similar spectral changes in all samples were followed over the time using principal components analysis (PCA). The PCA loading structure could be explained on the basis of absorptions from anthocyanins, tannins, phenolics, sugar and ethanol, the contents of which changed according to different fermentation time points. This study demonstrated the possibility of applying vis/NIR spectroscopy as an analytical tool for wine industry.

An optical, portable, experimental system (vis/NIR spectrophotometer) for non-destructive and quick prediction of ripening parameters of fresh berries and homogenized samples of grapes in the wavelength range 450-980 nm was built and tested in the past (Guidetti et al., 2010). Calibrations for technological ripening and for anthocyanins had good correlation coefficients ($r_{cv} > 0.90$). These models were extensively validated using independent sample sets. Good statistical parameters were obtained for soluble solids content ($r > 0.8$, SEP < 1.24 °Brix) and for titratable acidity ($r > 0.8$, SEP < 2.00 g tartaric acid L⁻¹), showing the validity of the vis/NIR spectrometer. Similarly, anthocyanins could be predicted accurately compared with the reference determination.

Other authors analyzed by vis/NIR spectroscopy different parameters commonly monitored in wineries (Urbano-Cuadrado et al., 2004). Coefficients of determination (R^2) obtained for the fifteen parameters were higher than 0.80 and in most cases higher than 0.90 while SECV values were close to those of the reference method. Authors said that these prediction accuracies were sufficient for screening purposes.

A study on the characterization and determination of the geographical origin of wines was presented in literature (Römisch et al., 2009). Three methods of discrimination and classification of multivariate data were

considered and tested: the classification and regression trees (CART), the regularized discriminant analysis (RDA) and the partial least squares discriminant analysis (PLS-DA). PLS-DA analysis showed better classification results with percentage of correct classified samples from 88 to 100%.

The goal of this study is to test an optical portable system (JAZ vis/NIR spectrophotometer by OceanOptics, USA) for non-destructive and quick prediction of ripening parameters of fresh berries of *Vitis vinifera* grape in the wavelength range of 400-1000 nm (Figure 1).



Figure 1. Images of spectral acquisition phases on fresh berries with the JAZ system

In order to validate the effectiveness of the system, this research focused on the evaluation of the correlation between the vis/NIR spectra and the classical ripeness parameters and phenolic ripening indices.

Materials and methods

Two distinct experimentations were carried out on red and white grapes (*Vitis vinifera* L., 'Cabernet sauvignon' and 'Sauvignon blanc' cultivar respectively) in vintage year 2011 in two different Chilean regions. The first grape cultivar was harvested in vineyard Valdivieso, which is located in the Curicó Valley in Maule Region, about 200 km south of Santiago. On the other hand, vineyard Cucha Cucha, where the white grape was grown, is located 300 km farther south, in Bío Bío Region. These areas correspond to the most prestigious and largest wine regions of Chile. Because of their morphology and geographical location, they have a pattern of rainfall similar to Mediterranean climate, with a warm dry summer and a mild winter during which the most of

precipitations accumulate. Both red and white grapes were investigated with spectral and classical destructive analyses.

Spectral acquisitions were realized on berries of grape directly in field using the optical portable system (JAZ vis/NIR spectrophotometer by OceanOptics, USA) operating in the wavelength range 400-1000 nm. The JAZ equipment is composed of a vis/NIR lighting system, a fiber optic probe for reflection measurement, a compact diode array spectrophotometer and a battery. The spectrophotometer was equipped with a diffractive grating for spectral measurements and a CCD sensor with a 2048 pixel matrix, corresponding to a nominal resolution of 0.3 nm. Spectra were acquired in reflectance: in this mode, the sample is hit by radiation produced by the lighting system, then the reflected component is measured by the spectrophotometer and registered through a dedicated software.

Classical ripeness parameters analyzed were total soluble solids content (TSS), titratable acidity, pH, weight of 200 berries, potential alcoholic degree and sugar/acidity ratio. Phenolic ripening parameters were extractable anthocyanins (EA), total anthocyanins (TA) and tannins.

Red Grape Sampling

A total of 86 fresh red grape samples were analyzed. Each sample was obtained by averaging the spectral acquisitions of 75 individual berries, for a total of more than 6400 spectral data. Samples were collected with a frequency of once a week during the last period of ripening just before harvest. They were drawn from different zones, throughout the entire vine area of the valley, in order to represent environmental variability.

White Grape Sampling

A total of 90 fresh white grape samples were analyzed with the same experimental protocol as for the red grape. In this case each sample was obtained by averaging the spectral acquisitions of 10 individual berries, for a total of 900 spectral data.

Data Processing

The following data processing was applied for both the studies.

All samples available were used for the creation of a chemometric regression model for each parameter considered. Chemometric analysis was performed using the Unscrambler software package (version 9.8, CAMO).

Smoothing treatment (15 points, Moving Average) was applied to the vis/NIR spectra to reduce spectral noise before building the calibration models.

Principal component analysis (PCA) was realized on vis/NIR spectra to examine sample groupings and identify outliers. Outliers were detected using score plots, residuals and leverages of PCA models.

The vis/NIR spectra were correlated with ripeness parameters and with phenolic ripening parameters using the partial least square (PLS) regression algorithm. To evaluate model accuracy, the statistics used were the coefficient of determination in calibration (R^2_{cal}), coefficient of determination in cross-validation (R^2_{cv}), root mean square error of calibration (RMSEC), and root mean square error of cross-validation (RMSECV).

Results and discussion

Results for Red Grape

Descriptive statistics for all ripeness parameters are shown in Table 1. Wide variability in composition was observed as a result of different sampling times before harvest.

Table 1. Descriptive statistics of red grape samples for ripeness parameters (TSS, titratable acidity, pH, weight of 200 berries, potential alcoholic degree and sugar/acidity ratio) and for phenol ripening parameters (TA, EA and tannins)

Parameter	Units	N°	Mean	SD	Min.	Max.
TSS	°Brix	86	22.7	3.0	10.9	27.2
Titratable acidity	$g_{\text{tart.acid}} \text{ dm}^{-3}$	86	6.4	2.2	3.6	18.3
pH		86	3.4	0.2	2.7	3.8
Weight of 200 berries	g	86	274.2	48.0	164.4	426.8
Potential alcoholic degree	% vol	86	12.4	1.9	5.1	15.3
Sugar/acidity		86	36.8	12.9	4.8	65.4
TA	mg dm^{-3}	86	1127.1	364.0	185.5	2107.0
EA	mg dm^{-3}	86	540.7	159.2	133.0	899.5
Tannins	mg kg^{-1}	86	1712.5	667.0	605.0	4744.0

Figure 2 shows vis/NIR spectra and the effect of preprocessing smoothing treatments, necessary for maximizing useful information and reducing spectral noise.

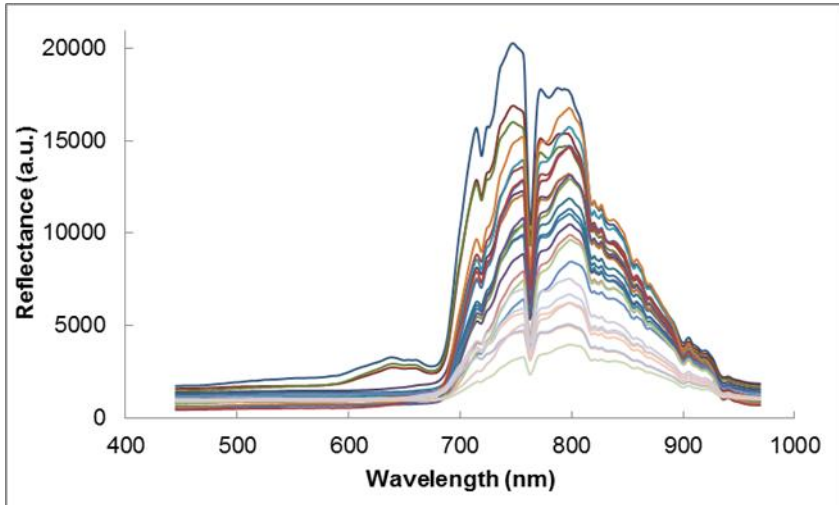


Figure 2. Examples of spectra of samples of fresh red grape berries after smoothing pretreatment

The absorption bands observed in the vis/NIR region arise from overtones of stretching vibrations involving functional groups with hydrogen atom or combinations involving stretching and bending modes of vibration of these groups. Most of the NIR bands observed involve CH, OH, NH and SH bonds. The absorption bands of water in the NIR region occur at 760 nm, 970 nm, 1190 nm, 1450 nm, and 1940 nm. In our case is visible the absorption band of water at 760 nm. Observed changes in the visible region spectra between 500 and 700 nm are due to changes in the amount of pigment during the ripening period. Particularly, around 675 nm is visible the peak of absorption of chlorophyll.

PCA was performed to provide partial visualization of the dataset in a reduced dimension, and three principal components with eigenvalues greater than one, accounting for more than 99% of the total variance, were obtained. The PCA results show no relevant samples grouping, regarding the different sampling zone and altitude. A significant sample grouping is visible for the different sampling times (Figure 3), particularly there is a trend regarding the sampling dates along the PC1 (91% of the explained variance), from positive to negative values. This is probably due to the different ripening stage of the samples in each sampling date.

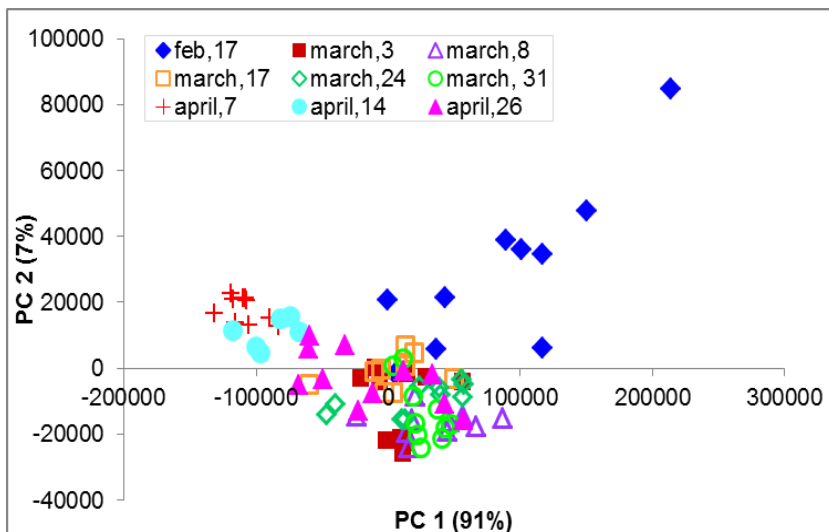


Fig. 3 PCA referred to different sampling dates on red grape samples: in the plot PC1 vs PC2 is reported, 91% and 7% of the total data variance respectively

Table 2 shows the statistics related to PLS models obtained by vis/NIR technology for the different parameter analyzed.

As an example, regression models for two of the most significant parameters analyzed (TSS as ripeness parameter and EA as phenolic ripening parameter) are shown in Figure 4. Each plot show calibration (blue) and cross-validation line (red). Target line (black) represent the line of perfect correlation between reference and predicted values.

Table 2. Results of PLS models for red grape samples. LV latent variables; R²_{cal} coefficient of determination in calibration; RMSEC root mean square error of calibration; R²_{cv} coefficient of determination in cross-validation; RMSECV root mean square error of cross validation

Parameter	Units	Number of samples	LV	Calibration		Validation	
				R ² _{cal}	RMSEC	R ² _{cv}	RMSECV
TSS	°Brix	76	5	0.78	1.0	0.74	1.1
Titrateable acidity	g _{tart. acid} dm ⁻³	78	4	0.73	0.84	0.70	0.89
pH		78	4	0.70	0.09	0.66	0.10
Weight of 200 berries	g	81	8	0.72	22.0	0.63	25.8
Potential alcoholic degree	% vol	76	5	0.78	0.65	0.75	0.72
Sugar/acidity		78	5	0.64	6.6	0.58	7.1
TA	mg dm ⁻³	76	5	0.64	176.8	0.59	193.1
EA	mg dm ⁻³	76	7	0.78	68.3	0.74	76.6
Tannins	mg kg ⁻¹	75	6	0.61	320.9	0.50	361.2

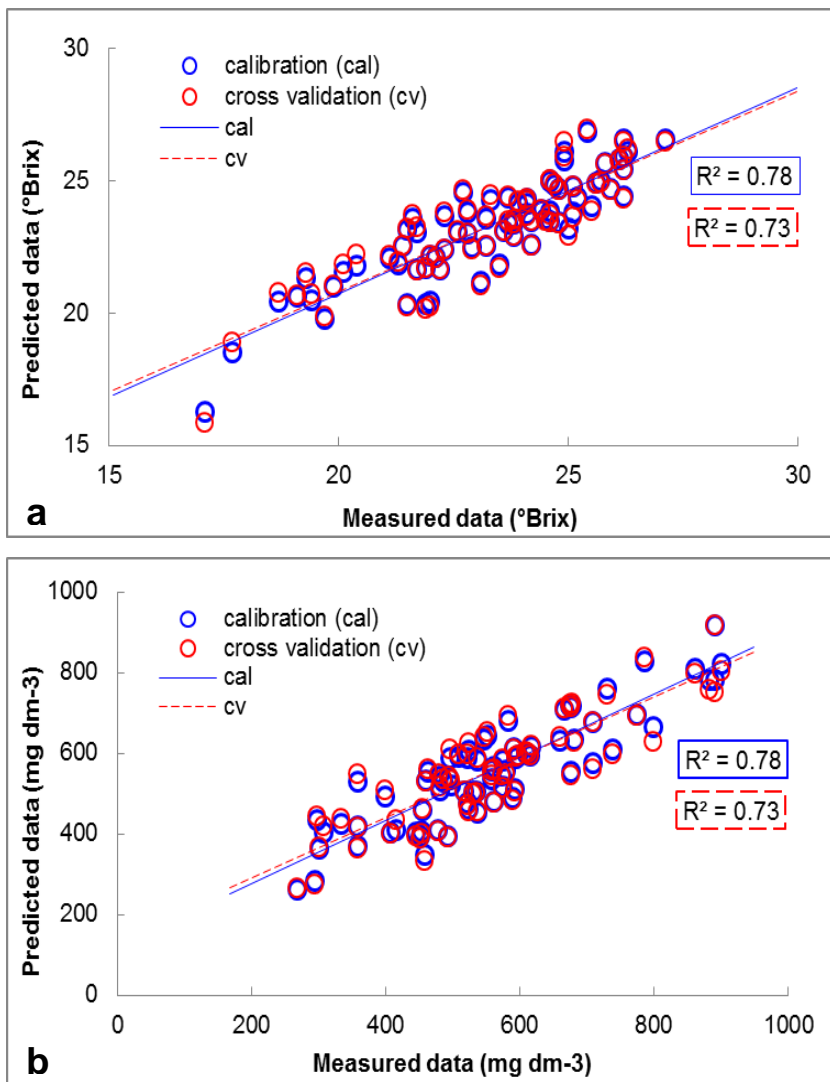


Figure 4. PLS model for TSS (°Brix) in (a) and for EA (mg dm⁻³) in (b) for red grape data

Good predictive skills were obtained for TSS, acidity and potential alcoholic degree, with R^2 values between 0.7 and 0.8 and low values of RMSE.

Regarding phenolic parameters, good results were achieved for extractable (EA, $R^2_{cv} = 0.74$); less accurate was the regression model elaborated for TA. Prediction of EA is important because directly correlated with the phenolic content and the colour of wine.

Models developed for the other parameters are acceptable and could be probably improved by using a larger number of samples.

Results for White Grape

Descriptive statistics for all ripeness parameters are shown in Table 3.

Table 3. Descriptive statistics of white grape samples for ripeness parameters (TSS, titratable acidity and pH)³

Parameter	N°	Mean	SD	Min.	Max.
TSS (°Brix)	90	22.8	1.8	18.1	26.7
Titratable acidity ($g_{\text{tart.acid}} \text{dm}^{-3}$)	90	9.5	1.7	4.8	13.9
pH	80	3.5	0.2	3.0	4.0

³SD = standard deviation, Min. = minimum, and Max. = maximum.

Similar to that seen for red grape, a significant sample grouping is visible in PCA for the different sampling times (Figure 5). Also in this case, this is probably due to the different ripening stage of the samples in each sampling date.

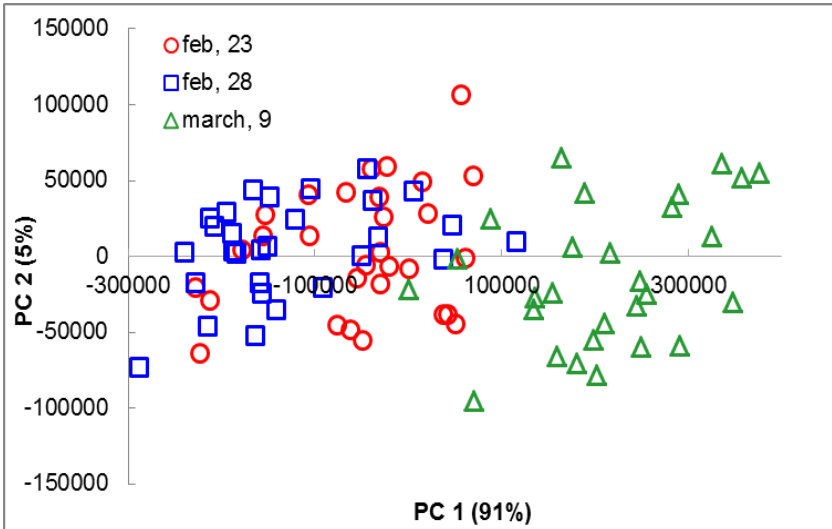


Figure 5. PCA referred to different sampling dates on white grape samples: in the plot PC1 vs PC2 is reported, 91% and 5% of the total data variance respectively

Figure 6 shows the regression model for TSS, among the most significant parameters analyzed.

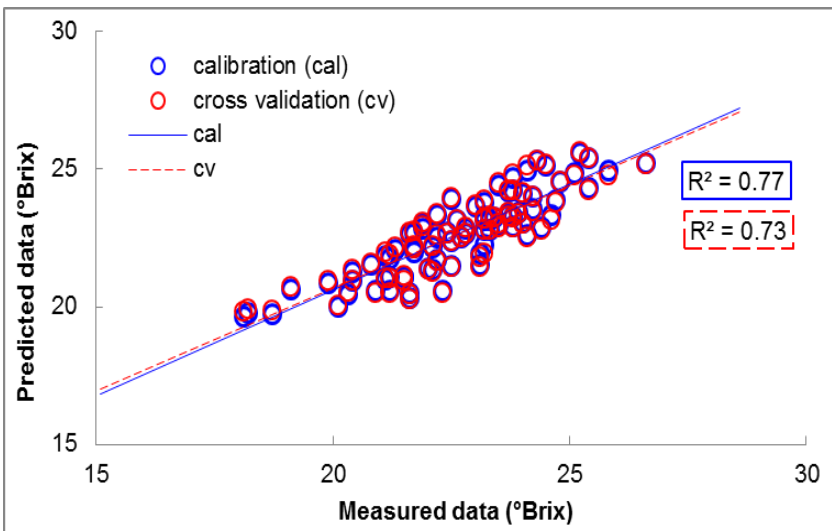


Figure 6. PLS model for TSS (°Brix) for white grape data

Table 4 shows the statistics related to PLS models obtained by vis/NIR technology for TSS, the different parameter analyzed.

Table 4. Results of PLS models for white grape samples⁴

Parameter	Units	N°	LV	Calibration		Validation	
				R ² _{cal}	RMSEC	R ² _{cv}	RMSECV
TSS	°Brix	82	5	0.77	0.8	0.74	0.9
Titratable acidity	g _{tart.acid} dm ⁻³	80	4	0.55	1.0	0.48	1.1
pH		73	4	0.61	0.11	0.59	0.12

N° number of samples; LV latent variables; R²_{cal} coefficient of determination in calibration; RMSEC root mean square error of calibration; R²_{cv} coefficient of determination in cross-validation; RMSECV root mean square error of cross validation.

Good results were obtained for TSS, with RMSE less than 0.9 °Brix. In literature are present works which demonstrate that a difference in sugar content of a fruit less than 1 °Brix is not perceptible by the consumer. Moreover it should be considered that the measurement error of digital refractometers used to measure the TSS content, and whose data are used as reference data for the development of PLS models, have a measurement error of about 0.5 °Brix.

Conclusions

In this work spectral data acquired with the portable system JAZ by OceanOptics during the experimental campaign 2011 in two different zones of Chile were correlated to the main ripeness parameters of grapes. Development of PLS regression models showed good results for some parameters, both technological and phenolic. The overall results are encouraging, especially considering the difficulties associated with the use of these tools directly in field, with difficult standardization of lighting conditions. Therefore, it is shown the ability to quick analyze grape maturity in a optical way. The robustness of the prediction models is certainly improvable, for example by expanding the available data set with samples from the vintage year 2012.

For the future a more detailed study of grape spectra could be done with the aim of investigate spectral modifications at particular bands in order to highlight and select the most informative wavelengths. In this way a more simple and inexpensive device could be realized to monitor

ripeness degree and classify samples entering the winery, for a better management of wine production process.

Acknowledgements

This research was carried out with the financial support of Regione Lombardia, SOQUIC research project and from Regione Lombardia and European Social Fund for a Post-doctoral Research Fellowship (“Progetto Dote Ricerca”).

References

- Carrara, M.; Catania, P.; Vallone, M. & Piraino, S. (2008). Mechanical harvest of grapes: Assessment of the physical-mechanical characteristics of the berry in order to improve the quality of wines. In Proc. Intl. Conf. on Agricultural Engineering: Agricultural and Biosystems Engineering for a Sustainable World (AgEng 2008). CIGR
- Cozzolino, D.; Parker, M.; Damberg, R.G.; Herderich, M. & Gishen, M. (2006). Chemometrics and visible-near infrared spectroscopic monitoring of red wine fermentation in a pilot scale. *Biotechnol. Bioeng.*, 95, 1101-1107
- Elbatawi, I.E. & Ebaïd, M.T. (2006). A new technique for grape inspection and sorting classification. *Arab Universities J. Agric. Sci.*, 14(2), 555-573
- Guidetti, R.; Beghi, R. & Bodria, L. (2010). Evaluation of Grape Quality Parameters by a Simple vis/NIR System. *Transaction of the ASABE*, 53(2), 477-484, ISSN: 2151-0032
- Römisch, U.; Jäger, H.; Capron, X.; Lanteri, S.; Forina, M. & Smeyers-Verbeke, J. (2009). Characterization and determination of the geographical origin of wines. Part III: multivariate discrimination and classification methods. *Eur. Food Res. Technol.*, 230, 31-45
- Urbano-Cuadrado, M.; de Castro, M.D.L.; Perez-Juan, P.M.; Garcia-Olmo, J. & Gomez-Nieto, M.A. (2004). Near infrared reflectance, spectroscopy and multivariate analysis in enology—Determination or screening of fifteen parameters in different types of wines. *Anal. Chim. Acta*, 527, 81-88

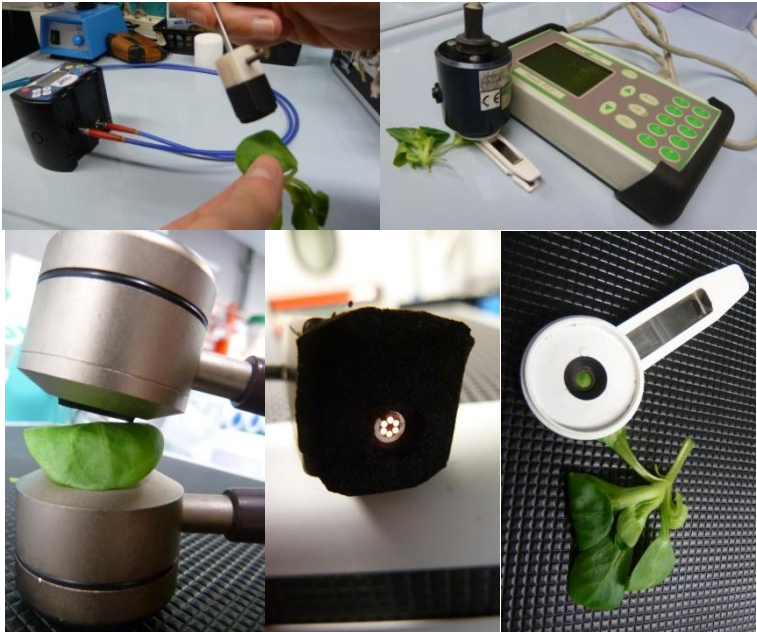
Section 5

Application of non-destructive techniques for the evaluation of fresh-cut Lamb's lettuce (*Valerianella locusta* Laterr.) shelf life: a preliminary study

Susanna Buratti¹, Valentina Giovenzana², Raffaele Civelli², Roberto Beghi², Riccardo Guidetti², Laura Piazza¹

¹Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, via Celoria 2, 20133 Milano

²Department of Agricultural and Environmental Sciences (DiSAA), Università degli Studi di Milano, via Celoria 2, 20133 Milano



Abstract

The aim of this work was to investigate the applicability of non-destructive techniques in monitoring freshness decay of fresh-cut Lamb's lettuce (*Valerianella locusta* Laterr.) during storage. Fresh-cut Lamb's lettuce samples were purchased in a local market the first day of commercialization and stored at two different temperatures (4 °C and 20 °C) up to 10 and 7 days respectively. The quality decay of samples was evaluated by chemical (pH and humidity), physical (texture) and nutritional (phenolic content and antioxidant power) parameters and by non-destructive sensory (electronic nose) and optical (vis/NIR and fluorescence) devices. Results demonstrated that electronic nose, vis/NIR technique and fluorescence could support the conventional techniques in the shelf-life assessment of fresh-cut Lamb's lettuce providing information useful for a better management of the product along the distribution chain.

Keywords *Valerianella locusta* Laterr, shelf life, fresh-cut vegetables, electronic nose, vis/NIR spectroscopy, fluorescence.

Introduction

Changes in human life styles have led consumers to move towards ready-to-eat products. Particularly in recent years, there has been an increase in the consumption of fresh-cut fruit and vegetables due to their ease of use and nutritional properties. The international Fresh-cut Produce Association (IFPA) defines fresh-cut products as “any fruit or vegetable or combination thereof that has been physically altered from its original form, but remains in a fresh state”.

A critical aspect relevant for this type of products is represented by their actual shelf life since manipulations such as trimming, peeling, cutting and or slicing increase the respiration rate and the ethylene production, and cause tissue disruption. These physiological changes may result in degradation of colour, texture, flavour and nutritional value (Rico, Martin-Diana, Barat & Barry-Ryan, 2007).

Several studies have been carried out to monitor the shelf life of fresh-cut fruit and vegetables by using chemical, physical and microbiological indices. These conventional methods are generally expensive, slow, require considerable analytical skill and are not suited to automation. Therefore rapid and non-destructive methods to investigate the freshness decay of fresh cut vegetables during or at the end of the distribution chain should be developed.

The aim of this work was to evaluate the quality changes of fresh-cut lamb's lettuce (*Valerianella locusta* Laterr.) during storage at two different temperature (4°C and 20 °C) by using non-destructive devices. In particular, a commercial electronic nose (EN) was used to monitor changes in volatile compound during storage, and two optical devices were applied in order to evaluate diffuse reflectance modifications in vis/NIR spectral range, as well as photosynthetic activity by measuring chlorophyll a fluorescence transients.

Materials and Methods

Three lots of fresh-cut Lamb's lettuce, supplied in a local market the first day of commercialization, were selected and analysed during storage at two different temperature, 4°C and 20 °C, up to 10 and 7 days

Section 5

Application of non-destructive techniques for the evaluation of fresh-cut Lamb's lettuce (*Valerianella locusta* Laterr.) shelf life: a preliminary study

respectively. The lettuce was packaged in polypropylene trays sealed with a polypropylene film of 100 g servings.

Electronic nose

The EN measurements were performed with a Portable Electronic Nose shown (PEN 2, Win Muster Airsense Analytics Inc., Germany, figure 1). It consists of a sampling apparatus, a detector unit containing an array of 10 Metal Oxide Semiconductor (MOS) type chemical sensors, and a pattern recognition software (Win Muster v.1.6) for data recording and elaboration. During sample storage the EN data were collected and elaborated by Principal Component Analysis (PCA).

Vis/NIR spectra

Spectral acquisitions were realized using an optical portable system (JAZ vis/NIR spectrophotometer, OceanOptics, USA, figure 1) operating in the wavelength range 400–1000 nm. The JAZ equipment is composed of a vis/NIR lighting system, a fiber optic probe, a compact diode array spectrophotometer and a battery. Spectra were acquired in reflectance: in this mode, the sample is hit by radiation produced by the lighting system, then the reflected component is measured by the spectrophotometer and registered through a dedicated software.

Every sampling day, 10 leaves were analyzed for both the temperatures realizing 3 acquisitions for leaf. A total of 240 spectra in 8 sampling dates (during the experimentation period of 10 days) were acquired for the lettuce stored at 4 °C. The rapid degradation of the lettuce stored at 20 °C, led to a total of 150 acquisitions in 5 sampling dates, during the first 7 days of study. Chemometric analysis was performed using the Unscrambler software package (version 9.8, CAMO, ASA, Norway). Spectra were pre-processed through smoothing treatments to improve the signal to noise ratio, and averaging them on every sampling date, in order to reduce the effects due to the physiological variability of leaves (Cattaneo & Ferrante 2010). Then PCA was applied to assess the evolution of the lamb's lettuce during its storage at the two temperatures.

Fluorescence

Chlorophyll *a* fluorescence transients were measured using a portable Handy Plant Efficiency Analyser (PEA, Hansatech, UK).

The measurements were taken on the leaves surface after illumination with a light intensity (LED with maximum emission peak at 650 nm) of 3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Baldassarre, Cabassi, & Ferrante, 2011). Every sampling date, 10 leaves were randomly taken from the stored packages and dark adapted with leaf clips for 30 minutes before of the acquisitions. Data analysis on fluorescence spectra was performed using SPSS 19.0: the chlorophylls were subjected to a two-way ANOVA, and differences among the means were calculated with Tukey's post-test.

Results and discussion

Electronic nose

The EN was applied in order to evaluate the evolution of the aroma profile of Lamb's lettuce during storage at 4 and 20 °C.



Figure 1. From left to right: Electronic Nose (PEN 2, Win Muster Airsense Analytics Inc., Germany); JAZ vis/NIR spectrophotometer, OceanOptics, USA; Handy Plant Efficiency Analyser (PEA, Hansatech, UK)

Section 5

Application of non-destructive techniques for the evaluation of fresh-cut Lamb's lettuce (*Valerianella locusta* Laterr.) shelf life: a preliminary study

The PCA score-plot (Figure 2) shows a clear distribution of samples along PC1 and PC2 (100.0% of explained variance) according to the storage temperature and time. In particular, samples stored at 4 °C are located at the right of the plot and their aroma profile is similar to that of the fresh product analyzed the first day of commercialization (T0). The lamb lettuce sample stored for 10 days at 4 °C is discriminated along the second principal component and its aroma profile is similar to that of sample stored at 20 °C for 3 days. A clear evolution of the aromatic profile of samples stored at 20 °C is evident along the first principal component from right to left. After 3 days of storage at 20 °C the aroma profile evolves and samples are completely discriminated from the fresh product (T0). Examining the loading plot (Figure 3) showing the relationship between the electronic nose variables and how they influence the system, the W5S, W2S and W1S sensors had the highest influence in the pattern file, but only one sensor (W5S) is relevant in the discrimination of lamb lettuce samples along the first principal component on the bases of their storage condition (temperature and time). This result is in agreement with those reported by other studies concerning the applicability of the electronic nose on the evaluation of fruits shelf life and ripeness. In these studies it has been demonstrated that W5S sensor (broad range) was particularly relevant in monitoring changes in volatile profile during fruits ripening (Benedetti, Buratti, Spinardi, Mannino & Mignani, 2008).

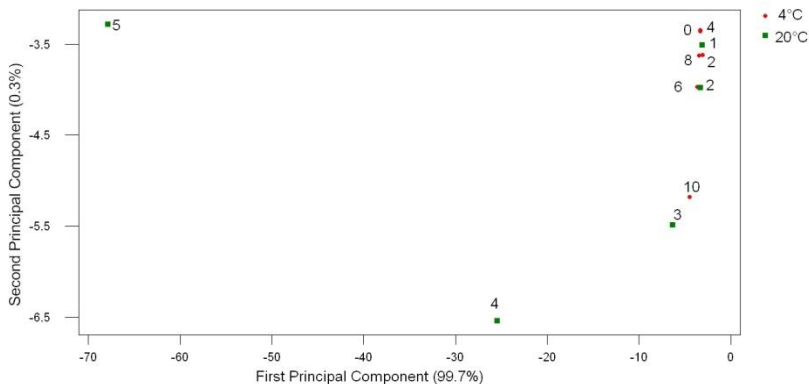


Figure 2. EN data. PCA-score plot of lamb lettuce samples stored at 4 °C and 20 °C. The numbers in score-plot represent the days of storage

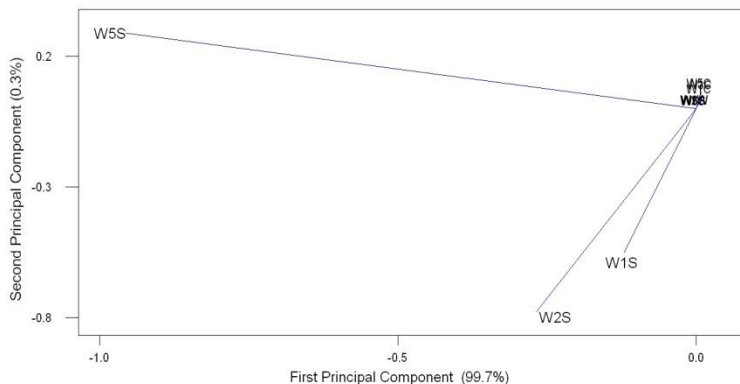


Figure 3. EN data. Loading plot (B) of lamb lettuce samples stored at 4 °C and 20 °C. The numbers in score-plot represent the days of storage

Vis/NIR spectra

Principal Components Analysis was performed on vis/NIR data to explore possible clustering of homogeneous samples belonging to the same shelf-life stage (Massart, Vandeginste, Buydens, De Jong, Lewi & Smeyers-Verbek, 1997; Naes, Isaksson, Fearn & Davies, 2002).

The PCA results for lamb's lettuce stored at 4 °C are shown in figure 4. Scores plot at the left side of the figure presents the two most significant PCs explaining 100% of total data variance (PC1: 96%; PC2: 4%). The evolution over time of the samples is well emphasized across PC2, while PC1 does not add any useful information in this purpose.

The main wavelengths contributing to PC2 are therefore the most significant in order to monitor shelf-life of lamb's lettuce. Wavelengths with high absolute values of PC2 are visible on PCs loadings plot at the right side of figure 5. In particular, two spectral bands at 680 nm and 740 nm were identified.

Similar results were obtained for lamb's lettuce stored at 20 °C.

Section 5

Application of non-destructive techniques for the evaluation of fresh-cut Lamb's lettuce (*Valerianella locusta* Laterr.) shelf life: a preliminary study

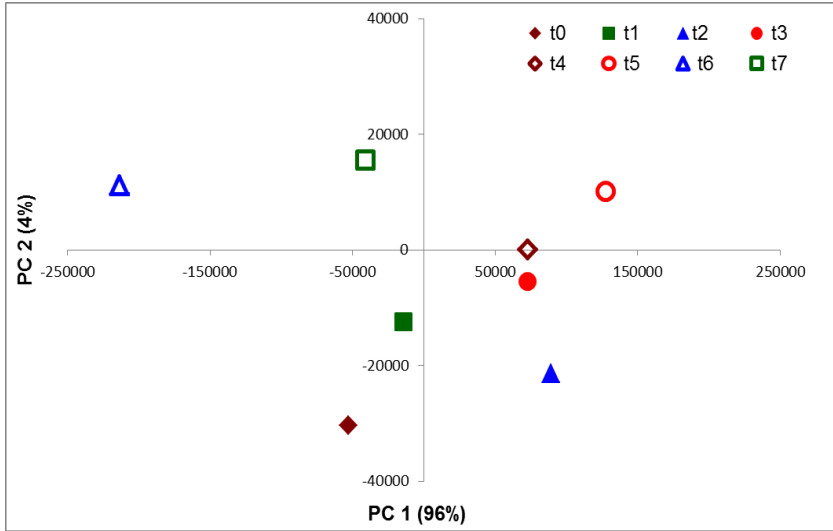


Figure 4. Vis/NIR data for samples stored at 4 °C. Scores plot of PC1 vs PC2 of averaged samples for each sampling date

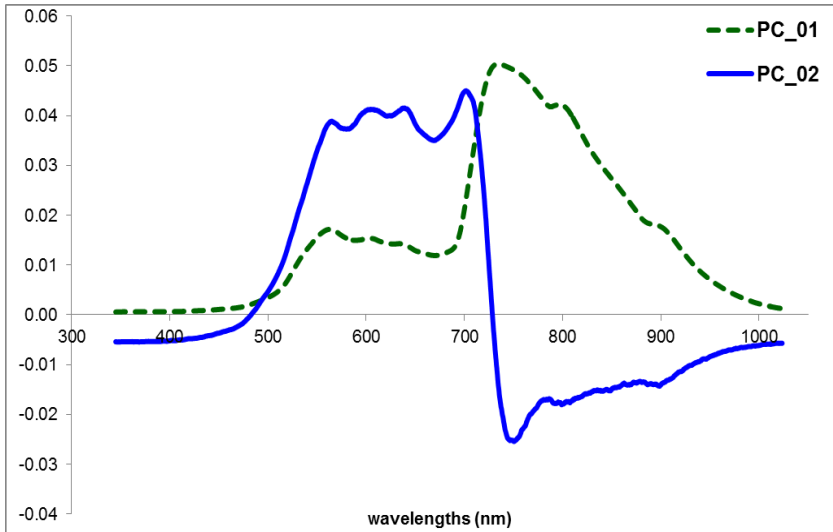


Figure 5. Vis/NIR data for samples stored at 4 °C. Loadings plot of the first two PCs

Fluorescence

Chlorophyll *a* fluorescence was used as a rapid diagnostic method to determine the health of lamb's lettuce leaves.

Indices derived from JIP analysis, performed on the intermediate data points, changed during storage for both temperatures.

The PI (performance index, shown in figure 6) is the parameter that better highlighted the differences between the two storage conditions. This index is a biophysical parameter and was useful in revealing differences in the response of PSII to dark stored leafy vegetables (Ferrante & Maggiore, 2007).

The mean initial PI value was about 3 and decreased to 1.4 (–53%) and 0.5 (–83%) in leaves stored at 20 and 4 °C, respectively. As expected, PI for stressed leaves at 20 °C decreased much more rapidly due to the extreme conditions of storage, indicating a decay in the efficiency of PSII photochemistry.

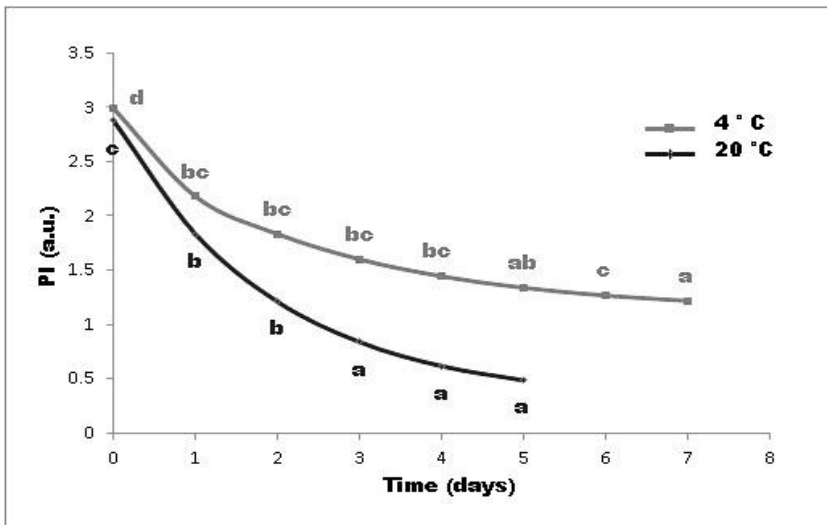


Figure 6. Chlorophyll *a* fluorescence data. Comparison in the evolutions of performance index for both the 4°C and 20 °C temperatures. Data were subjected to one-way ANOVA and different letters mean that values are statistically different (HSD di Tukey, $p < 0.05$)

Conclusions

Results of the present work demonstrate that electronic nose, vis/NIR techniques and fluorescence could be suggested as simple, rapid and non-destructive methods for evaluating the quality decay of Lamb's lettuce during storage.

Additional data could confirm the results. For example, an intermediate storage temperature of around 10 °C could be considered. A larger amount of data could also allow further processing, like the creation of chemometric regression models to correlate vis/NIR spectra with the significant parameters studied through the other techniques.

Moreover the information provided could be useful for a better management of the product along the distribution chain, ensuring the quality of the product to the consumer.

Acknowledgements

This study received financial support from AGER as “STAYFRESH - Novel strategies meeting the needs of the fresh-cut vegetable sector” research project and from Regione Lombardia and European Social Fund for a Post-doctoral Research Fellowship (“Progetto Dote Ricerca”).

References

- Baldassarre, V.; Cabassi, G. & Ferrante, A., 2011. Use of chlorophyll a fluorescence for evaluating the quality of leafy vegetables. *Australian Journal of Crop Science*, 5(6), 735-741
- Benedetti, S.; Buratti, S.; Spinardi, A.; Mannino, S. & Magnani, I., 2008. Electronic nose as a non-destructive tool to characterise peach cultivars and to monitor their ripening stage during shelf-life. *Postharvest Biology and Technology*, 47(2), 181-188
- Cattaneo T.M.P. & Ferrante A., 2010. Valutazione della qualità di ortaggi di IV gamma. Analisi non distruttive durante la shelf life, 55-70. ARACNE (ed). Italy ISBN: 978-88-548-2930-5
- Ferrante A. & Maggiore, T., 2007. Chlorophyll a fluorescence measurements to evaluate storage time and temperature of Valeriana leafy vegetables. *Postharvest Biology and Technology*, 45, 73-80
- Massart, D.L.; Vandeginste, B.G.M.; Buydens, L.M.C.; De Jong, S.; Lewi, P.J. & Smeyers-Verbeek, J., 1997. Handbook of Chemometrics and Qualimetrics: Part A. Elsevier, ISBN:0-444-89724-0, included in series: Data Handling in Science and Technology
- Naes, T.; Isaksson, T.; Fearn, T. & Davies, T., 2002. A user-friendly guide to multivariate calibration and classification. Chichester, UK: NIR Publications ISBN 0-9528666-2-5
- Rico, D.; Martin-Diana, A.B.; Barat, J.M. & Barry-Rian, C., 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: a review. *Trends in Food Science and Technology*, 18, 373-386

Section 6

Wavelengths selection with a view to a simplified handheld optical system to estimate grape ripeness.

Valentina Giovenzana, Roberto Beghi, Raffaele Civelli, Cristina Malegori, Riccardo Guidetti

Department of Agricultural and Environmental Sciences
Università degli Studi di Milano, via Celoria 2, Milano, 20133 Italy



Abstract

Simple devices in the oenological sector are important to support wine growers in monitoring ripening progress of grapes and plan harvest time. Vis/NIR spectroscopy devices nowadays available are, however, expensive and not suitable for small scale wineries.

The aim of this work was to identify the most significant wavelengths able to discriminate in the field the grapes ready to be harvested with a view to a simplified handheld and low-cost optical device.

The experimental plan monitored the Nebbiolo grape ripening process in Italy from July to October 2011. Non-destructive analyses were carried out on each sample using portable commercial vis/NIR spectrophotometer (Jaz Modular Optical Sensing Suite, Ocean Optics, Nederland).

Chemometric analyses were performed in order to extract the maximum useful information from spectral data and to select the most significant wavelengths, which could be used for a simplified device.

Correlation between normalized spectral data matrix and technological (Total Soluble Solids, TSS) and phenolic (polyphenols) parameters were carried out using Partial Least Square (PLS) regression in order to identifying relevant wavelengths sorted from the full vis/NIR data.

Standardized regression coefficients of PLS model were used to select the relevant variables, representing the most useful information of full spectral region. The standardization takes into account both standard deviation of reflectance, for each wavelength considered, and standard deviation of reference data (TSS and polyphenols).

To confirm the efficiency of the variable selection, qualitative evaluation of the average spectra and loading plot, deriving from Principal Component Analysis (PCA), were considered.

The three selected wavelengths were 670 nm, corresponding to chlorophyll absorption peak; 730 nm, equal to the maximum reflectance peak; and 780 nm, representing the third overtone of OH stretching. An additional band at 850 nm was chosen as normalization factor in a range of wavelengths where the contribution of all the PCs tends to cancel out.

The discriminating wavelengths were divided by the normalization one to obtain three normalized spectral ratios. PCA and Multiple Linear Regression (MLR) were applied to spectral ratios in order to verify the effectiveness of selected wavelengths.

This work highlighted the most important wavelengths (670 nm, 730 nm and 780 nm) for ripeness monitoring; an additional band at 850 nm was used as normalization factor.

Results demonstrate the feasibility of a simplified, low-cost handheld device for real-time assessment of grape ripeness in field.

This variable selection approach is suitable for other food matrices in order to isolate the useful information contained in vis/NIR spectral data.

Keywords: Grape, vis/NIR spectroscopy, non-destructive analysis, wavelengths selection, handheld device, Partial least squares (PLS), Multiple linear regression (MLR).

Introduction

In recent years, near-infrared (NIR) spectroscopy has gained wide acceptance in different fields, in particular in postharvest fruit and vegetables (Wang and Paliwal, 2007).

The main advantage of NIR technology, in fact, is the ability to record spectra for solid and liquid samples without any pretreatment. This characteristic makes it attractive speedy analysis of products. The cost savings of NIR measurements related to improved control and product quality are often achieved and can provide results significantly faster compared to traditional laboratory analysis. For instance, vine growers should be supported in their decisions by this non-destructive technology to objectively and more precisely assess grapes ripeness in field and to plan the best harvest time accordingly.

The success of NIRS technology have resulted in a good number of publications, dealing with different aspects of NIR technology. Wang and Paliwal (2007) synthesizes the status of NIRs in the agri-food industry in terms of hardware and software development as well as the direction in which the NIRs research is headed. Nicolai et al. (2007) gave a comprehensive overview of NIR spectroscopy for measuring quality attributes of fruit and vegetables. In particular, they paid attention to optics and chemometrics as well as applications, and identified areas where more research is required.

Many studies, reported in literature, evaluated the applicability of NIR spectroscopy to obtain a complete check of fruits. For example several applications to estimate ripeness parameters of different fruit species can be found on grapes (Cozzolino, 2006), pears (Sun et al., 2009), apples (Peirs et al., 2005), plums (Slaughter et al., 2003), oranges (Cayuela, 2008), peach fruits (Pérez-Marín et al., 2009; Ziosi et al., 2008), blueberries (Sinelli et al., 2008) and kiwifruits (Clark et al., 2004).

In recent years, the interest has shifted towards the development of portable systems which could be used also in orchards and during post-harvest.

Portable vis/NIR instruments were tested in controlled laboratory conditions or directly in field. Concerning laboratory applications, Puangsombut et al. (2012) investigated the feasibility of using vis/NIR

spectroscopic devices in transmission mode to predict the soluble solids content and total acidity of fresh-cut KaoNumpung pomelo; Antonucci et al. (2011) evaluated the ability of a portable single channel vis/NIR spectrometer to determine soluble solids content and total acidity of two thick peel mandarin cultivars while Camps and Christen (2009) showed that NIR portable devices could be applicable to evaluate apricot quality during post-harvest.

Regarding applications in uncontrolled field conditions, Bodria et al. (2004) used an optical techniques to estimate the ripeness of red-pigmented fruits; Larrain et al., (2008) developed a portable NIR instrument (640-1300 nm) for determining ripeness in wine grapes; Guidetti et al. (2008) tested a vis/NIR device working in reflectance configuration to predict blueberry ripeness; Guidetti et al. (2010) evaluated grape quality parameters by a portable vis/NIR system and finally Beghi et al. (2012) assessed apples nutraceutic properties through a visible and near-infrared portable system.

A NIRs instrument requires reliable hardware systems and complex mathematical techniques to explain chemical information encoded in spectral data (Cogdill and Anderson, 2005).

Chemometric techniques most used are i) spectral preprocessing, to remove any irrelevant information, ii) principal component analysis (PCA), to qualitative data analysis iii) Partial Least Square (PLS) regression analysis, to obtain quantitative prediction of interest parameters (Wold et al., 2001; Naes et al., 2002; Nicolai et al., 2007; Cen & He, 2007).

Chemometrics, in addition to applications mentioned above, can be used for selecting a few number of relevant variables, representing the most useful information of full spectral region (Xiaobo et al., 2010). This selection leads to two main advantages: eliminate variables containing mostly redundant information and spectral noise and reduce the cost of the potential miniaturized devices built only with the selected wavelengths. Generally, the selection of these optimal wavelengths reduce the size of the required measurement data while preserving the most important information contained in the data (Sun, 2010). Liu et al. (2009) proposed three effective wavelength selection methods combined with vis/NIR spectroscopy in order to determine the soluble

solids content of beer including successive projections algorithm (SPA), regression coefficient analysis (RCA) and independent component analysis (ICA). The maximum number of selected wavelengths, by SPA, ranged between 4 and 21 depending on different pretreating. They obtained 10 essential wavelengths both by RCA and by ICA.

Only a few examples of simplified non-destructive commercial devices are already available on the market. For example, the University of Bologna (Costa et al., 2011) patented innovative and simplified NIRs equipments (DA-Meter for apples and Kiwi-Meter for kiwifruits). These devices determine fruit maturity stage through indices based on absorbance differences between specific wavelengths. These indices are correlated with the main traditional parameters as well as with changes in flesh colour. Simple and portable instruments could be used directly on fruits on trees to monitor ripening evolution, and help to choose the best cultural management practices (such as pruning, thinning, nutrition, etc). It reduces the fruit ripening heterogeneity and simplify post-harvest fruit management. Another optical technique used to follow changes during grape ripening is the fluorescence. MultiPlex III (Force A, France) is a portable fluorescence detector provided on the market (Lurie et al., 2012).

Based on of these considerations the aim of this work was to identify the most significant wavelengths able to discriminate the grapes ready to be harvested with a view to a simplified handheld and low-cost optical device for field use which could support the small-scale producers.

The specific objectives of this research were: (1) establish the relationships between the vis/NIR spectra and grape fruit quality parameters, (2) obtain informative wavelength variables based on PLS-RCA variable selection method, (3) compare the prediction performances of calibration models established by PLS on the full vis/NIR spectra and Multiple Linear Regression (MLR) using the selected wavelengths.

Materials and methods

Sampling

The experimental plan monitored the grape ripening process in Valtellina viticultural area (Sondrio, north of Italy) during the last period of ripening just before harvest. Samples of red grape (Nebbiolo cultivar, ecotype Chiavennasca) were collected in four sampling date from September to October 2011. Nebbiolo is one of the most important red grape varieties in Italy. Samples were drawn from 17 different zones, throughout the entire wine area of the valley, in order to represent environmental variability and monitor the entire production region of DOC and DOCG wines. The samples were analyzed before wineries received the grapes.

For each sampling data (7th, 16th and 29th September and 12th October) at first non-destructive analysis were carried out on each sample using portable commercial vis/NIR spectrophotometer then destructive chemical analyses were performed.

A total of 68 samples were analyzed. Each sample was obtained by averaging the spectral acquisitions of 10 individual berries. For each berry 2 acquisitions, in their equatorial region, were performed. Considering the four sampling data, a total of 1360 spectral measurements were determined.

Portable vis/NIR device

Spectral acquisitions were realized on grape berries directly in field using Jaz (vis/NIR spectrophotometer by OceanOptics, USA), optical portable system operating in the wavelength range 400-1000 nm. The JAZ equipment consists of five components: 1) vis/NIR lighting system (halogen lamp); 2) fiber optic probe for reflection measurement; 3) spectrophotometer; 4) hardware for data acquisition and instrument control; 5) battery for power supplying.

Spectra were acquired in reflectance: light radiation was guided to the sample through a Y-shaped, bidirectional fiber optic probe (OceanOptics, USA). Y-shaped fiber allowed to guide light from halogen lamp to illuminate the sample while to collect simultaneously the radiation coming from the fruit and guide it back to the

spectrophotometer. The tip of the optical probe was equipped with a soft plastic cap to ensure contact with sample skin during measurements, while minimizing environmental light interference.

The integrated spectrophotometer was equipped with a diffractive grating for spectral measurements optimized in the range 400-1000 nm and a CCD sensor with a 2048 pixel matrix, corresponding to a nominal resolution of 0.3 nm.

Spectra measurements were taken the field on individual berries after artificial illumination with a probe tip. In order to reduce possible influence of environmental conditions, especially related to diurnal change of sunlight, spectral acquisitions were always done during few hours in the morning with the help of a plastic cap ensuring the contact between the probe tip and the measured berry. Specific tests conducted at different times of the day, confirmed the repeatability of measurements with artificial lighting and berry/probe contact configuration, which evidently were sufficient to cancel out the possible influence of ambient illumination on samples. Air temperature changes during acquisition were limited to range from 15 to 25 °C. Field dataset of spectra were assumed to randomly embed possible environment influencing factors in a representative way.

Chemical Analyses

Total soluble solids content (TSS) were measured using a digital pocket refractometer (DBX-55 ATAGO, Tokyo, Japan) while grape titratable acidity ($\text{g}_{\text{tartaric acid}} \text{dm}^{-3}$) was measured using an automatic titrator (TitroMatic KF 1S, Cison Instruments, Milan, Italy). The Glories method (Glories, 1984) was used to estimate grape phenolic content. According to the method, potential (PA) and extractable anthocyanins (EA), extracted at pH 1 and pH 3.2 respectively ($\text{mg}_{\text{anthocyanins}} \text{dm}^{-3}$), and total polyphenols (TP) were evaluated. The phenolic compounds quantification was based on the optical density (OD) measurement at 520 nm and 280 nm for anthocyanins and polyphenols, respectively, using a UV/vis spectrophotometer (7800, Jasco, Tokyo, Japan).

Data Processing

Spectral data

Chemometric analysis were performed using The Unscrambler® 9.6 software package (CAMO ASA, Norway) in order to extract the maximum usable information from spectral data and to select the most significant wavelengths, which could be used in a vision of a simplified device.

Collected spectra were pre-processed using smoothing (Moving-average, 15 nm window wide) and reduce techniques. Principal Components Analysis (PCA) was performed to explore possible clustering of samples spectra belonging to the sampling date. Loading plots obtained with the PCA were subsequently analyzed to confirm the variable selection realized using Regression Coefficient Analysis (RCA).

Correlation between normalized spectral data matrix and technological (Total Soluble Solids, TSS) and phenolic (polyphenols) parameters were carried out using Partial Least Square (PLS) regression algorithm, in order to identifying relevant wavelengths sorted from the full vis/NIR data. In this procedure, full cross-validation was used to develop a PLS regression model. To evaluate model accuracy, the statistical parameters used were coefficient of determination in calibration (R^2_{cal}), coefficient of determination in cross-validation (R^2_{cv}), root mean square error of calibration (RMSEC), and root mean square error of cross-validation (RMSECV). The best calibrations were selected based on minimizing the RMSECV. Percent errors in cross-validation (RMSECV%) were also calculated as: $RMSECV (\%) = RMSECV / \text{averaged reference values of each parameter}$ (Nicolai et al., 2007; Naes et al., 2004).

Wavelength selection

Regression Coefficient Analysis (RCA) was carried out for relevant variable selection, representing the most useful information of full vis/NIR spectral region (Xiaobo et al.,2010; Chong and. Jun 2005). In literature, this method was applied to select the most important wavelengths to determine TSS of beer (Liu et al., 2009), TSS and pH of

rice vinegars (Liu et al., 2008), TSS and pH in orange juice (Cen et al., 2006).

Standardized regression coefficients of PLS model were used for the elaboration. The standardization takes into account both standard deviation of reflectance, for each wavelength considered, and standard deviation of reference data (TSS and TP) (Frank and Todeschini, 1994).

Regression coefficients obtained by PLS model are used to calculate the Y-variables response value (TSS and polyphenols of grape) from the X-variables (grape spectra). Coefficients numerical size gave an indication of the different variables impact, on the response (Y). The final aim was to find which variables were important for predicting Y-variable. Large absolute values indicate the importance and the significance of the effect on the prediction of Y-variable preference. Hence, RCA could be used for essential wavelength selection.

Peaks and valleys represented the extremum of regression coefficients plot, and peaks and valleys were chosen by a visual selection criterion. This approach was used in previous studies (Liu et al., 2009; Liu et al., 2008).

Finally, qualitative evaluation of the average spectra and loading plot deriving from Principal Component Analysis (PCA) were considered to confirm the effectiveness of RCA variable selection. An additional band was chosen as normalization factor in a range of wavelengths where the contribution of all the PCs tends to cancel out. Spectral reflection intensity measured at selected wavelength ranges were finally used to create three normalized spectral ratios able to predict quality parameters (TSS and polyphenols) for the determination of different grape ripening stages.

Therefore, the three normalized ratios were employed as the input data matrix of PCA and for the elaboration of simple Multiple Linear Regression (MLR) models (Wu et al., 2010; Fernández-Novales et al., 2009; Li et al., 2007). Multiple linear regression (MLR) is a commonly used calibration algorithm which is simple and easy to be interpreted. This method, also, is well suited when variables are less than samples and are not affected by collinearity (Næs and Mevik, 2001). Verify the

prediction skills of MLR models was the final way to study the efficiency of selected wavelengths.

Results and discussion

Descriptive statistics for fruit technological indices (TSS and titratable acidity) and for nutraceutical parameters (potential anthocyanins, total anthocyanins and extractable anthocyanins) are shown in Tables 1. Average data, considering all 4 sampling dates, are shown. In Table 1 are also listed the statistics related to PLS models obtained by vis/NIR spectroscopy for grape qualitative parameters.

The model developed for TSS presents good determination coefficient ($R^2_{cal}=0.83$) and a low value of RMSEC (0.66 °Brix). In validation, R^2_{cv} was lower (0.77) and this value was followed by a slightly higher RMSECV (0.81 °Brix) with RMSECV% equal to 3.8%. Similar results were obtained by Guidetti et al. (2010), showing for fresh berry of red grape Nebbiolo (same conditions of this study), $R^2_{cv}=0.72$ and RMSECV=0.79 °Brix with a portable vis/NIR system, in the range 450-980 nm. Guidetti et al. (2008) tested this vis/NIR device (450-980 nm) also for the prediction of ripening indexes (TSS soluble solids content and total polyphenols) of fresh blueberries ('Brigitta' and 'Duke' varieties). Authors obtained regression models built for fresh berries, in validation, with $R^2=0.72$ and 0.66 for TSS and TP respectively. Concerning TP, regression line achieved in the present study reached $R^2_{cv}=0.74$ with RMSECV%=10.7%. Larrain et al. (2008) obtained also similar results using a portable NIR device (640-1300 nm) on wine grapes (RMSEV ranged from 1.01 to 1.27 °Brix) in field conditions. Beghi et al. (2012) applied portable vis/NIR device (450-980 nm) on the two apple varieties just before fruit harvest, obtaining similar results. For each variety, in fact, TSS estimation gave high R^2_{cv} values (0.72 for "Golden Delicious" and 0.77 for "Stark Red Delicious", in validation) with a low standard error (RMSECV=0.8 °Brix) testifying a high level of prediction performance.

Camps and Christen (2009) tested a commercial vis/NIR portable devices on fresh apricot, in laboratory conditions. PLS models obtained by authors for TSS and titratable acidity prediction, for different analysed varieties, RMSECV% ranged from 5.7 to 9.32 % and from 7.1 to 17.5% respectively.

Titratable acidity data show high coefficient in calibration ($R^2_{\text{cal}}=0.76$) and fair RMSECV value ($0.58 \text{ g}_{\text{titratable acid}} \text{ dm}^{-3}$). In validation, determination coefficient (0.62) is slightly lower, as well as RMSECV ($0.75 \text{ g}_{\text{titratable acid}} \text{ dm}^{-3}$) is lightly higher. The elaboration of a model for PA and EA displayed, in validation, the presence of low values of determination coefficients ($R^2_{\text{cv}}=0.41$ and 0.39) and levels of RMSECV% equal to 19.1% and 17.4% respectively. Guidetti et al. (2010) on fresh grape berries, operating with portable vis/NIR device obtained similar results to our findings. In validation, authors achieved, for titratable acidity, determination coefficient equal to 0.66 and a prediction error of $1.48 \text{ g}_{\text{titratable acid}} \text{ dm}^{-3}$; finally, for phenolic parameters PA and EA, $R^2=0.50$ and 0.46 respectively.

Obtained results show the possible application of the vis/NIR technology for the estimation of the most of measured ripening parameters, as widely shown in literature (Nicolai et al., 2007). The application of portable devices in field conditions is for sure more complex than laboratory experimentation. This is due to the uncontrolled environmental conditions such as ambient light and fluctuating temperatures, as highlighted by some published studies (Nicolai et al. 2007; Wang and Paliwal, 2007).

Table 1. Descriptive statistics and statistics of the PLS models to predict maturity indices and nutraceutical properties of Nebbiolo grape. TSS=Total Soluble Solid content; PA=potential anthocyanins; EA=extractable anthocyanins; TP=total polyphenols; OD=optical density; LV=latent variables

Ripening Parameters	Units	Number of samples	Calibration			Cross-validation				
			Mean	SD	LV	R ² _{cal}	RMSEC	R ² _{cv}	RMSECV	RMSECV%
TSS	°Brix	68	21.1	1.8	7	0.83	0.66	0.77	0.81	3.8%
Titrateable acidity	g _{tartaric acid} dm ⁻³	68	9.1	2.0	8	0.76	0.58	0.62	0.75	8.2%
PA	mg dm ⁻³	68	514.5	139.3	7	0.59	76.49	0.41	98.48	19.1%
EA	mg dm ⁻³	68	357.8	93.7	7	0.60	49.53	0.39	62.18	17.4%
TP	OD 280 nm	68	36.2	7.7	7	0.81	3.24	0.74	3.88	10.7%

Section 6

Wavelengths selection in a vision of a simplified handheld optical system to estimate grape ripeness

The best correlations were found for TSS and TP. Therefore the relevant wavelengths describing the features of spectra for the determination of TSS and TP were selected. The regression coefficients analysis (RCA) was performed starting from PLS models regarding these two parameters.

In particular, standardized regression coefficients of PLS model were used to select the relevant variables. The standardization takes into account both standard deviation of reflectance, for each wavelength of vis/NIR spectra, and standard deviation of reference data (TSS and TP).

Plots of standardized regression coefficients, for TSS and TP, vs vis/NIR wavelengths (400-1000 nm) are shown in figure 1. Trend and shape of regression coefficients plots were similar for TSS and TP. The three most relevant wavelengths were selected choosing the higher absolute regression values (Liu et al., 2008; Cen et al., 2006). Therefore, both for TSS and TP, the three selected wavelengths were 670, 730 and 780 nm.

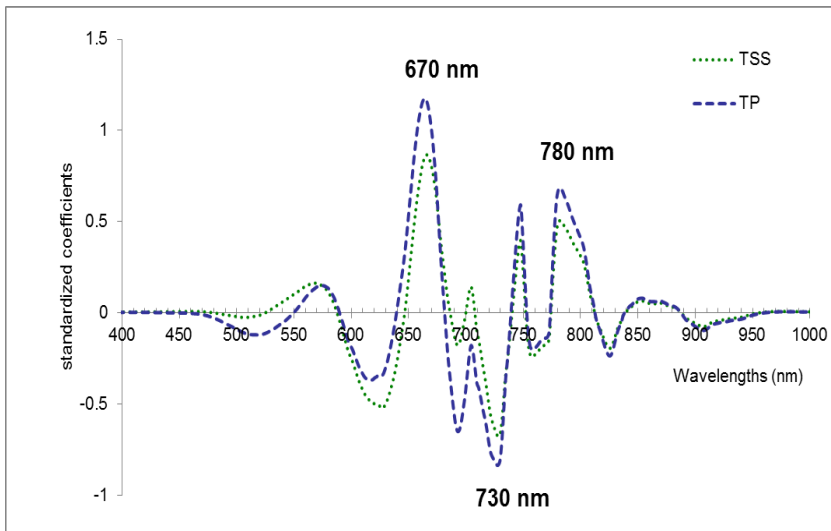


Figure 1. Standardized regression coefficients for total soluble solid content (TSS) and total polyphenols (TP)

Performance of RCA was confirmed using loading plot deriving from PCA and the visual inspection of average spectral curves (Sun, 2010).

The explorative PCA conducted on the full vis/NIR spectra results in three most significant PCs explaining 100% of total data variance (PC1: 97%; PC2: 2% and PC3: 1%). PCA scores plot (Figure 5) shows the evolution of the samples ripening in function of sampling date on PC1. PCs loadings (Figure 2) were analyzed in search of main wavelength bands contributing to PCs as candidate discriminators for the last stages of the ripening process.

With a close observation, PCA loadings plot (Figure 2) shows some positive and negative peaks at certain wavelengths. Corresponding to the three wavelengths (670 nm, 730 and 780 nm) sorted by RCA, loadings plot shows: i) at 670 nm maximum differences among PCs (high positive value for PC1 and high negative value of PC2), ii) at 730 nm the maximum PC1 peak explaining 97% of the total variance iii) at 780 nm a maximum positive value of PC2 couplet with fairly high positive value of PC1. An additional band at 850 nm was chosen as normalization factor in a range of wavelengths where the contribution of all the PCs tends to cancel out.

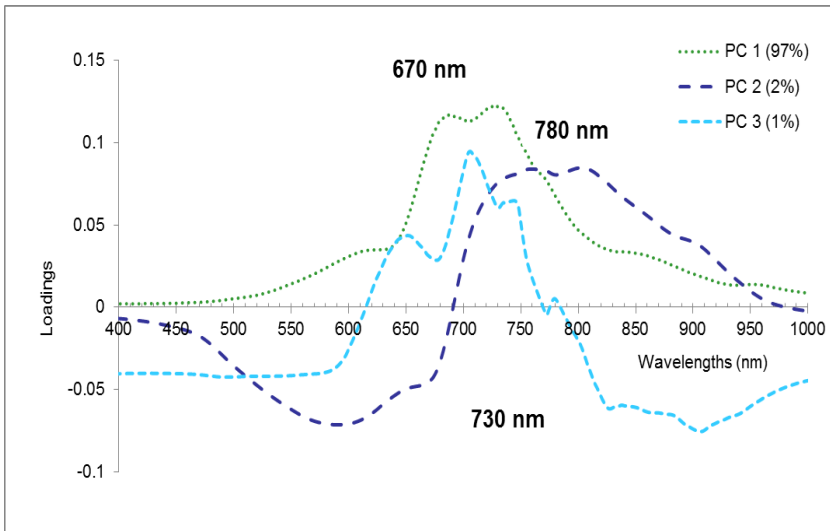


Figure 2. Loadings plot of the first three PCs

Average measured spectra of the four sampling date are shown in figure 3. As expected, spectra exhibit significant differences among sampling date corresponding to the last stages of the ripening process. Changes

Section 6

Wavelengths selection in a vision of a simplified handheld optical system to estimate grape ripeness

in spectra obviously reflect modifications in quality parameters during ripening. Observed changes in the visible region spectra between 500 and 700 nm are due to changes in the amount of pigment, especially linked to anthocyanins accumulation, during the ripening period. This leads to a decrease of reflectance in the visible band associated with anthocyanins absorption peak centered around 540 nm (Tamura & Yamagami, 1994).

The study of spectra evolution during time highlighted the wavelengths more sensible to grape variability during ripening: i) 670 nm, corresponding to chlorophyll absorption peak (McGlone et al., 2002), ii) 730 nm, equal to maximum reflectance peak, iii) 780 nm, representing the third overtone of OH stretching (Clement et al., 2008; Bertrand, 2000).

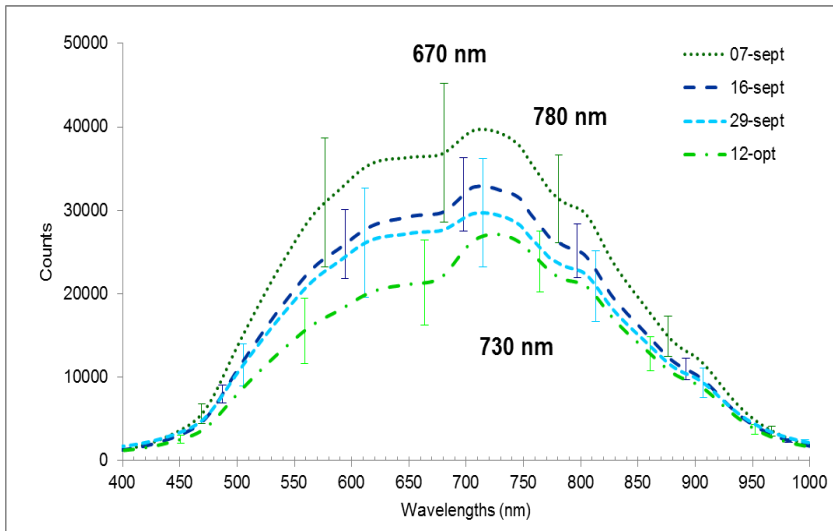


Figure 3. Average raw spectra of 1360 grape berries grouped in four sampling date corresponding to the last stages of the ripening process. Bars indicate the standard deviation within each group at different wavelengths

Reflection absorbance values of the three discriminating wavelengths were divided by diffuse spectral reflectance value of the normalization one (850 nm) to obtain three normalized spectral ratios.

An overview of the variable selection method is summarized in figure 4

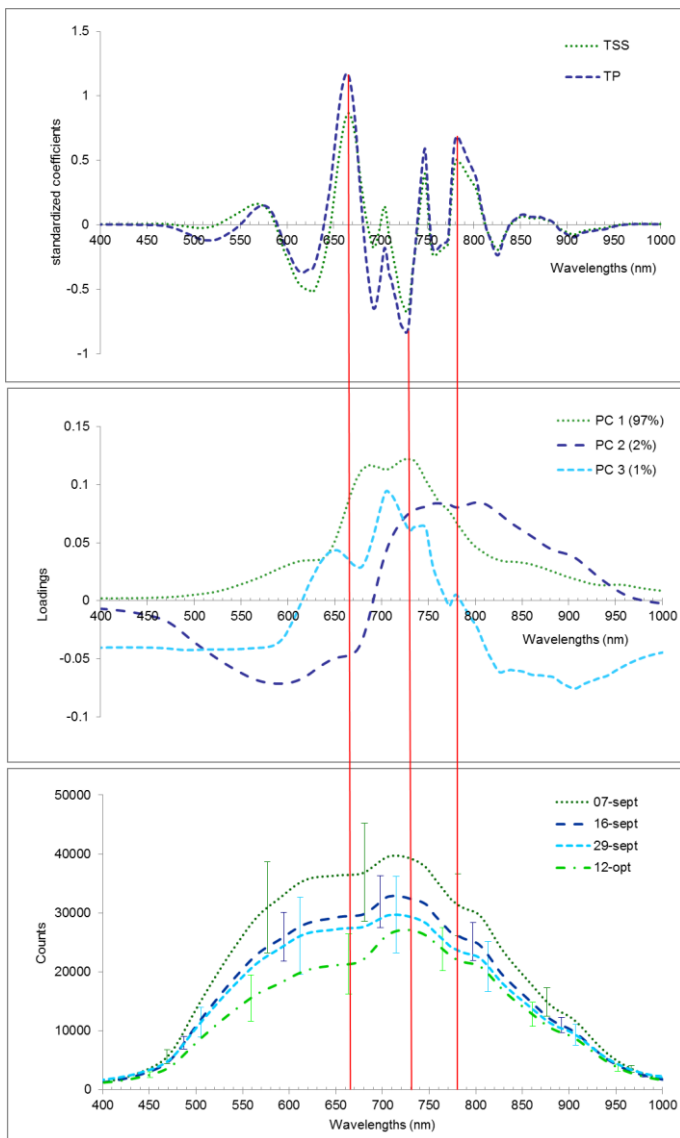


Figure 4. Overview of the used variable selection method

Section 6

Wavelengths selection in a vision of a simplified handheld optical system to estimate grape ripeness

Spectral reflection intensity measured at selected wavelengths were finally used to predict quality parameters (TSS and TP) for the determination of different grape ripening stages. Therefore, the three normalized ratios were employed as the input data matrix of PCA and for the elaboration of simple Multiple Linear Regression (MLR) models. Verify the prediction skills of MLR models was the final way to study the efficiency of selected wavelengths.

Comparison between PCA deriving from the initial full vis/NIR spectra (Figure 5) and PCA deriving from the only three normalized spectral ratios (Figure 6), consisting of sorted wavelengths, was carried out. In both graphs are represented the scores plot relatively to PC1 vs PC2. Regarding PCA scores plot of full spectra, ripening process is well fitted by PCA: spectral data corresponding to different sampling dates are sorted from negative PC1 and positive PC2 values to positive PC1 and negative PC2 values. The same behavior is displayed in PCA scores plot of PCA arising from normalized spectral ratios. In both cases PC1 reveals greatly accounts for separating grape in terms of ripening stages.

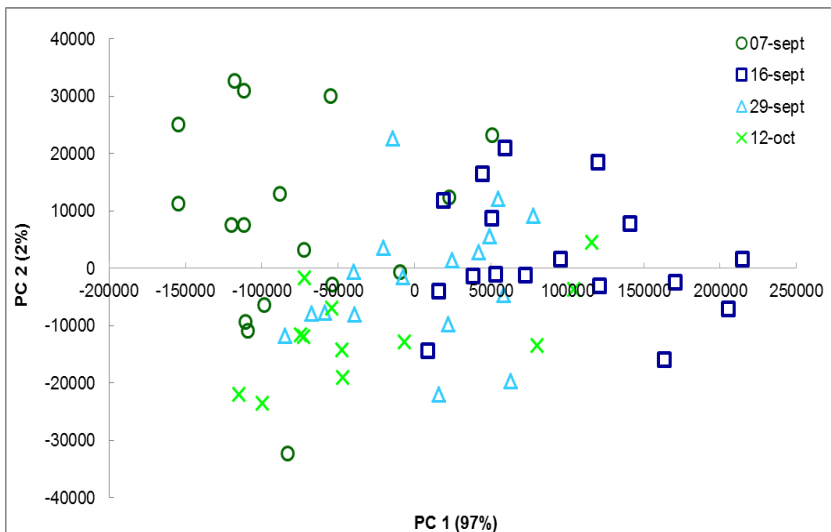


Figure 5. PCA deriving from the initial full vis/NIR spectra

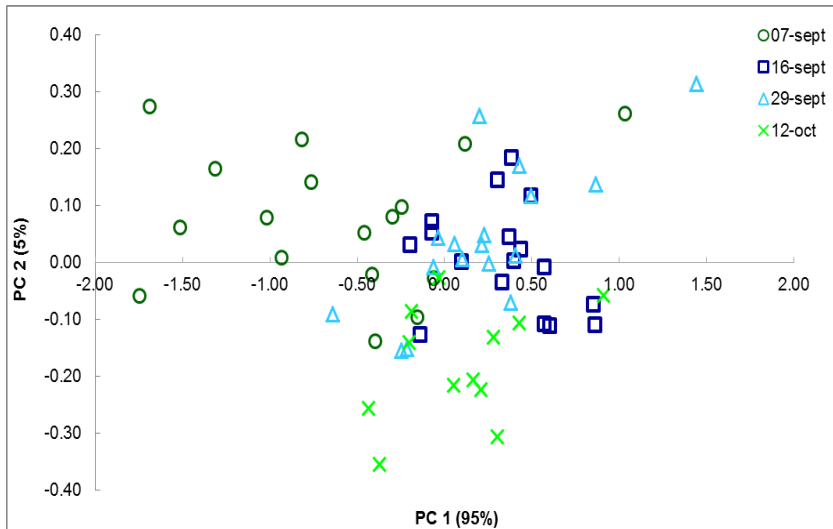


Figure 6. PCA deriving from the only three normalized spectral ratios

According to the variable selection methods stated above, normalized spectral ratios were employed as the inputs for MLR models elaboration on TSS and TP. Results are shown in table 2.

For TSS and TP prediction, statistics of MLR models are equal to $R^2_{CV} = 0.71$ and 0.70 , $RMSECV = 0.83$ °Brix and 4.3 OD_{280} respectively.

Wu et al. in 2010 tested a similar selection approach on transmittance spectra (325-1075 nm) for the investigation of TSS and pH of grape juice beverages. $RMSECV$ obtained by authors, in validation, are 0.360 and 0.054 for TSS and pH respectively. Results are better than our findings but experimental setting with controlled laboratory conditions and the homogeneous matrix of the juice helped these excellent outcomes.

Good results were also obtained by Li et al., (2007) on tea soft drink and by Liu et al., (2009) on beer, using analogues variable selection and validation approaches.

Table 2. Descriptive statistics and statistics of the MLR models to predict on Nebbiolo grape ripeness parameters. TSS=Total Soluble Solid content; TP=total polyphenols; OD=optical density; LV=latent variables

Ripening Parameters	Units	Number of samples	Calibration				Cross-validation		
			Mean	SD	R ² _{cal}	RMSEC	R ² _{cv}	RMSECV	RMSECV%
TSS	°Brix	68	21.1	1.8	0.75	0.77	0.71	0.83	3.9%
TP	OD 280 nm	68	36.2	7.7	0.74	4.0	0.70	4.3	11.9%

Comparison between PLS deriving from full vis/NIR spectra and MLR arising from the only three normalized spectral ratios, was carried out on TSS and TP.

The overall calibration and prediction results of the MLR models, both for TSS and TP prediction, are satisfactory, although the performance of MLR models were slightly worse than the good PLS models. Obtained RMSECV values are similar for PLS and MLR models (0.81 °Brix, 3.9 OD₂₈₀; 0.71 °Brix and 4.3 OD₂₈₀ for PLS models and MLR models, respectively). This mean that only a small information loss is noticeable between PLS model calculated using 2048 wavelengths and MLR models obtained employing only three effective variables. Moreover, the samples are distributed closely to the regression line, which shows an excellent spectral analysis performance of PLS-RCA-MLR method, figures 7 and 8 for TSS and figures 9 and 10 for TP.

Wu et al. in 2010 applied similar selection approach on transmittance spectra for the investigation of TSS and pH of grape juice beverages. Authors obtained optimal determination coefficients, in validation, for both TSS that for pH ranged from 0.89 to 0.97 and 0.91 to 0.96 respectively, relating PLS models, deriving from the full spectra (325-1075 nm). MLR analysis was applied to verify results of wavelengths selection. Regarding TSS, authors gained very good results compared with PLS models with $R^2_{cv}=0.97$ and 0.98 for 5 and 9 variables selected and. For pH they achieved analogous results with determination coefficients, equal to 0.96 and 0.97 respectively.

Concerning reducing sugar content on red grape must, Fernández-Navales et al., (2009) found slight decline between PLS model calculated on the full spectra (800-1050 nm) and MLR based on 4 sensitive wavelengths (RMSEP_{PLS}=12.20 g dm³; RMSEP_{MLR}=20.51 g dm³).

Section 6

Wavelengths selection in a vision of a simplified handheld optical system to estimate grape ripeness

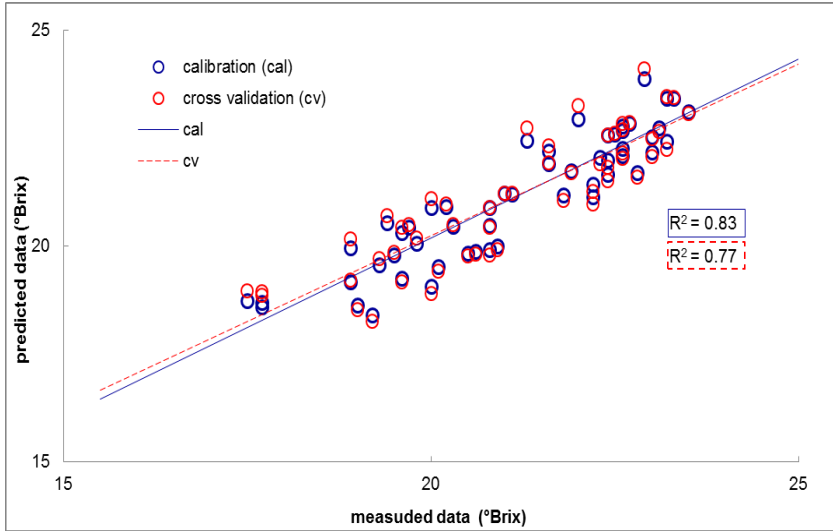


Figure 7. PLS total soluble solid content (TSS) model deriving from the initial full vis/NIR spectra

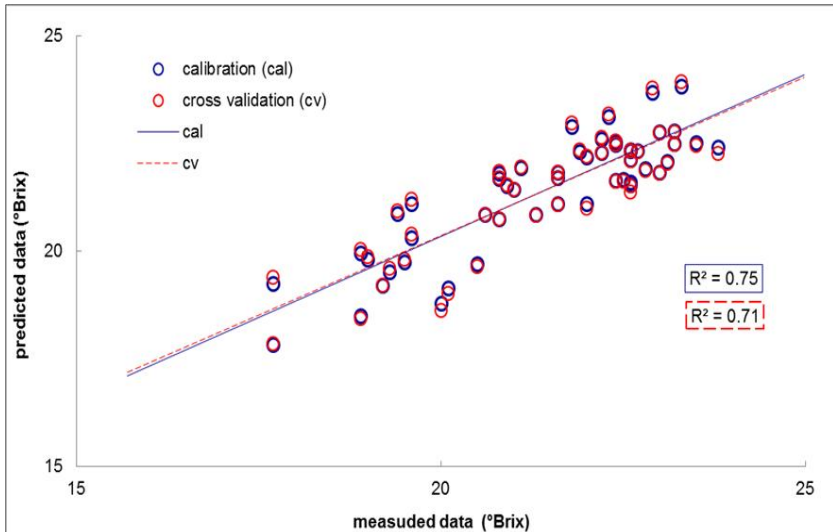


Figure 8. MLR total soluble solid content (TSS) model deriving from the only three normalized spectral ratios

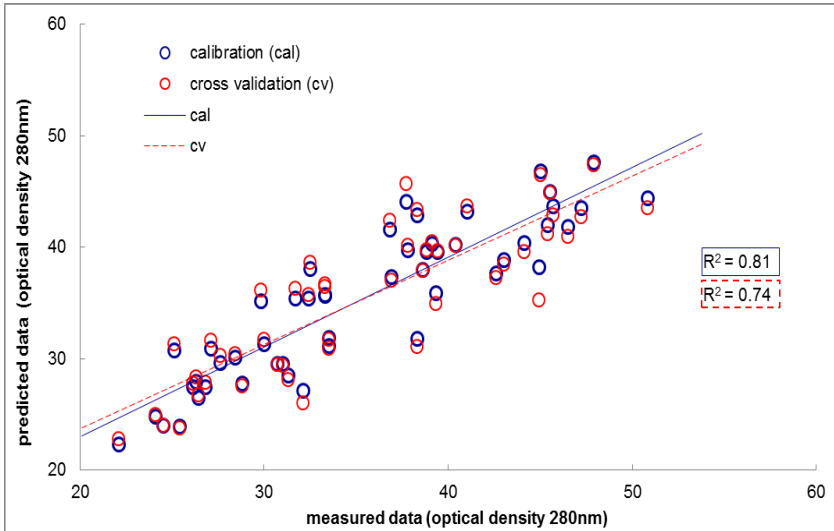


Figure 9. PLS total polyphenols (TP) model deriving from the initial whole vis/NIR spectra

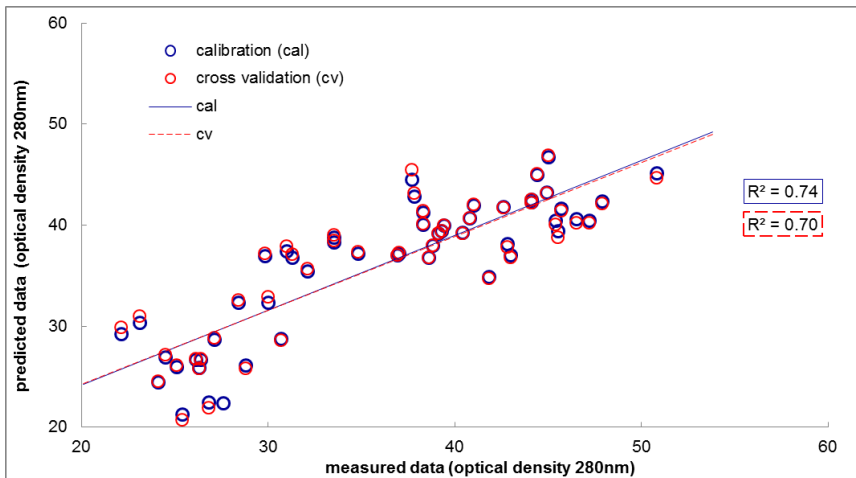


Figure 10. MLR total polyphenols (TP) model deriving from the only three normalized spectral ratios

Conclusions

In this work, a variable selection methodology has been proposed in order to select a reduced set of features that are effective in the detection of grape ripening using vis/NIR spectroscopy.

The aim was to identify, using PLS-RCA variable selection method, the effective wavelengths able to discriminate the grapes ready to be harvested with a view to a simplified handheld and low-cost optical device for field use which could support the small-scale producers.

The three selected candidate wavelengths were 670 nm, 730 nm and 780 nm, with an additional normalization band at 850 nm.

Reflection absorbance of the effective discriminating wavelengths and the normalization one were combined to obtain three normalized spectral ratios. PCA and MLR were applied to ratios in order to validate prediction skills, compared with PCA and PLS models on the full spectra, and verify the effectiveness of selected wavelengths. Both qualitative and quantitative analysis highlighted good results showing a good samples separation during ripening and confirmed the right wavelengths choice.

The potential of vis/NIR to predict TSS and TP was ascertained, and the fingerprint spectra, which were strongly related with these indices, were obtained through multivariable analysis. These individual fingerprint wavelengths could be used for the design of a simplified, low-cost handheld device which would allow real-time assessment of grape ripeness in field.

In particular this device will be based on measurement and processing of diffuse spectral reflectance at a few appropriately selected wavelengths. These type of optical tools could be suitable for small scale wineries thanks to non-complex data in order to avoid specific chemometric analyses and trained personnel.

References

- Antonucci, F.; Pallottino, F.; Paglia, G.; Palma, A.; D'Aquino, S. & Menesatti, P. 2011. Non-destructive estimation of Mandarin maturity status through portable VIS-NIR spectrophotometer. *Food and Bioprocess Technology*, 4(5), 809-813
- Beghi, R.; Spinardi, A.; Bodria, L.; Mignani, I. & Guidetti, R., 2012. Apples nutraceutic properties evaluation through a visible and near-infrared portable system. *Food and Bioprocess Technology* DOI 10.1007/s11947-012-0824-7
- Bertrand, D., 2000. Spectroscopie de l'eau. In *La Spectroscopie Infrarouge et ses Applications Analytiques*; Bertrand, D., Dufour, E., Eds.; TEC & DOC: Paris, France, 93-105
- Bodria, L.; Fiala, M.; Guidetti, R. & Oberti, R., 2004. Optical techniques to estimate the ripeness of red-pigmented fruits. *Trans. of ASAE* 47, 815-820
- Camps, C. & Christen, D., 2009. Non-destructive assessment of apricot fruit quality by portable visible-near infrared spectroscopy. *Food Science and Technology*, 42(6), 1125-1131
- Cayuela, A., 2008. Vis/NIR soluble solids prediction in intact oranges (*Citrus sinensis* L.) cv. Valencia Late by reflectance. *Postharvest Biol. Technol.* 47(1), 75-80
- Cen, H. & He, Y., 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. *Trends in Food Science & Technology*, 18, 72-83
- Cen, H.; He, Y. & Huang M., 2006. Measurement of Soluble Solids Contents and pH in Orange Juice Using Chemometrics and Vis-NIRS. *J. Agric. Food Chem.* 2006, 54, 7437-7443
- Chong, I.G. & Jun, C.H., 2005. Performance of some variable selection methods when multicollinearity is present. *Chemom. Intell. Lab. Syst.*, 78, 103-112
- Clark, C.J.; McGlone, V.A.; De Silva, H.N.; Manning, M.A.; Burdon, J. & Mowat, A.D., 2004. Prediction of storage disorders of kiwifruit

(*Actinidia chinensis*) based on visible-NIR spectral characteristics. *Postharvest Biol. Technol.* 32, 147-158.

Clement, A.; Dorais, M. & Vernon, M., 2008. Nondestructive Measurement of Fresh Tomato Lycopene Content and Other Physicochemical Characteristics Using Visible-NIR Spectroscopy. *J. Agric. Food Chem.*, 56, 9813-9818

Cogdill, R.P. & Anderson, C.A., 2005. Efficient spectroscopic calibration using net analyte signal and pure component projection methods. *J. Near Infrared Spectrosc.* 13(3), 119-132

Costa, G.; Bonora, E.; Fiori, G. & Noferini, M., 2011. Innovative non-destructive device for fruit quality assessment. *Acta Hort. (ISHS)* 913, 575-581

Cozzolino, D.; Damberg, R.G.; Janik, L.; Cynkar, W.U. & Gishen, M., 2006. Analysis of grapes and wine by near infrared spectroscopy. *Journal of Near Infrared Spectroscopy*. Chichester, UK. 14, 279-289.

Fernández-Navales, J.; López, M.I.; Sánchez, M.T.; Morales, J. & González-Caballero, V., 2009. Shortwave-near infrared spectroscopy for determination of reducing sugar content during grape ripening, winemaking, and aging of white and red wines, *Food Research International*, 42(2), 285-291

Frank, I.E. & Todeschini, R. (1994). *The Data Analysis Handbook*. Elsevier. ISBN 0-444-81659-3, included in series: *Data Handling in Science and Technology*.

Glories Y., 1984. The colour of red wines. *Connaissance de la Vigne et Du Vin*, 18, 195-217

Guidetti, R.; Beghi, R. & Bodria, L., 2010. Evaluation of Grape Quality Parameters by a Simple Vis/NIR System. *Trans. of the ASABE* 53(2), 477-484

Guidetti, R.; Beghi, R.; Bodria, L.; Spinardi, A.; Mignani, I. & Folini, L., 2008. Prediction of blueberry (*Vaccinium corymbosum*) ripeness by a portable Vis-NIR device. *Acta Horticulturae* 310, ISBN 978-90-66057-41-8, 877-885

- Larrain, M.; Guesalaga, A.R.; Agosin, E.; 2008. A Multipurpose Portable Instrument for Determining Ripeness in Wine Grapes Using NIR Spectroscopy. *Instrumentation and Measurement, IEEE Transactions* 57(2), 294-302
- Li, X.; He, Y.; Wu C. & Sun, D.W., 2007. Nondestructive measurement and fingerprint analysis of soluble solid content of tea soft drink based on Vis/NIR spectroscopy. *Journal of Food Engineering* 82, 316.323
- Liu, F.; He, Y. & Wang, L., 2008. Comparison of calibrations for the determination of soluble solids content and pH of rice vinegars using visible and short-wave near infrared spectroscopy. *Anal. Chim. Acta* 610, 196-204
- Liu, F.; Jiang, Y. & He, Y., 2009. Variable selection in visible/near infrared spectra for linear and nonlinear calibrations: A case study to determine soluble solids content of beer. *Analytica Chimica Acta*, 635, 45-52
- Lurie, S.; Friedman, H.; Dagar, A.; Weksler, A.; Rot, I.; Kaplunov, T.; Zutahy, Y.; Nyasordzia, J. & Lichter, A., 2012. Non-destructive Tools for Determining Harvest Dates of Deciduous Fruits. CIGR Section VI International Technical Symposium on "Innovating the Food Value Chain" Postharvest Technology and Agri-Food Processing. Stellenbosch, South Africa, 25-28 November, 2012.
- McGlone, V.A.; Jordan, R.B. & Martinsen, P.J., 2002. Vis/NIR estimation at harvest of pre- and post-storage quality indices for 'Royal Gala' apple. *Postharvest Biology and Technology*, 25(2), 135-144
- Næs, T. & Mevik, B.H., 2001. Regression models with process variables and parallel blocks of raw material measurements. *J. Chemometr.* 15 413-426
- Naes, T.; Isaksson, T.; Fearn, T. & Davies, T., 2002. A user-friendly guide to multivariate calibration and classification. Chichester, UK: NIR Publications ISBN 0-9528666-2-5
- Nicolai, B.M.; Beullens, K.; Bobelyn, E.; Peirs, A.; Saeys, W.; Theron & K.I., Lammertyna J., 2007. Non-destructive measurement of fruit and

- vegetable quality by means of NIR spectroscopy: A review. *Postharvest Biology and Technology*, 46, 99-118
- Peirs, A.; Schenk, A. & Nicolaï, B.M., 2005. Effect of natural variability among apples on the accuracy of VIS-NIR calibration models for optimal harvest date predictions. *Postharvest Biology and Technology* 35(1), 1-13
- Pérez-Marín, D.; Sánchez, M.T.; Paz, P.; Soriano, M.A.; Guerrero, J.E. & Garrido-Varo, A., 2009. Non-destructive determination of quality parameters in nectarines during on-tree ripening and postharvest storage. *Postharvest Biology and Technology* 52(2), 180-188
- Puangsoambut A.; Pathaveerat S.; Terdwongworakul A. & Puangsoambut K., 2012. Evaluation of internal quality of fresh-cut pomelo using vis/NIR transmittance. *Journal of texture studies*, 43, 445-452
- Sinelli, N.; Spinardi, A.; Di Egidio, V.; Mignani, I. & Casiraghi, E., 2008. Evaluation of quality and nutraceutical content in blueberries (*Vaccinium corymbosum* L.) by near and mid-infrared spectroscopy. *Postharvest Biol. Technol.*, 50(1), 31-36.
- Slaughter, D.C.; Thompson, J.F. & Tan, E.S., 2003. Non-destructive determination of total and soluble solids in fresh prune using near infrared spectroscopy. *Postharvest Biol. Technol.*, 28, 437-444
- Sun, D.W., 2010. *Hyperspectral imaging for food quality analysis and control*. Edited by Da-Wen Sun. Elsevier. ISBN 978-0-12-374753-2
- Sun, T.; Lin, H.; Xu, H. & Ying, Y., 2009. Effect of fruit moving speed on predicting soluble solids content of 'Cuiguan' pears (*Pomaceae pyrifolia* Nakai cv. Cuiguan) using PLS and LS-SVM regression. *Postharvest Biology and Technology*, 51(1), 86-90
- Tamura H. & Yamagami A, 1994. Antioxidative Activity of Monoacylated Anthocyanins Isolated from Muscat Bailey A Grape. *J. Agric. Food Chem.* 42, 1612-1615
- Wang, W. & Paliwal, J., 2007. Near-infrared spectroscopy and imaging in food quality and safety. *Sens. & Instrumen. Food Qual.* 1,193-207
- Wold, S.; Sjöström, M. & Eriksson, L., (2001). PLS-regression: a basic tool of chemometrics. *Chemom. Intell. Lab. Syst.* 58, 109-130

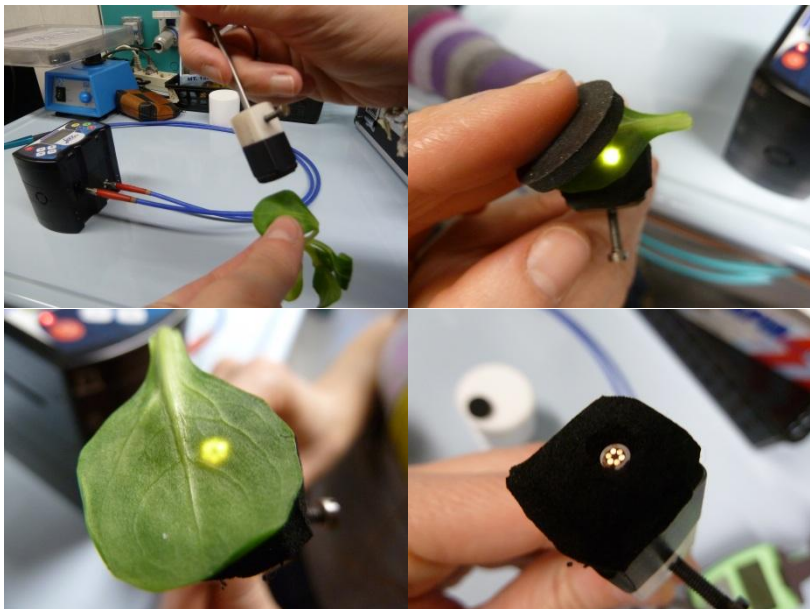
- Wu, D.; He, Y.; Nie, P.; Cao, F. & Bao, Y., 2010. Hybrid variable selection in visible and near-infrared spectral analysis for non-invasive quality determination of grape juice, *Analytica Chimica Acta*, 659(1-2), 229-237
- Xiaobo, Z.; Jiewen, Z.; Povey, M.J.W.; Holmes, M. & Hanpin M., 2010. Variables selection methods in near-infrared spectroscopy. *Analytica Chimica Acta* 667, 14-32
- Ziosi, V.; Noferini, M.; Fiori, G.; Tadiello, A.; Trainotti, L.; Casadoro, G. & Costa, G., 2008. A new index based on vis spectroscopy to characterize the progression of ripening in peach fruit. *Postharvest Biology and Technology*, 49(3), 319-329

Section 7

Feasibility of a simplified handheld optical system to evaluate fresh-cut *Valerianella locusta* Laterr.

Valentina Giovenzana, Roberto Beghi, Raffaele Civelli, Riccardo Guidetti

Department of Agricultural and Environmental Sciences
Università degli Studi di Milano, via Celoria 2, Milano, 20133 Italy



Abstract

Fresh-cut fruit and vegetable sector could be greatly helped by new analytical methods that are accurate, rapid and integrated into production process to meet consumer demand.

The aim of this work was to study the feasibility of a simplified handheld and low-cost optical device, based on measurement and processing of diffuse reflectance at a few wavelengths appropriately selected. This study was focused on identifying the most significant wavelengths able to discriminate freshness levels during shelf life of fresh-cut Lamb's lettuce (*Valerianella locusta* Laterr.).

For this aim vis/NIR spectra, in the range 340-1000 nm, were acquired for Lamb's lettuce stored at 4 °C and 20 °C up to 7 and 5 days respectively. A total of 140 samples were analyzed and divided into two categories: before (C1) and after (C2) commercial expiration date.

Principal Component Analysis (PCA) highlighted two principal spectral bands (at 680 nm and 740 nm) in which differences between C1 and C2 samples spectra were maximized. Reflectance values at these wavelengths were used to define specific normalized spectral ratios. Based on these relations, a simple classification index was proposed.

Results demonstrated the feasibility of a simplified and low-cost handheld device, based on the use of only three wavelengths (680 nm, 740 nm and 850 nm), able to quickly monitoring shelf life of fresh-cut Lamb's lettuce.

Keywords *Valerianella locusta* Laterr, shelf life, fresh-cut vegetables, vis/NIR spectroscopy, non-destructive analysis

Introduction

Changes in human life styles have produced a demand for a wider range of products, and have led people to spend less time cooking at home and to eat out more often. Such trends have been reflected in an increase of minimally-processed convenience foods that are ready-to-eat (Abadias, Usall, Anguera, Solsona, & Viñas, 2008).

Particularly in recent years, there has been an increase in the consumption, in European countries, of fresh-cut or minimally processed fruit and vegetables due to their ease of use and nutritional properties.

Fresh-cut products are fruit and vegetables that have been washed, trimmed, peeled and/or cut into 100% usable products, and packaged in the right size.

These products are often subjected to rapid loss of colour, organic acids, vitamins and other compounds that determine flavour and nutritional value.

Usually the shelf life of these products is much shorter than that of unprocessed vegetables. The selling period on the shelf of markets is limited to 5-7 days for most leafy vegetables.

A critical aspect relevant for this type of products is represented by their actual shelf life, which is often lower than the expiring date reported on the label by the producer.

In literature, shelf life of fresh-cut fruit and vegetables is often studied by the analysis of chlorophyll fluorescence (Baldassarre, Cabassi, & Ferrante, 2011; Schofield, DeEll, Murrc & Jenni, 2005; Moshou, Wahlen, Strasser, Schenk, De Baerdemaeker, & Ramon, 2005). In *Valerianella* leafy vegetables stored at 4 and 10°C, Ferrante & Maggiore in 2007 analysed some key parameters of chlorophyll *a* fluorescence and some derived indexes to describe the progression of senescence and loss of product quality.

Colour is one of the most widely measured product quality attributes in postharvest handling and in the food processing research and industry. Colour is an important quality attribute and it influences consumer's choice and preferences (Pathare, Opara & Al-Julanda Al-Said, 2012).

Ferrante, Incrocci, Maggini, Serra & Tognoni, in 2004 studied colour changes of fresh-cut leafy vegetables during storage time.

Light conditions during conservation of fresh-cut vegetables could influence important quality characteristics. Zhana, Lia, Hub, Panga & Fana in 2012 studied the effect of continuous high intensity light, low intensity light and darkness on browning and quality of fresh-cut romaine lettuce upon 7 days of cold storage.

In food industry the interest in image analysis techniques is growing, since food appearance contains a variety of information directly related to the quality of the product itself. These characteristics are difficult to be measured through classical analysis methods. In addition, image analysis techniques provide information much more accurate than human vision, are objective and continuous over time and offer the great advantage of being non-destructive. An imaging process to classify spinach leaves according to colour degradation during storage was proposed by Lunadei, Diezma, Lleó, Ruiz-Garcia, Cantalapiedra & Ruiz-Altisent in 2012. Løkke, Seefeldt, Skovb & Edelenbos in 2013 published a study regarding the application of multispectral imaging for the analysis of important attributes for the perception of freshness of wild rocket like green colour and texture.

Among the non-destructive techniques, the optical analysis in the region of near infrared (NIR) and visible-near infrared (vis/NIR), based on the use of information arising from the interaction between the structure of food and light, has met a significant development in the last 20 years.

Vis/NIR spectroscopy measures the spectral components of light emerging from sample after penetrating its surface and being partly absorbed and partly backscattered by the first internal layers. Measuring the spectral modifications of the radiation after interaction with the sample is possible to establish a relationship between spectral reflectance (or transmittance) and specific components concentration in the product. Reflectance measurement technique in vis/NIR is well suited to investigate the main components of leafy samples or the first representative layers of fruit pulp, while it may give limited results when investigating most inner part of fruits and vegetables (Guidetti, Beghi & Giovenzana, 2012). Portable vis/NIR instruments are often used in controlled laboratory conditions (Antonucci, Costa, Pallottino, Paglia,

Rimatori, De Giorgio et al., 2011). However, this acquisition technique is particularly versatile and is suitable to design compact, portable, handheld devices, to be directly used everywhere to monitor quality parameters (Beghi, Spinardi, Bodria, Mignani & Guidetti, 2012; Bodria, Fiala, Guidetti & Oberti, 2004).

The aim of this work was to study the feasibility of a simplified handheld and low-cost optical device, based on measurement and processing of diffuse reflectance at a few wavelengths appropriately selected. This study was focused on identifying the most significant wavelengths able to discriminate freshness levels during shelf life of fresh-cut Lamb's lettuce (*Valerianella locusta* Laterr.).

Materials and methods

Vis/NIR spectra were acquired in December 2011 on Lamb's lettuce cultivated in Lombardy, north of Italy, and stored at 4 °C and 20 °C up to 7 and 5 days respectively. A total of 140 samples were analyzed and divided into two categories: before (C1) and after (C2) commercial expiration date. Spectra measurements were taken on individual leaves in three different points after artificial illumination with a probe tip.

Vis/NIR device

Spectral acquisitions were realized on samples using an optical portable system (JAZ vis/NIR spectrophotometer by OceanOptics, USA) operating in the wavelength range 340-1000 nm. The JAZ equipment is composed of five components: a) vis/NIR lighting system; b) fiber optic probe for reflection measurement; c) spectrophotometer; d) hardware for data acquisition and instrument control; e) battery for power supplying.

Spectra were acquired in reflectance: light radiation was guided to the sample through a Y-shaped, bidirectional fiber optic probe (OceanOptics, USA). Y-shaped fiber allowed to guide light from halogen lamp to illuminate the sample while to collect simultaneously the radiation coming from the leaf and guide it back to the spectrophotometer. The tip of the optical probe was equipped with a soft plastic cap to ensure contact with sample skin during measurements, while minimizing environmental light interference (Figure 1).

Section 7

Feasibility of a simplified handheld optical system to evaluate fresh-cut *Valerianella locusta* Laterr.

The spectrophotometer was equipped with a diffractive grating for spectral measurements optimized in the range 340-1000 nm and a CCD sensor with a 2048 pixel matrix, corresponding to a nominal resolution of 0.3 nm.

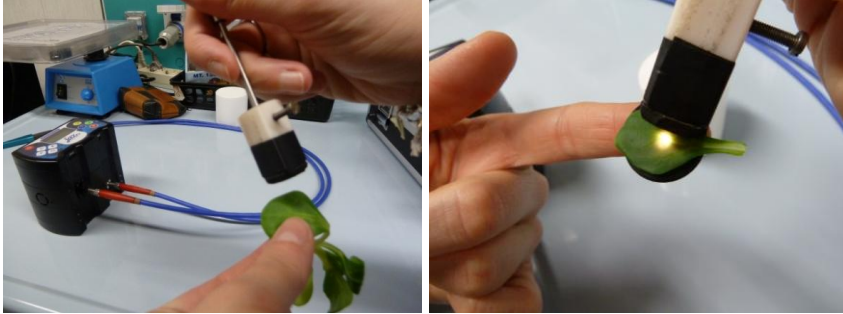


Figure 1. Particular of spectral acquisitions on Lamb's lettuce with JAZ vis/NIR system

Data processing

Chemometric analysis of spectra was based on Unscrambler 9.6 software package (CAMO ASA, Norway). Moving-averaged smoothed spectra were used for Principal Components Analysis (PCA) to explore possible clustering of homogeneous samples spectra belonging to the same shelf-life class (Massart, Vandeginste, Buydens, De Jong, Lewi & Smeyers-Verbek, 1997; Naes, Isaksson, Fearn & Davies, 2000).

Scores and loading plots obtained by the PCA were analyzed to determine a few wavelength ranges able to better discriminate among different classes, with particular emphasis on differences between class C1 and class C2.

Reflectance values measured at these few selected wavelength ranges were subsequently applied to map the dataset of spectra and to define a linear classifier able to optimally discriminate C1 from C2 Lamb's lettuce samples.

Results and discussion

Results for 4 °C storage temperature

Average raw spectra of the different shelf-life classes at storage temperature of 4 °C in the considered vis/NIR range are shown in figure 2.

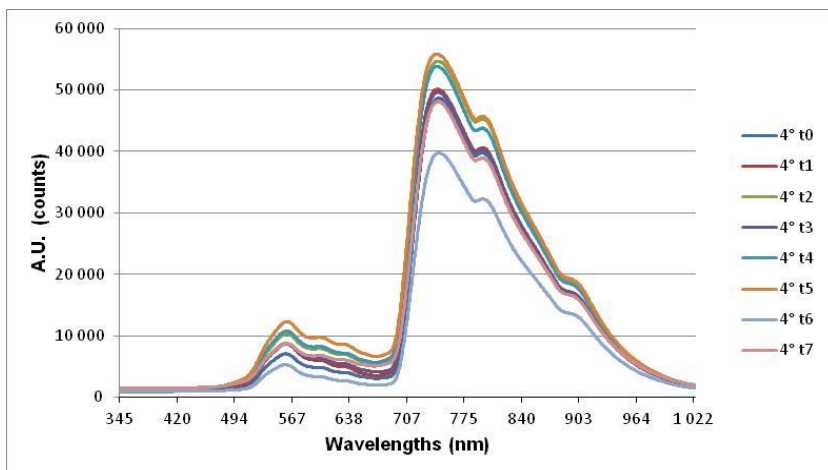


Figure 2. Average spectra of Lamb's lettuce at different storage time (from 0 to 7 days) at 4 °C in the range 340-1000 nm

As expected, spectra exhibit differences as time went by. In particular, the figure shows relevant differences in the reflectance peak centered around 540 nm in the visible band associated with green samples reflectance peak. The same differences could be noticed also around 680 nm, associated, in this case, with the chlorophyll absorption peak. In the NIR region a maximum reflectance peak is shown around 740 nm.

The explorative PCA conducted on 80 spectra stored at 4 °C resulted in two most significant PCs explaining 100% of total data variance (PC1: 96%; PC2: 4%).

Scores plot of PC1 vs PC2 (Figure 3) emphasizes an evolution over time of the samples mainly across PC2.

For this reason on PCs loadings plot (Figure 4) the main wavelengths contributing to PC2 were identified as candidate discriminators for

Section 7

Feasibility of a simplified handheld optical system to evaluate fresh-cut *Valerianella locusta* Laterr.

monitoring shelf-life. Wavelengths with high positive or negative values of PC2 were chosen combined with, when possible, simultaneously low values of the most important component (PC1), which however is not useful for the purpose of separating samples during shelf-life.

Two spectral bands at 680 nm and 740 nm were identified, with an additional band at 850 nm chosen as normalization factor in a range of wavelengths where the contribution of all the PCs tends to be cancelled out.

The candidate discriminating wavelengths were combined to obtain two normalized spectral ratios between the light intensity I at the two different wavelengths: the ratio $S1 = I_{680} / I_{850}$ and the ratio $S2 = I_{740} / I_{850}$. For each measured leaf, the value of $S1$ and $S2$ can be calculated from the intensity of sample's spectrum at the three selected wavelengths.

The spectral ratios $S1$ and $S2$ were expected to discriminate shelf-life stages, and in particular, to discriminate the critical stages around commercial expiration date with special reference to C1 and C2.

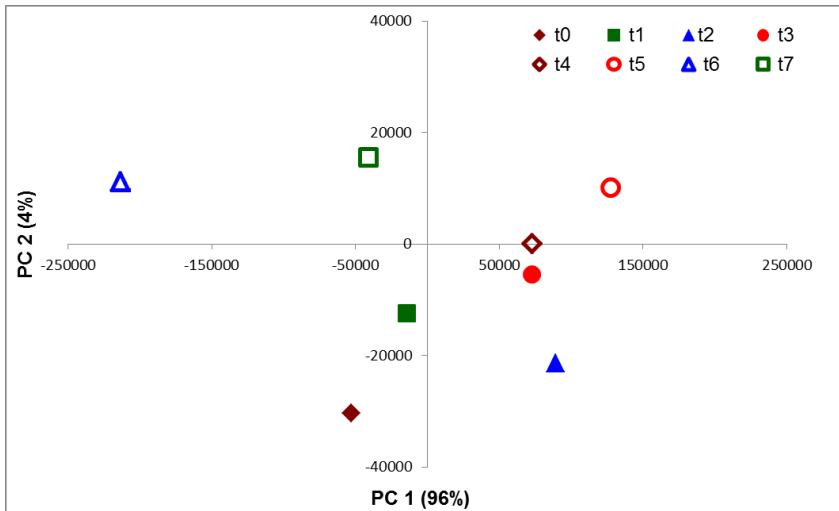


Figure 3. Samples stored at 4 °C. Scores plot for PC1 vs PC2 of averaged samples for each sampling time during shelf-life

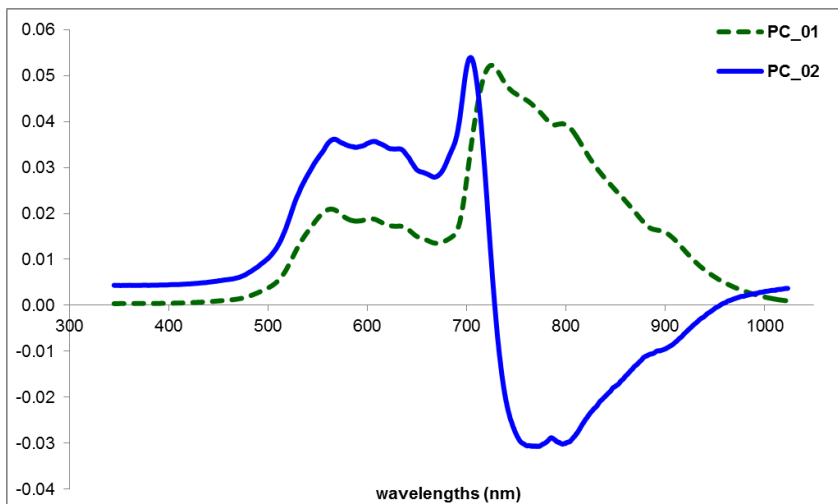


Figure 4. Samples stored at 4 °C. Loadings plot for PC1 and PC2

With this representation of early senescence process during days of shelf-life (Figure 5), the specific problem of discriminating samples belonging to class C1 from those of class C2 can be solved by defining an appropriate subdivision of the S1/S2 plane by a linear discriminant function (LD_4) able to maximize the separation between time 3 and 4 (corresponding to commercial expiration date).

The “C2 domain” can be defined as the region to the right of the separation line LD_4 in S1/S2. Graphically LD_4 represents a specific shelf-life threshold and its equation is:

$$S2 = -1.5 \cdot S1 + 2.218$$

Based on this relation, a Shelf-Life Index for samples stored at 4 °C (SLI_4) was proposed with a threshold value of 2.218:

$$SLI_4 = 1.5 \cdot S1 + S2$$

The SLI_4 value was calculated for each Lamb’s lettuce sample and thanks to this simple classification algorithm, it was possible to classify leaves with $SLI_4 < 2.218$ as fresh samples and samples with $SLI_4 > 2.218$ as no more fresh leaves.

Section 7

Feasibility of a simplified handheld optical system to evaluate fresh-cut *Valerianella locusta* Laterr.

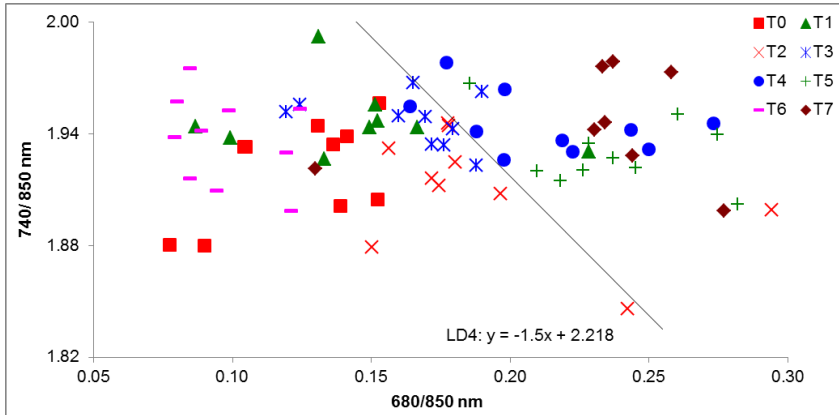


Figure 5. Bidimensional plot of the different shelf-life stages for samples stored at 4 °C. Linear discriminant equation (LD₄) could be identified as the best separation line between C1 and C2 samples

Table 1. Classification according to SLI₄ for samples stored at 4 °C

Classification according to SLI ₄			
	Shelf-life stage	Fresh (C1)	No more fresh (C2)
SLI ₄ (Threshold value 2.218)	C1	37/39 (95%)	2/39 (5%)
	C2	13/41 (32%)	28/41 (68%)

Results regarding classification according SLI₄ gave good percentage of correct classified samples. An interesting positive predictive value (PPV) of 95% was achieved for C1 and PPV of 68% for C2 (Table 1).

Regarding C2 classification results, a wrong classification of all the samples of the time 6 of sampling was highlighted. This could be due to an error during sampling in that day. For this reason, these samples should be considered as outliers, and consequently not taken into account in the classification. In this case the PPV for C2 samples would reach the value of 90% instead of 68%.

Results for 20 °C storage temperature

The proposed analysis for Lamb's lettuce stored at 4 °C was also carried out on samples stored at 20 °C. In this case, the sampling has

been carried out daily for 5 days (condition of evident senescence of leaves).

In this case too, the explorative PCA conducted on 60 spectra stored at 20 °C resulted in two most significant PCs explaining 100% of total data variance (PC1: 99%; PC2: 1%).

Scores plot of PC1 vs PC2 emphasizes also in this case an evolution over time across PC2 of the samples (less evident respect to leaves stored at 4 °C).

For this reason on PCs loadings plot (Figure 6), the main wavelengths contributing to PC2 were identified as candidate discriminators for monitoring shelf-life. Due to a similar trend of loadings respect to 4 °C, the two spectral bands previously identified (680 nm and 740 nm), with the additional band at 850 nm, were chosen again for monitoring shelf-life at 20 °C.

Similarly, the candidate discriminating wavelengths were combined to obtain two normalized spectral ratios: the ratio $S1 = I_{680} / I_{850}$ and the ratio $S2 = I_{740} / I_{850}$. For each measured leaf, the value of S1 and S2 can be calculated from the intensity of sample's spectrum at the three selected wavelengths.

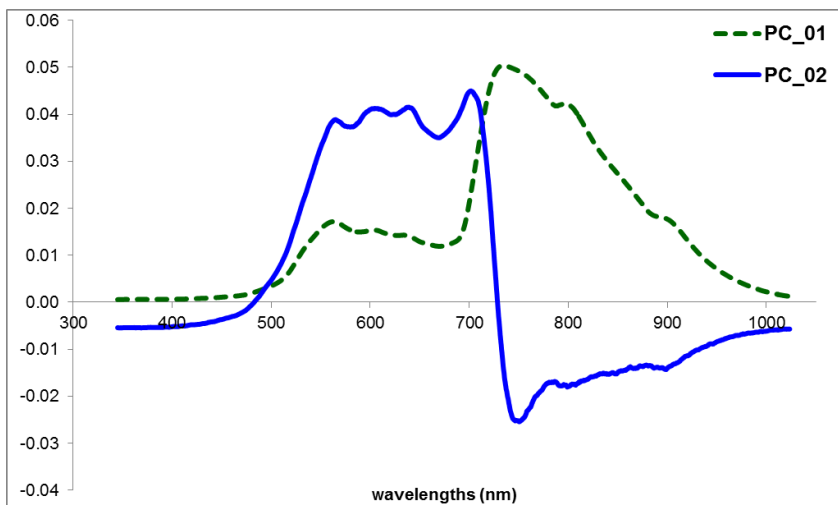


Figure 6. Samples stored at 20 °C. Loadings plot for PC1 and PC2

Section 7

Feasibility of a simplified handheld optical system to evaluate fresh-cut *Valerianella locusta* Laterr.

Also in this case the goal was to discriminate samples belonging to class C1 from those of class C2 by defining an appropriate subdivision of the S1/S2 plane by a linear discriminant function (LD_{20}) able to maximize the separation between time 3 and 4 (corresponding to commercial expiration date).

The “C2 domain” can be defined as the region to the right of the separation line LD_{20} in S1/S2. Graphically LD_{20} represents a specific shelf-life threshold and its equation is (Figure 7):

$$S2 = -1.8 \cdot S1 + 2.266$$

Based on this relation, a Shelf-Life Index for samples stored at 20 °C (SLI_{20}) was proposed with a threshold value of 2.266:

$$SLI_{20} = 1.8 \cdot S1 + S2$$

The SLI_{20} value was calculated for each Lamb's lettuce sample and thanks to this simple classification algorithm, it was possible to classify leaves with $SLI_{20} < 2.266$ as fresh samples and samples with $SLI_{20} > 2.266$ as no more fresh leaves.

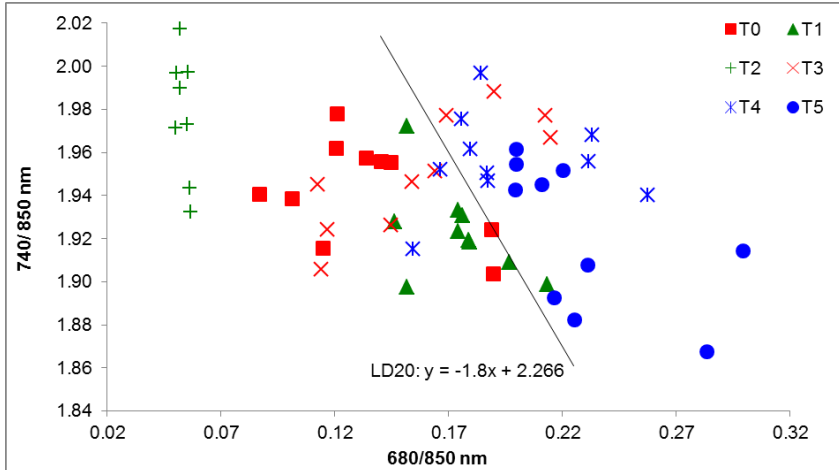


Figure 7. Bidimensional plot of the different shelf-life stages for samples stored at 20 °C. Linear discriminant equation (LD_{20}) could be identified as the best separation line between C1 and C2 samples

Table 2. Classification according to SLI_{20} for samples stored at 20 °C

Classification according to SLI_{20}			
	Shelf-life stage	Fresh (C1)	No more fresh (C2)
SLI_{20} (Threshold value 2.266)	C1	30/37 (81%)	7/37 (19%)
	C2	1/23 (4%)	22/23 (96%)

Results regarding classification according to SLI_{20} gave good percentage of correct classified samples. An interesting PPV of 96% was this time achieved for C2 samples and PPV of 81% for C1 (Table 2).

In this case, regarding C1 classification results, samples belonging to time two of sampling were correctly classified but with an anomalous behavior. For this reason, additional validation tests of the classification algorithm should be carried out in future to understand these anomalous behaviors of some sampling times.

Conclusions

The aim of this work was to study the feasibility of a simplified handheld optical device, based on measurements and processing of diffuse reflectance of Lamb's lettuce leaf at a few wavelengths appropriately selected.

Three wavelengths (680, 740 and 850 nm) based on PCA analysis were selected. A Shelf-Life classification Index for two different storage temperatures (SLI_4 and SLI_{20}) based on these three wavelengths was elaborated and tested with different separating thresholds.

Lamb's lettuce grading based on the proposed indices showed a high ability in discriminating fresh samples before commercial expiration date (C1) from no more fresh samples (C2).

Results of this study demonstrate the feasibility of a simplified and low-cost handheld device, based on the use of only three wavelengths, able to quickly estimate Lamb's lettuce freshness degree, with special reference to most sensitive stages of shelf-life time.

Section 7

Feasibility of a simplified handheld optical system to evaluate fresh-cut *Valerianella locusta* Laterr.

Moreover, the classification algorithm can be set also in a more restrictive way according to the needs of the user.

According to results an instrument based on LED (light emitting diodes) technology can be designed. This kind of device would be a compact, pocket-size, inexpensive and easy to use system. Producers, sellers and consumers could employ it for a quickly evaluation of shelf life of fresh-cut Lamb's lettuce.

Acknowledgements

This study received financial support from AGER as “STAYFRESH - Novel strategies meeting the needs of the fresh-cut vegetable sector” research project and from Regione Lombardia and European Social Fund for a Post-doctoral Research Fellowship (“Progetto Dote Ricerca”).

References

- Abadias, M.; Usall, J.; Anguera, M.; Solsona, C. & Viñas, I., 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology*, 123, 121-129
- Antonucci, F.; Costa, C.; Pallottino, F.; Paglia, G.; Rimatori, V.; De Giorgio, D. & Menesatti, P., 2011. Quantitative method for shape description of almond cultivars (*Prunus amygdalus* Batsch). *Food and Bioprocess Technology*. doi:10.1007/s11947-010-03892
- Baldassarre, V.; Cabassi, G. & Ferrante, A., 2011. Use of chlorophyll a fluorescence for evaluating the quality of leafy vegetables. *AJCS*, 5(6), 735-741.
- Beghi, R.; Spinardi, A.; Bodria, L.; Mignani, I. & Guidetti, R., 2012. Apples nutraceutic properties evaluation through a visible and near-infrared portable system. *Food and Bioprocess Technology*, DOI 10.1007/s11947-012-0824-7
- Bodria, L.; Fiala, M.; Guidetti, R. & Oberti, R., 2004. Optical techniques to estimate the ripeness of red-pigmented fruits. *Trans. of ASAE*, 47, 815-820
- Ferrante, A. & Maggiore, T., 2007. Chlorophyll a fluorescence measurements to evaluate storage time and temperature of *Valeriana* leafy vegetables. *Postharvest Biol Technol.*, 45, 73-80
- Ferrante, A., Incrocci, L., Maggini, R., Serra, G., & Tognoni, F., 2004. Colour changes of fresh-cut leafy vegetables during storage. *J Food Agric Environ.*, 2, 40-44
- Guidetti, R.; Beghi, R. & Giovenzana, V., 2012. Chemometrics in Food Technology, *Chemometrics in Practical Applications*, Dr. Kurt Varmuza (Ed.), ISBN: 978-953-51-0438-4, InTech, Available from: <http://www.intechopen.com/books/chemometrics-in-practical-applications/chemometrics-in-food-technology>
- Løkke, M.M., Seefeldt, H.F., Skovb, T. & Edelenbos, M., 2013. Color and textural quality of packaged wild rocket measured by

multispectral imaging. *Postharvest Biology and Technology*, 75, 86-95

Lunadei, L.; Diezma, B.; Lleó, L.; Ruiz-Garcia, L.; Cantalapiedra, S. & Ruiz-Altisent, M., 2012. Monitoring of fresh-cut spinach leaves through a multispectral vision system. *Postharvest Biology and Technology*, 63(1), 74-84

Massart, D.L.; Vandeginste, B.G.M.; Buydens, L.M.C.; De Jong, S.; Lewi P.J. & Smeyers-Verbeek, J., 1997. *Handbook of Chemometrics and Qualimetrics: Part A*. Elsevier, ISBN: 0-444-89724-0, included in series: *Data Handling in Science and Technology*

Moshou, D.; Wahlen, S.; Strasser, R.; Schenk, A.; De Baerdemaeker, J. & Ramon, H., 2005. Chlorophyll fluorescence as a tool for online quality sorting of apples. *Biosyst Eng* 91, 163-172

Naes, T.; Isaksson T.; Fearn, T. & Davies, A.M.C., 2000. *A User-Friendly Guide to Multivariate Calibration and Classification*. NIR Publication, Chichester, UK

Pathare, P.B.; Opara, U.L. & Al-Julanda Al-Said F., 2012. Colour Measurement and Analysis in Fresh and Processed Foods: A Review. *Food and Bioprocess Technology* 2012, DOI: 10.1007/s11947-012-0867-9

Schofield, R.A.; DeEll, R.D.; Murrc, D.P. & Jenni, S., 2005. Determining the storage potential of iceberg lettuce with chlorophyll fluorescence. *Postharvest Biol Technol.*, 38, 43-56

Zhana, L.; Lia, Y.; Hub, J.; Panga, L. & Fana, H., 2012. Browning inhibition and quality preservation of fresh-cut romaine lettuce exposed to high intensity light. *Innovative Food Science & Emerging Technologies*, 14, 70-76

Section 8

Derivation of a blueberry ripeness index with a view to a low-cost, handheld optical sensing device for supporting harvest decisions

Roberto Beghi, Valentina Giovenzana, Anna Spinardi, Luigi Bodria, Riccardo Guidetti, Roberto Oberti

Department of Agricultural and Environmental Sciences – Production, Landscape, Agroenergy
Università degli Studi di Milano, via Celoria 2, Milano, 20133 Italy



Abstract

Berry fruit farming and marketing have widely increased in recent years in marginal areas of Italy, especially in response to consumers' growing interest in adhering to a bioactive and health-protecting diet.

The ripening stage of berry fruits, on which their quality and nutraceutical attributes depend, is typically estimated by growers through visual inspection based on the growers' experience. Low-cost, handheld, user-friendly devices could assist growers in the field in determining the optimal harvest date in accordance with the desired ripeness of berries. In order to explore the technical feasibility of such a system, this study focused on defining a simple ripeness index for blueberries by identifying the main spectral changes accompanying their ripening process, with special attention paid to the last and most relevant stages. With this aim, spectral measurements in the range of 445-970 nm were carried out on *Vaccinium corymbosum* (cv. "Brigitta") during two different growing seasons. Spectra on individual berries were acquired in the field at different dates. Measurements were accompanied by weekly samplings, with picked fruits divided into four ripeness grades according to commercial growers' classification.

A principal component analysis of 942 fruit spectra highlighted that two main wavebands (680 nm and 740 nm) can maximize the differences between fully ripe samples and those close to ripeness or unripe. Hence, spectral values at 680, 740 and 850 nm (the latter being an additional normalization waveband) were used to define a blueberry ripeness index (BRI) as a linear combination of two spectral ratios $S1 = \log\left(\frac{I_{680}}{I_{850}}\right)$ and $S2 = \frac{I_{740}}{I_{850}}$.

The definition of specific ripeness thresholds for the BRI according to more or less selective criteria was illustrated in an application example, and the ripeness classification capability was then assessed on a separate validation set of 471 berries.

When applying a less selective threshold approach, the BRI correctly classified as ripe 85% of manually graded fully ripe berries, whereas 13% of close but not yet ripe validation samples were misclassified as fully ripe and ready to harvest. Comparatively, when a more demanding

ripeness threshold was applied, the amount of nearly ripe berries misclassified as ripe decreased to 8%, but, the amount of fully ripe berries not identified as ripe rose to 25%. In both cases, none of the unripe validation samples was erroneously classified as a ripe fruit.

These results, which were obtained with a BRI defined by spectral measurements at just three discrete wavelengths, point to the feasibility of a simple, microcontroller-based, handheld optical device able to implement the BRI to quickly assess the ripeness of sets of berries during the last and most delicate stages of the ripening process.

Keywords: berry fruits, blueberry ripening, Vis/NIR spectroscopy, ripeness sensing device.

Introduction

In recent years, consumers' interest in berry fruits has increased together with the growing demand for health-producing foods (Gosch 2003), owing to the berries' bioactive and health-protecting properties (Szajdek and Borowska 2008). Indeed, blueberries, blackberries, and raspberries are a rich source of antioxidant compounds, particularly of flavonoids and anthocyanin (Wang and Lin 2000). Moreover, the increasing demand for these fruits may represent an important income-generating opportunity for marginal rural areas, because their cultivation is particularly suited for small farms and mountain regions.

The maturity stage is the major factor influencing the compositional quality of fruits and vegetables (Prior et al. 1998). During fruit ripening, physiological, biochemical, and structural modifications occur that determine fruit quality. Nowadays, fruit quality has become the primary issue for a rapidly increasing number of consumers who are concerned about the healthy aspects of food. Indeed, the current concept of fruit quality is gradually being broadened to include nutritional parameters and health-protecting components (Tromp 2005).

At present, blueberry (family Ericaceae, genus *Vaccinium*) ripening stage and harvest time are typically estimated by visual assessment based on growers' experience, which can result in unsatisfactory homogeneity of the fruit lots and large variability from farm to farm. Growers are in need of simple and portable devices that can provide a quick and direct ripeness assessment of a large set of samples in the field to help them to determine the best harvest time.

In recent years, extensive research has been devoted to applying non-destructive techniques to evaluating a fruit's ripening stage (Nicolai et al. 2007; Saranwong and Kawano 2006.). In particular, visible and near-infrared (vis/NIR) and near-infrared (NIR) spectroscopy have been widely used to assess fruit quality parameters, which are classically determined using complex and costly analytical methods. These alternative techniques provide fast and reliable information on the internal characteristics of many fruit species (Ozaki et al. 2007). Vis/NIR spectroscopy measures the spectral components of light emerging from a sample after penetrating its surface and being partly absorbed and partly backscattered by the first internal layers (skin and flesh). By

measuring the spectral modifications of the radiation after interaction with the fruit, it is possible to establish a relationship between the spectral data and the concentration of specific components in the sample. This technique is well suited for investigating the main components of the first representative layers of fruit pulp.

Following the pioneering works of Dull et al. (1992), Kawano et al. (1993), and Slaughter (1995), among others, several applications for estimating the ripeness parameters of different fruit species can be found in more recent literature. For example, studies have been published on apples (Peirs et al. 2005), pears (Sun et al. 2009), plums (Slaughter et al. 2003), oranges (Cayuela 2008), peaches (Pérez-Marín et al. 2009; Ziosi et al. 2008), grapes (Cozzolino 2006), and kiwifruits (Clark et al. 2004). Sinelli et al. (2008) studied the application of Fourier transform-NIR spectroscopy to estimate the quality parameters of blueberries. This type of bench-top instrumentation, however, is obviously not suitable for field use.

Portable vis/NIR instruments have been tested both in controlled laboratory conditions (Antonucci et al. 2011; Camps and Christen 2009; Xing et al. 2005) and in the field (Beghi et al. 2012; Costa, et al. 2011; Guidetti et al. 2010; Bodria et al. 2004). Guidetti et al. (2008) tested a vis/NIR device that uses reflectance configuration to predict blueberry ripeness.

These approaches, nevertheless, always require a complex processing of data to build calibration and prediction models (Guidetti et al. 2012; Cen and He 2007; Williams and Norris 2002).

The aim of this study was to identify the main spectral changes accompanying the ripening process of blueberries, with a special emphasis on the last and most relevant stages. In particular, the spectral information is analyzed to select a few significant wavelengths, allowing the definition of a simple ripeness index for blueberries. The objective is to assess the feasibility of a simplified, handheld optical device based on spectral measurements at a few selected wavelengths and straightforward data processing. The possible final application would be a low-cost and user-friendly device that supports the grower in determining the optimal harvest date according to the desired ripening degree of berries.

Materials and Methods

Blueberries and sampling plan

A total of 942 Vis/NIR fruit spectra in the range of 445-970 nm were acquired for *Vaccinium corymbosum* ("Brigitta" cultivar) during two different growing seasons (2009 and 2010), cultivated in different farms in the area of Valtellina, Italy (approx. 46.2° N, 9.9° E). Spectra measurements were taken in the field on individual berries along their equator region after artificial illumination with a probe tip. In order to reduce the possible influence of environmental conditions, especially related to diurnal changes in sunlight, spectral acquisitions were always performed in the morning with the help of a plastic cap to ensure contact between the probe tip and the measured berry. Specific tests conducted at different times of the day confirmed the repeatability of measurements with artificial lighting and berry/probe contact configuration, which evidently were sufficient to cancel out the possible influence of ambient illumination on the samples. Air temperature changes during acquisition were limited to a range of 15 to 25 °C. Field dataset of spectra were assumed to randomly embed possible environment influencing factors in a representative way.

Field spectra acquisitions of berries were carried out at weekly intervals. Just after measurement of the plants, fruits samples were picked and divided into four ripeness classes according to the criteria used by expert growers (mainly relying on size and surface color distribution). According to commercial growers' classification, blueberry fruits were graded as follows (see Fig. 1):

- Ripeness grade 1 (R1 = totally unripe): green, small-sized berries, needing 3-5 weeks to commercial harvest;
- Ripeness grade 2 (R2 = unripe): green skin over more than half of the surface, medium-sized berries, needing 1.5-3 weeks to commercial harvest;
- Ripeness grade 3 (R3 = close to ripeness): almost completely blue-pigmented (except the stem's end), nearly full or full-sized berries, needing 1 week or less to commercial harvest;

- Ripeness grade 4 (R4 = fully ripe): completely blue-pigmented (included stem's end), full-sized berries, ready to be harvested.



Figure 1. Left: blueberries grouped into four ripeness grades according to commercial growers classification. Right: illustrative example of spectral measurement on a freshly detached berry; in this study, the acquisition of berries' spectra was done with fruits on the plant

Berries harvested and classified were immediately brought to a laboratory to be analyzed in order to determine their main qualitative parameters indicating the ripening stage.

As shown by Figure 1, totally unripe and unripe fruits (grades R1 and R2) can be clearly distinguished by their appearance from fully ripe fruits. Contrarily, it is much more difficult, especially in the field, to discriminate between blueberries close to ripeness (R3) and those that are fully ripe (R4), because the differences in appearance are limited to changes in skin color in a small area around the stem's end. Nevertheless, discerning between these two grades is a crucial task during these last stages of ripening that involve substantial modifications in fruit quality and flavor. Due to this, the present study was particularly focused on discriminating between spectra of R3 and R4 graded berries, which constitutes the most interesting applicative case for supporting growers in the field.

Field spectra acquisition

Blueberry spectra were acquired in the vis/NIR range (445-970 nm) using a portable system. The field system consisted of a) a lighting system; b) a fiber optic probe; c) a spectrophotometer; d) a notebook PC for data acquisition and instrument control; and e) a battery for

Section 8

power supplying. All the components were housed in a backpack for easiness of use during field measurements.

Berry samples were illuminated by the radiation produced by the lighting system, and the back-scattered component was guided by an optical fiber to be measured by the spectrophotometer. Spectra and other field logs data were saved in the PC memory by custom software.

The light source was a 50 W halogen lamp (Decostar Coolblue, Osram, Germany) with a peak in the emission spectrum at 500 nm. This lamp was specifically chosen because its emission peak overlapped with anthocyanin's absorption range. The light source was embedded in a metal holder and attached to the optical fiber with a SMA connector, specifically designed to focus the halogen spotlight on the illuminating optical fiber.

Light radiation was guided to the fruit sample through a Y-shaped, bidirectional fiber optic probe (FCR-19IR200-2-ME-S1, Avantes, the Netherlands). The Y-shaped fiber allowed the light from the lamp to be guided to illuminate the sample and simultaneously collected the radiation coming from the berry pulp and guided it back to the spectrophotometer. The tip of the optical probe was equipped with a soft plastic cap to ensure contact with the sample skin during measurement while minimizing environmental light interference.

The spectrophotometer (AvaSpec-2048, Avantes, the Netherlands) mounted a diffractive grating for spectral measurements optimized in the range of 445-970 nm and a CCD sensor with a 2048 pixel matrix, corresponding to a nominal resolution of 0.3 nm.

The software controlled acquisition parameters and data logging, as well as instrument operation and illumination. Thus, after lamp stabilization, measured samples were illuminated only for the time necessary to acquire the spectrum, allowing the battery power source to last longer in the field. Typically, the system was able to measure more than 300 samples without any detectable decrease in illumination intensity.

Laboratory analysis of quality parameters

Total soluble solids (TSS) of harvested samples were determined from the berry juice obtained from batches of 5 berries blended for 2 minutes

with a blender (Waring Blender, Waring Laboratory, USA) and using a digital portable refractometer (Atago-PR-32, Japan). A few drops of the juice were placed on the refractometer sensor, previously calibrated with distilled water, which gave the result directly in Brix degrees. Titrable acidity expressed as meq/L was measured using an automatic sample titrator (Crison, Titromatic 2S-3B). The total phenolic content of blueberry samples was determined spectrophotometrically at 700 nm according to the Folin-Ciocalteu method (Waterhouse 2005). The results were expressed as mg catechin per g of fresh weight. Total anthocyanin content was estimated as malvidin 3-glucoside at 520 nm and expressed as mg per g of fresh weight. For ascorbate analysis, 7.5 g of blueberries were homogenized in cold 6% (w/v) metaphosphoric acid at 4 °C. After centrifugation and filtration through a 0.2 µm nylon filter, an aliquot of supernatant was analyzed using high performance liquid chromatography (HPLC) equipped with an Inertsil ODS-3 (5 µm; 4.6 mm x 250 mm) GL Science column at 20 °C. The mobile phase was 0.02 M orthophosphoric acid, and ascorbic acid was detected at 254 nm with an UV-975 UV/VIS detector (Jasco, UK).

Data analysis

For quality parameters, namely TSS, total anthocyanin, total phenols, total flavonoids, and ascorbic acid, the statistical significance of differences between blueberries of ripening grades R3 and R4 was computed using the t-Student test, $p < 0.05$ (SPSS 19.0 for Windows).

The chemometric analysis of spectra was based on the Unscrambler 9.6 software package (CAMO ASA, Norway). Moving-average (3 nm window wide) smoothed spectra were used in a principal component analysis (PCA) to explore the possible clustering of sample spectra belonging to the same ripening grade. Scores and loading plots obtained with the PCA were subsequently analyzed to select the most characteristic wavelength ranges, allowing for better discrimination among different ripening stages, with a particular focus on differences between grade R3 and grade R4.

Finally, spectral reflection intensity measured at selected wavelength ranges was used to map the dataset of spectra and to derive a ripeness

index for blueberries particularly able to pinpoint the last and most relevant stages of the ripening process.

Results and Discussion

Field spectra and quality parameters evolution with ripening

Average measured raw spectra of the four ripening grades are shown in Figure 2.

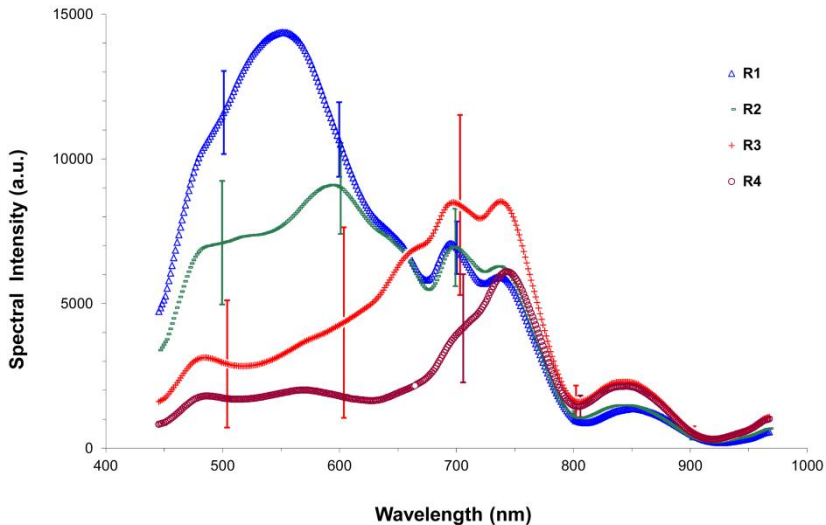


Figure 2. Average raw spectra of 942 blueberries grouped in four ripening grades (R1 = totally unripe, R2 = unripe; R3 = close to ripeness; R4 = fully ripe). Bars indicate the standard deviation within each ripening group at different wavelengths

As expected, the spectra exhibit significant differences among ripening classes, with dramatic changes in the visible band occurring from unripe berries (R1) to the last ripening stages (R3 and R4), especially linked to anthocyanin accumulation during ripening. This leads to a strong decrease in reflectance in the visible band associated with the anthocyanin absorption peak centered around 540 nm.

Changes in spectra obviously reflect modifications in quality parameters during ripening. Figure 3 summarizes the measured differences for grade R3 and grade R4 blueberries in terms of the main quality and

nutraceutical parameters (total soluble solids, total anthocyanin, total phenols, total flavonoids, and ascorbic acid content).

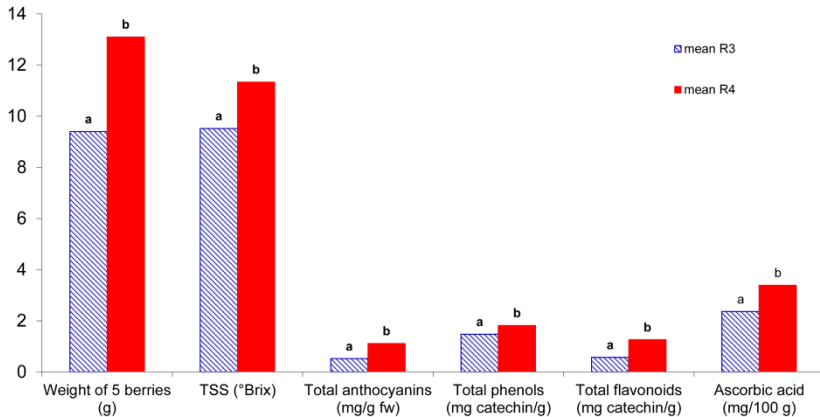


Figure 3. Evolution with ripening of the main quality and nutraceutical parameters measured for close to ripeness (R3) and fully ripe (R4) blueberries. Statistical significance of differences between the two ripeness grades was computed with t-Student test, $p < 0.05$ (a, b)

Relevant increases were especially observed for TSS, with 9.5 ± 0.6 °Brix for R3 and 11.3 ± 0.6 °Brix for R4, as well as for total anthocyanin, with 0.52 ± 0.12 mg/g for R3 and 1.12 ± 0.15 mg/g for R4, and for total flavonoids, with 0.57 ± 0.13 mg catechin/g for R3 and 1.26 ± 0.16 mg catechin/g for R4.

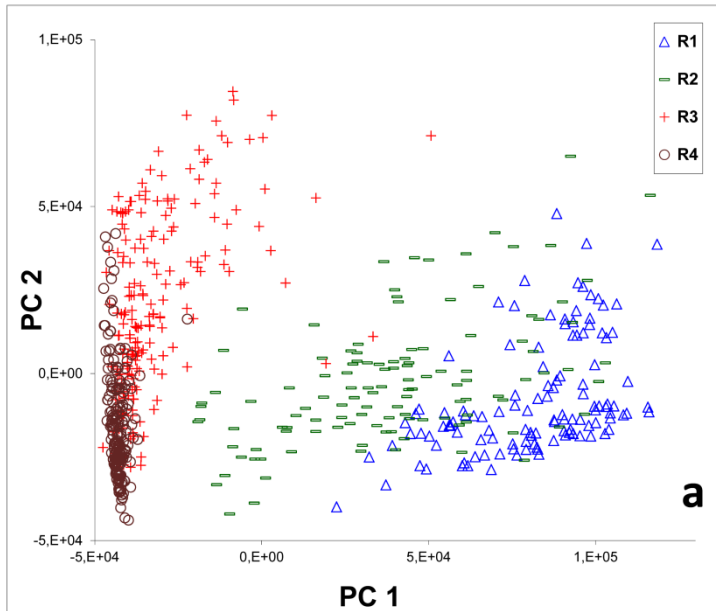
Spectra analysis

The explorative PCA conducted on 942 spectra of blueberries resulted in three most significant PCs explaining 98% of total data variance (PC1: 76%; PC2: 18%; and PC3: 4%). The scores plot of PC1 vs. PC2 (Fig. 4a) reveals that PC1 greatly accounts for separating grades R1 and R2 (unripe) from grades R3 and R4 (close to ripeness or fully ripe). On the contrary, grades R3 and R4 are almost completely overlapping along the PC1 axis, whereas PC2 shows only a partial discriminating capability between the two last ripening stages. This behavior is explained by the dominant loadings of PC1 around 540 nm, i.e., corresponding to anthocyanin absorption. Therefore, PC1 enables a direct discrimination between unripe samples and already blue-pigmented samples.

Section 8

Derivation of a blueberry ripeness index with a view to a low-cost, handheld optical sensing device for supporting harvest decisions

Less obvious is the distinction between grades R3 and R4 highlighted by the scores plot of PC2 vs. PC3 (Fig. 4b), which shows that a combination of these two PCs enables a fair separation of grade R3 from grade R4 spectra. Hence, PCs loadings (Fig. 4c) were analyzed in search of main wavelength bands contributing to PC2 and PC3 as candidate discriminators for the last stages of the ripening process. Two wavelengths were then identified, namely 680 nm and 740 nm, with an additional one at 850 nm chosen as a normalization band in a particular range of wavelengths where the contribution of all the PCs tends to cancel out.



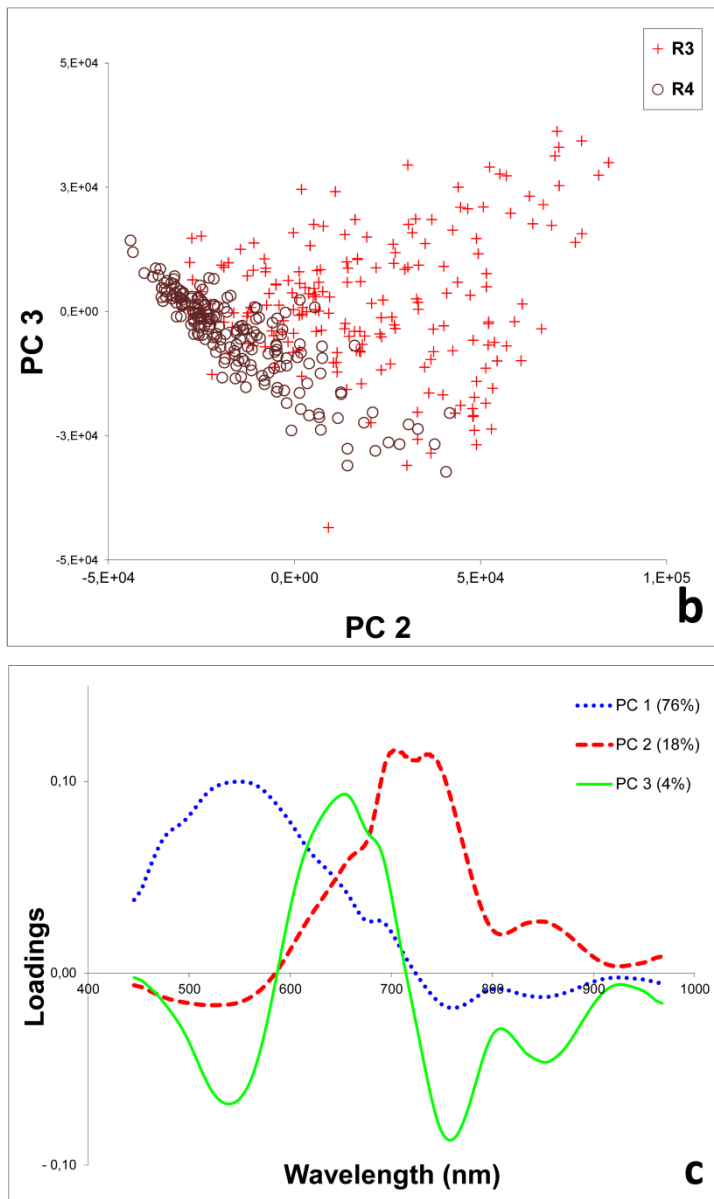


Figure 4. Results of principal components analysis on berries' spectra of the calibration set: a) scores plot on PC1 vs PC2; b) scores plot on PC2 vs PC3; c) loadings plot of the first three PCs

Definition of a blueberry ripeness index (BRI)

The candidate wavelengths, especially intended to identify the last ripeness stages of blueberry, were combined to obtain two normalized spectral ratios: the logarithm ratio $S1 = \log\left(\frac{I_{680}}{I_{850}}\right)$ and the simple ratio

$$S2 = \frac{I_{740}}{I_{850}}$$

To test the ripeness classification capability of spectral ratios S1 and S2, the field measured spectra were divided in calibration and validation sets each consisting of 471 spectra, where all the four grades were homogeneously represented. For each sample, the values of S1 and of S2 were then computed from the berry's spectral intensity at 680 nm, 740 nm and 850 nm (averaged on a window of ± 5 nm).

Figure 5a shows how the 471 berries of the calibration set are mapped by the two variables S1 and S2 according to their ripening stage. The samples appear distributed along a narrow linear band in the plot, with unripe berries (grades R1 and R2) close-clustered on the right-end of the band. As ripening progresses (grades R3 and R4), the samples tend to be distributed toward the left end of the band, appearing more and more separately differentiated at advanced ripeness stages.

Such a distribution gives a graphical representation of blueberry's ripening process as a gradual shift from higher (S1, S2) values towards lower values which occurs along a "ripening line" having general equation:

$$\frac{I_{740}}{I_{850}} - m \cdot \log\left(\frac{I_{680}}{I_{850}}\right) = c \quad [1]$$

where the parameters m and c of such "ripening line" to be computed from a linear fit of known (S1, S2) pairs' values measured for the berries in the calibration set.

With this simplified model, the S1-S2 plane can be separated in regions, each assigned to a different ripeness grade. As identifying boundaries of these regions we can assume specific lines perpendicular to ripening line [1], which separate the plane in contiguous rectangular bands corresponding, from right to left, to grades from R1 to R4 respectively (Figure 5b).

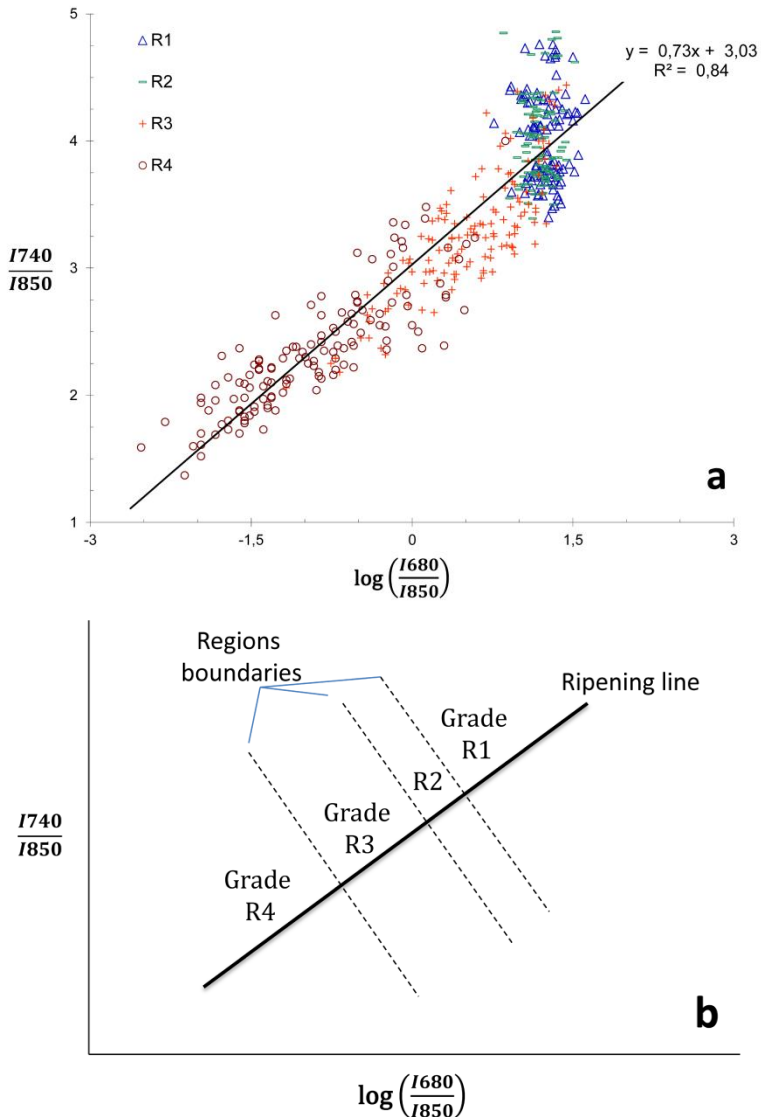


Figure 5. **a**) Plot of the spectral indexes $S1 = \log\left(\frac{I_{680}}{I_{850}}\right)$ and $S2 = \frac{I_{740}}{I_{850}}$ computed for 471 berries (the calibration set) at four ripening grades: totally unripe R1 (Δ); unripe R2 ($-$); close to ripeness R3 ($+$); fully ripe R4 (\circ). The linear fitting equation is the ripening line [Eq. 1, in the text]. **b**) Separation of the $S1$ - $S2$ plane into different ripeness grade regions by boundaries lines perpendicular to the ripening line

Section 8

Derivation of a blueberry ripeness index with a view to a low-cost, handheld optical sensing device for supporting harvest decisions

Being the slope m^* of all boundary lines defined, by condition of perpendicularity, from the parameter m in Eq.1, the position where one ripeness region ends and the contiguous starts is specified by the y-intercepts of each boundary. In other words, the value of y-intercepts of boundaries correspond to the specific thresholds (T_i) defining the ripeness regions in the plane S1-S2.

Then, with reference to Figure 5b, one berry belongs to a specific grade region if its spectral indexes S1 and S2 identify a point within the lower and upper boundary lines of that region. This corresponds to the condition:

$$T_{i+1} < \frac{1740}{1850} - m^* \cdot \log\left(\frac{1680}{1850}\right) < T_i \quad [2]$$

where T_i and T_{i+1} the ripeness thresholds defining the position of the grade region's boundaries in the plane S1-S2. As a consequence, it can be noticed that the term within the inequalities in [2] assumes the meaning of a blueberry ripeness index:

$$BRI = \frac{1740}{1850} - m^* \cdot \log\left(\frac{1680}{1850}\right) \quad [3]$$

and its value computed for one sample and compared to the boundary ripeness thresholds (i.e. by verifying whether $BRI < T_i$), assigns the sample to a ripeness grade according to spectral intensities measured at 680 nm, 740 nm and 850 nm.

Example of application and validation of BRI

Within the described approach and with a focus on the particularly interesting case of selecting fully ripe grade (i.e. grade R4) ready to be harvested, these berries can be discriminated in term of the blueberry ripeness index [3] by applying the condition $BRI < T_{R4}$.

In order to discuss this specific case as an example of application, an explicit expression of the BRI and of the ripeness threshold T_{R4} for grade R4 were defined on the basis of measured data for the berries of the calibration set and subsequently tested on the validation set.

First, the ripeness line parameters in [1] were estimated by a linear fit of 471 pairs of indexes (S1,S2) of the calibration set, resulting in $m=0.73$ and $c=3.03$. Directly from the former, the value of m^* in [2] could be

computed as $m^* = -1/m$, leading to an explicit expression of BRI [3] for the specific measurement setup and cultivar considered in this study:

$$\text{BRI} = \frac{1740}{1850} + 1.37 \log\left(\frac{1680}{1850}\right) \quad [3B]$$

Concerning the choice of a ripeness threshold for grade R4, because contiguous grades appear widely overlapped in S1-S2 plane (Figure 5a), the identification of a value T_{R4} able to sharply separate berries of grade R4 from those of R3 is not possible. A compromise value relying on grower's choice criterion has to be adopted.

A possible approach can be to choose a T_{R4} value such that the condition $\text{BRI} < T_{R4}$ leaves out from the fully ripe region (R4) a number of calibration berries known to be R4 grade which equals the erroneously included calibration berries known to be R3, that is:

Criterion A)

T_{R4} such that: n of R4 misclassified as R3 = n of R3 misclassified as R4

From BRI values computed for berries graded R3 and graded R4 in our calibration dataset, the threshold value fulfilling criterion A resulted $T_{R4} = 2.75$.

Alternatively, a more demanding approach to choose T_{R4} may be adopted to obtain higher quality and nutraceutical attributes levels in the harvested lot, by lowering the samples close but not yet fully ripe (grade R3) erroneously included by the condition $\text{BRI} < T_{R4}$. For example, T_{R4} can be chosen such that the number of samples close to ripeness (R3) misclassified as fully ripe (R4) is penalized by a factor of three, compared to the failures to include fully ripe berries in R4 region, that is:

Criterion B)

T_{R4} such that: n of R4 misclassified as R3 = $3 n$ of R3 misclassified as R4

From BRI values of berries graded R3 and graded R4 in our calibration dataset, the threshold value fulfilling criterion B resulted $T_{R4} = 2.13$.

The two alternative full ripeness conditions, defined on the base of criterion A and B respectively, were tested on a separate validation set, consisting of spectra of 471 blueberries samples not used yet for the derivation of the ripeness index. Operatively, based on spectral

Section 8

Derivation of a blueberry ripeness index with a view to a low-cost, handheld optical sensing device for supporting harvest decisions

intensities measured at 680 nm, 740 nm and 850 nm, the BRI [3B] of samples was computed and berries were classified as fully ripe and ready for harvest, depending on whether or not the condition $BRI < 2.75$, or the more demanding $BRI < 2.13$, were satisfied.

In table 1 the manual grading of the validation samples according to expert growers reference, is compared to the classification obtained by applying the two BRI thresholds.

Table 1. Classification of 471 validation samples obtained with the derived blueberry ripeness index BRI (Eq.3B in the text). A less and more demanding ripeness thresholds, defined on the base of criterion A and B (see in paragraph *Definition of a blueberry ripeness index*) respectively, are considered. BRI classification is compared with manual grading according to growers reference.

Ripening grade (manual classification)	Blueberry ripeness index classification			
	BRI<2.75 (criterion A)		BRI<2.13 (criterion B)	
	Ripe	Not ripe yet	Ripe	Not ripe yet
R1 (n=100)	0%	100%	0%	100%
R2 (n=100)	0%	100%	0%	100%
R3 (n=137)	13.1%	86.9%	8.0%	92.0%
R4 (n=134)	85.1%	14.9%	74.6%	25.4%
Total (n=471)	28.0% (n=132)	72.0% (n=339)	23.6% (n=111)	76.4% (n=360)

R1= totally unripe; R2= unripe; R3= close to ripeness; R4= fully ripe.

In the first case ($BRI < 2.75$), the ripeness index correctly classified as “ripe” 85% of R4 graded (fully ripe) validation samples, whereas it erroneously rejected the remaining 15% as “not ripe yet.” Besides, it included as false positives also 13% of grade R3 (close to ripeness) validation samples, with a total of 28% classified as “ripe.” Interestingly, it can be noticed that the number of R3 validation samples classified as ripe (18 of 137) is quite close to the number of R4 validation samples not included in the ripe class (20 of 134), which fully reflects the criterion A used on the calibration set to establish the threshold value 2.75.

When the more demanding threshold was applied ($BRI < 2.13$), the amount of R3 validation samples misclassified as ripe decreased to 8%, but the amount of grade R4 berries positively included was also reduced to less than 75%. As a result, less than 24% of the total sample was

classified as “ripe” and ready for harvest when the more restrictive condition for BRI was applied. It can be noticed here that the number of R4 samples not classified as ripe (34 of 134) is three times the number of R3 samples erroneously included (11 of 137), which reflects the criterion B used to establish the threshold value 2.15. Nevertheless, with both criteria, none of the unripe validation samples (grades R1 and R2) were erroneously classified as a ripe fruit.

As expected, the more demanding BRI threshold example, based on applying criterion B to the calibration set, resulted in a more selective classification of the validation berries compared to that obtained by applying the less demanding criterion A. Indeed, the total number of samples classified as “ripe” and then considered as ready for harvest with the condition $BRI < 2.13$ was 16% lower than that obtained by $BRI < 2.75$ (111 against 132 berries, respectively), whereas the positively selected samples contained 90% of grade R4 berries for the former case (100 of 111), but just 85% for the latter (114 of 132).

Possible implementation of BRI in a simple optical device

A possible main application of the introduced BRI is to support berry growers in planning the harvest or even in defining the selective harvesting of plots according to specific ripeness thresholds. Indeed, by applying the approach described above to a representative calibration sample of fruit in the field, a grower may determine a threshold value according to his/her commercial objectives with a trade-off between the amount of harvested produce and its ripeness stage which, in turn, will reflect its quality and nutraceutical attributes.

Based on the intensity of light reflected at three discrete wavelengths (680, 740, and 850 nm), the implementation of the BRI classifier using a simple, microcontroller-based, handheld optical device can be envisaged. Figure 6 illustrates a possible functional scheme of such a device, in which samples' illumination at the specified wavelengths with high-power LEDs (light emitting diodes) and monochromatic read-outs of reflected light with filtered photodiodes can be obtained. From the intensity signals sensed at the three wavelengths, the microcontroller can then compute the BRI value, display it to the user, or/and classify the measured fruit according to the thresholds set by the grower.

Section 8

Derivation of a blueberry ripeness index with a view to a low-cost, handheld optical sensing device for supporting harvest decisions

Considering the components and possible design, the envisaged system would likely result in a compact-sized, low-cost, and easy-to-use device, allowing the grower in the field to quickly and precisely evaluate the ripening stage of representative sets of blueberries.

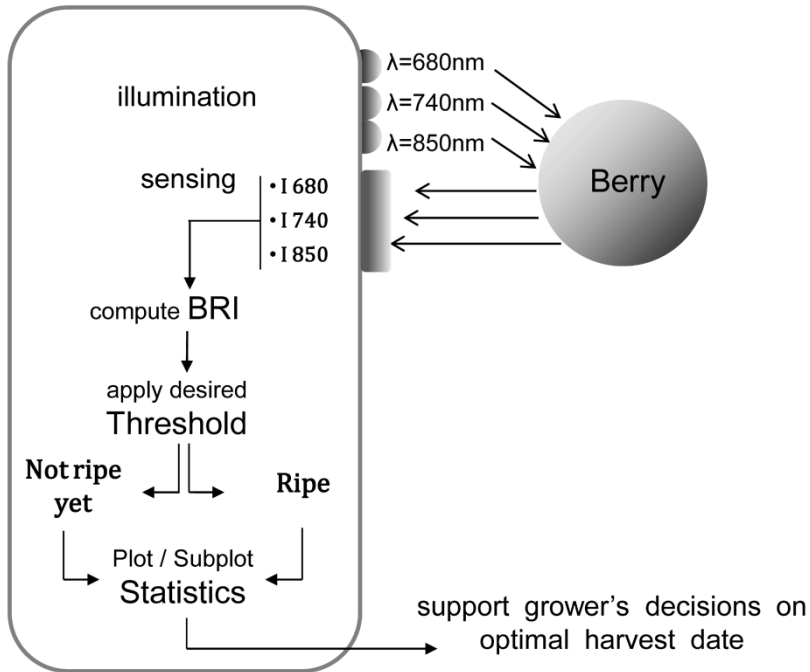


Figure 6. Functional scheme of a simple, handheld optical device implementing discrete wavelengths spectral measurements and BRI-based classification to support berry growers in planning optimal or selective harvest according to desired ripeness thresholds

Conclusions

This study aimed to derive a blueberry ripeness index (BRI) especially able to discern the last stages of the ripening process, i.e., to distinguish fully ripe berries from those close to ripeness. In addition, the technical feasibility of a simplified handheld optical device implementing the elaborated ripeness index to be used in the field was explored. The derivation of the BRI was based on an analysis of the main changes in blueberries' optical spectra measured during ripening, and it led to an index having the general form [3] and resulting from a combination of

spectral intensities measured at three wavelengths: 680, 740, and 850 nm.

The definition of specific ripeness thresholds for the BRI according to more or less selective criteria was then illustrated in an application example, and the ripeness classification capability was assessed on a separate validation set of berries manually graded according to expert growers' reference. When applying a less selective threshold approach, the BRI correctly classified as "ripe" 85% of the manually graded fully ripe berries, while failing to classify the remaining 15%, but also erroneously including 13% of close but not yet ripe validation samples. Comparatively, when a more demanding ripeness threshold was adopted, the amount of nearly ripe berries misclassified as ripe decreased to 8%, but the amount of fully ripe berries not included rose to 25%. In both cases, none of the unripe validation samples was erroneously classified as a ripe fruit.

The satisfactory results obtained with a BRI defined by the measurements of the spectral intensity at just three discrete wavelengths point to the feasibility of a simple, microcontroller-based, handheld optical device able to implement the BRI to assist the grower in the field to quickly assess the ripening stage of sets of berries and to plan optimal harvest dates according to the desired final ripening.

Acknowledgements

This study received financial support from Regione Lombardia as "VALORVÌ" research project and from Regione Lombardia and European Social Fund for a Post-doctoral Research Fellowship ("Progetto Dote Ricerca").

References

- Antonucci, F., Pallottino, F., Paglia, G., Palma, A., D'Aquino, S., & Menesatti, P., 2011. Non-destructive estimation of Mandarin maturity status through portable VIS-NIR spectrophotometer. *Food and Bioprocess Technology*, 4(5), 809-813
- Beghi, R., Spinardi, A., Bodria, L., Mignani, I., Guidetti, R., 2012. Apples nutraceutical properties evaluation through a visible and near-infrared

Section 8

Derivation of a blueberry ripeness index with a view to a low-cost, handheld optical sensing device for supporting harvest decisions

portable system. Food and Bioprocess Technology DOI 10.1007/s11947-012-0824-7

Bodria, L., Fiala, M., Guidetti, R., Oberti, R., 2004. Optical techniques to estimate the ripeness of red-pigmented fruits. Trans. of ASAE, 47, 815-820

Camps, C., & Christen, D., 2009. Non-destructive assessment of apricot fruit quality by portable visible-near infrared spectroscopy. Food Science and Technology, 42(6), 1125-1131

Cayuela, A., 2008. Vis/NIR soluble solids prediction in intact oranges (*Citrus sinensis* L.) cv. Valencia Late by reflectance. Postharvest Biol. Technol., 47(1), 75-80

Cen, H., He, Y., 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. Trends Food Sci. Technol., 18, 72-83

Clark, C.J., McGlone, V.A., De Silva, H.N., Manning, M.A., Burdon, J., Mowat, A.D., 2004. Prediction of storage disorders of kiwifruit (*Actinidia chinensis*) based on visible-NIR spectral characteristics. Postharvest Biol. Technol., 32, 147-158

Costa, G., Bonora, E., Fiori, G., Noferini, M., 2011. Innovative non-destructive device for fruit quality assessment. Acta Hort. (ISHS), 913, 575-581

Cozzolino, D., Damberg, R.G., Janik, L., Cynkar, W.U., Gishen, M., 2006. Analysis of grapes and wine by near infrared spectroscopy. Journal of Near Infrared Spectroscopy. Chichester, UK. 14, 279-289.

Dull, G.G., Leffler, R.G., Birth, G.S., Smittle, D.A., 1992. Instrument for nondestructive measurement of soluble solids in honeydew melons. Transactions of the ASAE, 35(2):735-737

Gosch, C., 2003. Antioxidative effects of blueberries (*Vaccinium* sp): an overview. Erwerbs-Obstbau 45, 117-124.

Guidetti, R., Beghi, R., Bodria, L., 2010. Evaluation of Grape Quality Parameters by a Simple Vis/NIR System. Trans. of the ASABE, 53(2), 477-484

- Guidetti, R., Beghi, R., Bodria, L., Spinardi, A., Mignani, I., Folini, L., (2008). Prediction of blueberry (*Vaccinium corymbosum*) ripeness by a portable Vis-NIR device. *Acta Horticulturae* 310, ISBN 978-90-66057-41-8, 877-885
- Guidetti, R., Beghi, R., Giovenzana, V., 2012. Chemometrics in Food Technology. In Chemometrics. InTech: E-Publishing, ISBN: 978-953-51-0438-4. 10, 217-252
- Kawano S, Fujiwara T, Iwamoto M., 1993. Non-destructive determination of sugar content in satsuma mandarin using near infrared (NIR) transmittance. *Journal of the Japanese Society for Horticultural Science*, 62, 465-470
- Nicolaï, B.M., Beullens K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K.I., Lammertyn, J., 2007. Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. *Postharvest Biol. Technol.* 46, 99-118
- Ozaki, Y., McClure, W.F., Christy, A.A., 2007. *Near-Infrared Spectroscopy in Food Science and Technology*, Wiley Interscience, Hoboken, New Jersey, USA
- Peirs, A., Schenk, A., Nicolaï, B.M., 2005. Effect of natural variability among apples on the accuracy of VIS-NIR calibration models for optimal harvest date predictions. *Postharvest Biology and Technology*, 35(1), 1-13
- Pérez-Marínb, D., Sáncheza, M.T., Paza, P., Soriano, M.A., Guerrero, J.E., Garrido-Varob, A., 2009. Non-destructive determination of quality parameters in nectarines during on-tree ripening and postharvest storage. *Postharvest Biology and Technology*, 52(2), 180-188
- Prior, R.L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G., Mainland, C.M., 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *J. Agric. Food Chem.*, 46, 2686-2693
- Saranwong, S., Kawano, S., 2006. Fruits and Vegetables. In: Y. Ozaki, W. F. McClure, and A.A. Christy (eds.), *Near Infrared Spectroscopy*

Section 8

Derivation of a blueberry ripeness index with a view to a low-cost, handheld optical sensing device for supporting harvest decisions

in Food Science and Technology. Wiley-Interscience, Chichester, UK., 219-245

- Sinelli, N., Spinardi, A., Di Egidio, V., Mignani, I., Casiraghi, E., 2008. Evaluation of quality and nutraceutical content in blueberries (*Vaccinium corymbosum* L.) by near and mid-infrared spectroscopy. *Postharvest Biol. Technol.*, 50(1), 31-36
- Slaughter, D.C., 1995. Nondestructive determination of internal quality in peaches and nectarines. *Transactions of the ASAE*, 38(2):617-623
- Slaughter, D.C., Thompson, J.F., Tan, E.S., 2003. Nondestructive determination of total and soluble solids in fresh prune using near infrared spectroscopy. *Postharvest Biol. Technol.*, 28, 437-444
- Sun, T., Lin, H., Xu, H., Ying, Y., 2009. Effect of fruit moving speed on predicting soluble solids content of 'Cuiguan' pears (*Pomaceae pyrifolia* Nakai cv. Cuiguan) using PLS and LS-SVM regression. *Postharvest Biology and Technology*, 51(1), 86-90
- Szajdek, A., Borowska, E.J., 2008. Bioactive Compounds and Health-Promoting Properties of Berry Fruits: A Review. *Plant Foods Hum. Nutr.*, 63,147-156
- Tromp, J., 2005. Fruit ripening and quality. In: J. Tromp, A.D. Webster, and S.J. Wethaim (eds.), *Fundamentals of Temperate Zone Tree Production*. Backuys Publishers, Leiden, NL
- Wang, S.Y., Lin, H.S., 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.*, 48, 140-146
- Waterhouse, A.L., 2005. Determination of total phenolics. In: Wrolstad, R.E. (Ed.), *Handbook of food analytical chemistry-pigments, colorants, flavors, texture and bioactive food components*. John Wiley and Sons, New York, US, 463-464
- Williams, P., Norris, K., 2002. *Near-Infrared Technology in the Agricultural and Food Industries*, 2nd Edition. American Association of Cereal Chemist, St. Paul, Minnesota, USA
- Xing, J., Bravo, C., Jancsok, P.T.; Ramon, H., De Baerdemaeker, J., 2005. Detecting Bruises on 'Golden Delicious' Apples using

Hyperspectral Imaging with Multiple Wavebands. *Biosystems Engineering*, 90(1), 27-36

Ziosi, V., Noferini, M., Fiori, G., Tadiello, A., Trainotti, L., Casadoro, G., Costa, G., 2008. A new index based on vis spectroscopy to characterize the progression of ripening in peach fruit. *Postharvest Biology and Technology*, 49(3), 319-329

Section 9

Design of a simplified LED based system for a quick monitoring of grape ripening

Valentina Giovenzana, Roberto Beghi, Raffaele Civelli, Ezio Naldi, Roberto Oberti, Cristina Malegori, Riccardo Guidetti.

Department of Agricultural and Environmental Sciences
Università degli Studi di Milano, via Celoria 2, Milano, 20133 Italy



Abstract

Among non-destructive techniques, the optical analysis in the region of near infrared (NIR) and visible-near infrared (vis/NIR) has met a significant development in the last 20 years. NIR and vis/NIR analyses are based on the use of spectral information arising from the interaction between food matrix and light.

NIR spectroscopy has proved to be one of the most efficient and advanced tools to monitor product quality and for the process controlling of food industry. A lot of works were published on this topics.

Portable vis/NIR instruments are often used in controlled laboratory conditions. However, this acquisition technique is particularly versatile and is suitable for designing of compact, portable, handheld devices, to be directly used in field to monitor the ripening process or quality parameters.

Data complexity arising from vis/NIR spectroscopy requires specific statistical analysis and qualified staff. Moreover, nowadays available devices are expensive and therefore not suitable for small scale wineries.

The aim of this work was to design and test a simplified optical device to estimate grape ripening directly in field. This device allowed non-destructive and quick prediction of grape ripeness degree and quality parameters evaluation. It was developed according to results arising from previous study based on wavelengths selection (section 6).

LED (light emitting diodes) technology was chosen as light source in order to design a simplified and low-cost optical device. Spectral acquisition based on reflectance at the wavelengths of interest (670, 730, 780 and 850 nm) was proposed.

The integration of a simple processing algorithm in the microcontroller software would allow to visualize real time values of relative reflectance. Being immediately comparable and interpretable, these would represent a particularly useful information for the user.

The development stage of the prototype has reached a satisfactory level. An evolution and an engineering of the system will be desirable in order to obtain a compact, pocket-size, inexpensive and easy to use

device. Farmers could employ it directly in field for a quick evaluation of grapes ripening level.

The design of the prototype of the simplified optical device was realised with particular attention to versatility and modularity. The possibility to adjust light sources with a specific choice of wavelengths for LEDs, makes it possible to use the same simplified optical device for many different applications. This modular design allows an easy adjustment for different objective (i.e. ripeness evaluation, chemicals and physical properties prediction or shelf life analysis), and for different kind of food sample matrix.

Keywords: portable optical device, non-destructive analysis, grape, reflectance, ripening.

Introduction

Fruit ripeness monitoring in the orchard may provide valuable data to control quality of final foodstuff. For the most part of fruits, in fact, establishing ripeness at harvest is a crucial issue since fruit quality and potential shelf life are closely related to it. Also, monitoring along the entire food chain is essential for developing methods to assess the impact of process factors on the product quality.

Ripening is a complex process which is routinely characterized by measurement of quality parameters, essential for each food matrix, e.g. soluble solids, acidity, phenols, changes in colour and firmness. Except for colour evaluation, these are destructive measurements and are performed on a small sample of the entire production (Lelievre, et al., 1997).

The search for non-destructive methods which could explore a larger sample and give a more comprehensive overview of ripening is an ongoing process. Food industry increasingly requires process automation, for this reason since some time there is a growing interest in non-destructive technologies that can be integrated in the processes. For instance in the food production, pigment changes in fruit and vegetables can be assessed on the plant, and subsequently checked in the entire supply chain (El-Masry et al., 2008; Nicolai et al., 2007; Wang and Paliwal, 2007).

Currently, image analysis, visible near infrared (vis/NIR) and near infrared NIR (NIRs) spectroscopy and multi-hyperspectral techniques are the three techniques most applied in the food sector (Guidetti et al., 2012).

Since the appearance of a food contains a variety of information directly related to product quality and these characteristics are difficult to measure through use of classical methods of analysis, machine vision systems is a widespread technology in the food sector. Vision systems may be used in real time on the process lines, allowing on-line control and automation of sorting and classification within the production process (Brosnan and Sun, 2004).

Traditional image analysis, based on a computer system, had a strong development in food sector with the aim of replacing human eye

resulting in costs saving and efficiency, speed and accuracy improvement. Appearance characteristics (colour, shape, etc.) are easily detectable with conventional image analysis, but computer vision technology is not able to select between objects of similar colour, make complex classifications, predict quality characteristics (e.g. chemical composition) or detect internal defects (Du and Sun, 2006; Zheng et al., 2006).

In the food industry, among non-destructive techniques, the optical analysis in the region of NIRs has met a significant development in the last 20 years (McClure, 2003). NIRs analyses are based on the use of spectral information arising from the interaction between food matrix and light. NIRs spectroscopy has proved to be one of the most efficient and advanced tools to monitor product quality and for the process controlling of food industry.

Since the quality of a food is not an individual attribute but it contains a number of inherent characteristics of the food itself, to measure the optical properties of food products has been one of the most studied non-destructive techniques for the simultaneous detection of different quality parameters. The light reflected from the food contains information about constituents in the inner layers of the sample and at the surface of the foodstuff also. NIRs analyses allow measures only in a limited area of the sample. Therefore it is especially suitable for homogeneous products.

Hyperspectral imaging (or imaging spectroscopy) has emerged as a technology with great potential for effective and non-destructive quality and safety evaluation, and inspection in the area of food processing. Among the three mentioned technologies, the hyperspectral analysis is still the technique less widespread because of its complexity (Sun, 2010).

The combination of image analysis technology and spectroscopy is the chemical imaging spectroscopy that allows to get spatial and spectral information for each pixel of the foodstuff. This technology allow to know the location of each chemical component in the scanned image, but the analysis regarding only the sample surface (Firtha et al. 2008).

During fruit ripening biochemical changes occur at skin level but also in the pulp and the changes are homogeneous for the whole fruit. NIRs analyses allow measures in the inner layers of the sample.

For these reasons, in order to evaluate ripeness in the orchard and postharvest quality characteristics of fruits, NIRs spectroscopy resulted as the most suitable technique for this purpose and was chosen in this paper.

Three main types of NIRs systems are available: i) laboratory systems for applications in research centers or in large industries laboratories, ii) sorting and sizing devices designed specifically for the fruit and vegetable industries, e.g. in warehouses, iii) simplified systems for the analysis directly in the field to support the small-scale producers. Table 1 shows the main differences between the three groups of NIRs systems.

Table 1. Characteristics of the three main categories of NIRs systems

	Application area	Flexibility of use	Applicability	Measurement accuracy and reproducibility	Cost
Laboratory systems	Research	Modifiable according to requirements	Fixed system	Optimal	Average/high
Sorting and sizing	Industry	Specific categories of products	Fixed system	Good	Average/high
Simplified systems	In field, to support small-scale producers	Dedicated for individual products	Portable/handheld	Good	Low

A review of literature reveals that the NIRs techniques (vis/NIR and NIR) were applied to a wide array of agri-food applications. It is obvious that NIRs has simplified and helped automation of a variety of routine inspection tasks, including postharvest handling of fruit and vegetables. In literature, the feasibility of NIRs spectroscopy to measure quality attributes of fruit and vegetables has been shown for many products (Nicolai et al., 2007). Data complexity arising from NIRs requires specific statistical analysis and qualified staff. For example, nowadays available devices are expensive and therefore not suitable for small scale producers.

For every system categories, in fact, calibration models to be used in practice should be based on large datasets, encompassing several orchards, climate conditions, seasons and operational conditions (temperature) and optimised towards robustness by incorporating appropriate preprocessing methods (Nicolai et al., 2007; Wang and Paliwal, 2007). Also sample presentation is a crucial step in NIRs analysis. Specialized sampling probes, liquid cells and accessories have been manufactured to meet measurement demands in function of where they will be performed (in a laboratory, at a production line, or in the field) (Osborne et al., 1993; Wang and Paliwal, 2007).

The development of calibration models and the extraction of useful information present in the spectra data is possible thank to multivariate analysis. Chemometrics, in fact, is an essential part of NIRs spectroscopy applications in food sector. These techniques must always be complemented with chemometric analysis to enable the extraction of useful information present in the spectra separating it both from not useful information to solve the problem and from spectral noise. Chemometric techniques most used are the Principal Component Analysis (PCA) as a technique for qualitative analysis of the data and Partial Least Square (PLS) regression analysis as a technique to obtain quantitative prediction of the parameters of interest (Naes et al., 2002; Wold et al., 2001; Nicolai et al., 2007; Cen & He, 2007).

Today, a wide selection of spectroscopic devices is available and there are about 60 NIR spectrometer manufacturers around the globe (McClure and Tsuchikawa, 2007).

Research groups in cooperation with industry developed sorting lines using spectroscopy in the visible and near-infrared wavelength ranges, e.g., for grading according to fruit pigment and soluble solids contents. So far the most part of applications have been carried out in controlled laboratory conditions. For industrial applications, the first grading line with a NIR sensor in reflectance mode was introduced in 1989 by Mitsui Minig Co., Ltd. (Japan) to sort peaches based on TSS (Kawano, 1998). Nowadays grading lines equipped with NIRs sensors are now commercially available from Aweta (IQA, <http://www.aweta.nl>), Greefa (iFA, <http://www.greefa.nl>), Mitsui-Kinzoku (<http://www.mitsuikinzoku.co.jp>), Sacmi (F5, <http://www.sacmi.it>), TasteMark ([---

241](http://www.taste-</p></div><div data-bbox=)

technologies.com), Color Vision Systems (Insight, <http://www.cvs.com.au>), UNITEC spa (<http://www.unitec-group.com>) and others.

The development of portable devices is more complex than laboratory applications, due to the uncontrolled environmental conditions such as ambient light and fluctuating temperatures. These phenomena should be either minimised by appropriate data processing (Nicolai et al., 2007).

The availability of low cost miniaturised spectrophotometers has opened up the possibility of portable devices which can be used in the orchard for monitoring the fruit maturity.

In literature, some studies regarding portable NIRs are reported. In the most cases relatively poor prediction accuracy was obtained. This might be due to issues affecting robustness, such as temperature fluctuations, uncontrolled lighting conditions, the limited wavelength range, or the fact that the devices were still at the prototype stage.

There is certainly a large demand of NIRs portable devices, so more research is required in this area (Nicolai et al., 2007).

Portable vis/NIR instruments were tested in controlled laboratory conditions by Antonucci et al. (2011) for the evaluation of mandarin maturity status and by Camps and Christen (2009) for assessing apricot quality. In uncontrolled field conditions, instead, a portable vis/NIR device was tested in order to estimate apples nutraceutical properties (Beghi et al. 2012), to evaluate grape quality parameters (Guidetti et al. 2010), to assess the ripeness of red-pigmented fruits (Bodria et al. 2004) and to predict blueberry ripeness (Guidetti et al. 2008).

Research and innovations have enabled NIRs devices to further decrease their physical size while increase dimensions of collected data. Therefore, new NIRs instrumentation tends to be more compact and portable (Wang and Paliwal, 2007; McClure and Tsuchikawa, 2007).

Few device are commercially already available, for examples FANTEC Fruit Tester 20 was used (Saranwong et al., 2003), Jaz Modular Optical Sensing Suite, Ocean Optics

(<http://www.oceanoptics.com/Products/jaz.asp>) and QS_300, UNITEC spa (<http://www.unitec-group.com>).

The use of these portable NIRs devices, however, require skilled operator. Moreover, these applications always require a complex data processing to build calibration of prediction models (Guidetti et al. 2012; Cen and He, 2007).

For this reason, in order to support the small-scale producers, simplified, easy to use, low-cost devices for real-time assessment of fruit ripeness in field are desirable.

To reach this goal, recently, considerable effort has been directed towards developing and evaluating different procedures for an objective identification of variables which contain useful information and the elimination of variables containing mostly noise (Xiaobo et al., 2010). Liu et al. (2009) proposed three effective wavelength selection methods combined with vis/NIR spectroscopy in order to determine the soluble solids content of beer.

Only a few examples of simplified non-destructive commercial devices are already available on the market. For example, the University of Bologna (Costa et al., 2011) patented innovative and simplified NIRs equipments (DA-Meter for apples and Kiwi-Meter for kiwifruits). These devices determine fruit maturity stage through indices based on absorbance differences between specific wavelengths. These indices are correlated with the main traditional parameters as well as with changes in flesh colour. Simple and portable instruments could be used directly on fruits on trees to monitor ripening evolution, and help to choose the best cultural management practices (such as pruning, thinning, nutrition, etc). It reduces the fruit ripening heterogeneity and simplify post-harvest fruit management. Another optical technique used to follow changes during grape ripening is the fluorescence. MultiPlex III (Force A, France) is a portable fluorescence detector provided on the market (Lurie et al., 2012).

The design of this kind of simplified NIRs system is a process in which a number of factors must be taken into consideration, e.g., sample features, working environmental operating conditions, sampling time, operators skill levels and, first of all, an accurate wavelengths selection.

Other crucial spectrometric parameters for the performance of these NIRs instruments are: spectral resolution, system linearity, sensitivity, and signal to noise ratio (Wang and Paliwal, 2007).

Based on these considerations the aim of this work is to design and realize preliminary test of a simplified optical device, able to quick evaluate grape ripeness degree directly in field. It was developed according to results arising from previous study based on wavelengths selection (section 6).

The handheld device

LED (light emitting diodes) technology was chosen as light source in order to design a prototype of a simplified optical device (Wang et Paliwal, 2007). The system is based on reflectance spectral acquisition at the wavelengths of interest. Wavelength variables selected in the previous study (Section 6) were 670, 730, 780 and 850 nm.

Due to problems of availability on market, we were forced to buy LEDs of different wavelengths from those selected. Therefore, wavelengths used for the development of the simplified optical system are: 630 and 690, near chlorophyll absorption peak; 750, the unique LED available on market in the 730 - 780 nm range, nevertheless with high absolute regression coefficient values (Figure 1 in Section 6).

Modularity

The design of the prototype of the simplified optical device was realised with particular attention to versatility and modularity. The possibility to adjust light sources with a specific choice of wavelengths for LEDs, makes it possible to use the same simplified optical device for many different applications. This modular design allows an easy adjustment for different objective and for different kind of food sample matrix.

Description of the device

Figure 1 shows a diagram of the instrument, with the relationships between its functional units. In particular the figure shows:

1. the control and processing unit;
2. the input/output unit;
3. the analog-to-digital and the digital-to-analog converters;

4. the LEDs and optical filters modules;
5. the photodiodes;
6. the eight-arm optical fiber;
7. the battery.

1. The control/processing unit

This is the main board of the device, equipped with a PICTM microcontroller (Programmable Integrated Controller, PIC18F series, Microchip Corporation, USA), (1 in Figure 1). When turned on, the microcontroller automatically runs a preloaded firmware and manages the inputs entered by the user. Based on these, it verifies the state of the system, sends signals to the other units, decides and coordinates all the following operations.

2. The input/output unit

The user can control the instrument through a four buttons keyboard, which is connected to the main board (2 in Figure 1) and represents the input unit of the system. Using the keyboard, the operator can perform various tasks, i.e. he can start the calibration procedure, make a new measurement or delete the last one.

The output unit consists of an alphanumeric four lines LCD display (2 in Figure 1). The measured data and other messages are displayed in real time, giving the user an instant feedback to verify if the instrument is working correctly.

At the same time, the data are sent to a PC through a wireless Bluetooth card (2 in Figure 1). In this way, it is possible to follow the work session on the PC monitor too, and save the data for any post processing. To realize this, a specific application was written in C# language using the Microsoft® Visual Studio development environment.

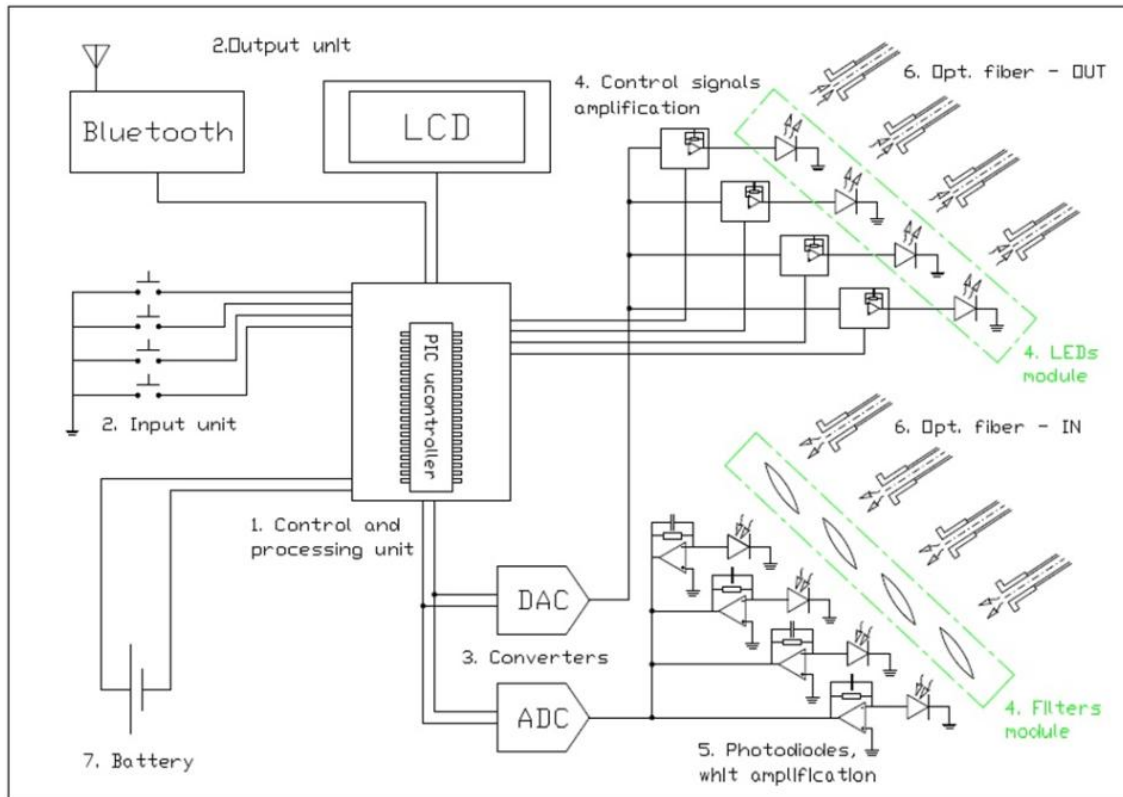


Figure 1. Block diagram of the main components of the prototype

1. The converters

The ADC and DAC modules (Analog to Digital Converter and Digital to Analog Converter) convert a voltage/current signal from analog form to digital one, and vice versa (3 in Figure 1). This conversion makes possible the communication between a digital component, such as the microcontroller, with transducers (LEDs and photodiodes) which instead require DC signals.

2. The LEDs and optical filters modules

A LED (light emitting diode) is an optoelectronic component based on a p-n junction semiconductor element, appropriately doped. When subjected to a voltage, this diode emits energy in the form of optical radiation, whose wavelength depends on the semiconductor material and on the doping elements.

The instrument is equipped with four LEDs (ELJ series, Roithner Lasertechnik GmbH, Austria) which have a semiconductor element in AlInGaP (Aluminium-Gallium-Indium-Phosphide) or GaAlAs (Gallium-Aluminum-Arsenide) material, suitable for light emission in the spectral range of interest ($\lambda = 630, 690, 750, 850$ nm). The use of LED technology presents the following advantages:

- Precise emitted radiation at well-defined wavelength.
- Reduced response time with the possibility to use a current signal to adjust the light intensity. This aspect make a LED particularly suitable to be driven by the microcontroller.
- Radiation is emitted according to a narrow optical cone, which simplifies its collimation towards the optical fiber and reduces the effects of crosstalk between different channels.
- Good efficiency of the ELJ series, which means a high intensity of emitted radiation associated with a relatively small consumption of electric power (Nicolai et al., 2007).

Four optical filters (NT65-NT67, Edmund Optics Ltd, UK), one for each LED, were added at the end of the optical fiber input arms (see paragraph *the eight-arm optical fiber*), just before the photodiodes. The introduction of a filter at the same wavelength of the corresponding LED

cuts down the wide range environmental noise during the acquisition, improving the signal to noise ratio.

LEDs and relative filters represent the module unit of the instrument (4 in Figure 1) that can be change according to the specific application (see paragraph *Modularity*).

3. The photodiodes

A photodiode is a transducer which detects optical radiation and converts it into a proportional electrical signal. The sensing element is based on a p-n junction semiconductor material, with an asymmetric doping.

Four photodiodes, one for every LED, were used (IQ 800 series, Roithner Lasertechnik GmbH, Austria). These are silicon-based photodiodes with a good response in the vis/NIR spectral range, and with integrated low noise JFET-amplifier. However, a dedicated stage of filtering and amplification was designed for every photodiode in order to further improve the signal to noise ratio, before the analog-digital conversion (5 in Figure 1).

4. The eight-arm optical fiber

An optical fiber is a dielectric waveguide, composed of glass or plastic thin filaments transmitting electromagnetic radiation. Fibers are flexible, immune to electrical noise and weather conditions and insensitive to temperature variations.

A customized quartz optical fiber (HCL-600 quartz type) was specifically designed and realized for the application by a leader industry (Fort Fibre Ottiche, Italy), in order to optimize radiation transmission and the optical couplings. The fiber consists of eight independent arms, all converging in a single probe. This is the element to put in contact with the sample during a measurement. Four arms (output arms in Figure 1) are connected to the LEDs, collecting their emitted radiation towards the probe and then to the sample. At the same way, the other four arms (input arms in Figure 1) collect the radiation coming back from the sample and transfer it to the four photodiodes.

5. The battery

The prototype is powered by a single internal battery of 12 V. This makes the instrument portable and suitable for field use.

How it works

When the user presses the acquisition button (2 in Figure 1), the microcontroller runs a specific routine, pre-loaded in its own integrated memory, which controls the turning on of the LEDs according to the correct sequence. With reference to Figure 1, it is possible to schematically describe the operations composing the algorithm of the acquisition routine.

1. The microcontroller (1) sends the driving signal to turn on the first LED. Before reaching it, the signal is converted by the DAC (3) in analogue form and modulated by the amplification stage (4). In general each LED has, in fact, its own driving current intensity value, predetermined during the calibration phase.
2. As soon as the LED turns on, the emitted light radiation is brought to the sample through the respective output arm of the optical fiber, and then reflected back in the input arm (6).
3. At the same moment, the microcontroller enables the detection on the corresponding photodiode (5) for a set integration time. The light is thus translated into an electrical signal, which is amplified and converted into digital form by the ADC (3), in order to be returned to the microcontroller.
4. For statistical purposes, four measurements are taken in rapid succession on the same LED and then averaged in a single value by the processor of the microcontroller.
5. Once this procedure is completed for all the LEDs, data are ready to be shown on the display (2). In the same way, they are sent to the Bluetooth card (2) and then to the computer.

The overall acquisition algorithm takes no more than half second, after which the device is ready for a new acquisition.

Preliminary tests of linearity

Materials and Methods

The prototype is designed to perform a direct measurement of the reflected light intensity, i.e. a measure of the absolute reflectance, expressed in an arbitrary unit coming from the electronics of the system (on a scale of values between 0 and 32767). Thanks to a specific calibration procedure, based on the acquisition of standard references with known reflection coefficients, an indirect estimation of the relative reflectance is possible. At the moment, this is realized in post-processing through the application of simple ratios and normalization factors. The relative reflectance is the real significant variable, which allows comparisons between acquisitions made at different times and under different conditions.

The quality of the direct measurement is a prerequisite to obtain a correct estimation of the relative reflectance. The choice of the device setting parameters, that affect this measure, therefore becomes crucial. This should ideally ensure the linearity of the response and be appropriate to the specific application, by expressing an adequate sensitivity: the linearity to allow immediate comparisons; the sensitivity to be able to describe phenomena characterized by small dynamics and to exploit the whole available measurement scale.

In particular, measuring the same sample under the same conditions, the instrumental parameters which can be set and that influence the light detected on a certain channel are two:

- the stimulation level *Stimulus* of the LED drive signal, which modulates the intensity of the incident light and, consequently, of the reflected light, too;
- the amplification factor *Gain* of the signal detected by the photodiode, which defines the numerical value actually read within the available measurement scale.

In order to simplify the use of the instrument, the full capability of setting for these parameters is not given to the user. The identification of their suitable operating values, in fact, is in general not immediate.

The optimal value of amplification *Gain* for each channel must therefore be determined and pre-set at a fixed value.

On the other hand, the calibration procedure automatically sets the value of *Stimulus* every time the instrument is switched on. According to this algorithm, an initial acquisition of a standard reference must be realized. The level of *Stimulus* that induces a detected light equal to $\frac{1}{2}$ of the available measurement scale is determined through successive signal increments. If calibrating with appropriate standard reference, this threshold value should avoid the risk of saturation that could be caused by more reflective samples. At the same time, measurements of a sufficiently large order of magnitude should be ensured also for samples less reflective. The value of *Stimulus* which is defined in calibration for each channel is then used to drive the corresponding LED during the whole working session, until the instrument is switched off. In this way, the user has an indirect effect on the determination of the optimal *Stimulus* with the choice of the standard reference to be used in calibration, depending on the specific experimentation.

The aim of this part of the work was to study the behaviour of the device as a function of the *Stimulus* and *Gain* parameters. A special test software has been implemented and loaded into the microcontroller to explore automatically the totality of the parameter space. With this configuration of the device, it was possible to evaluate the response for all the admissible (*Stimulus*, *Gain*) couples (with integer values of *Stimulus* in the range $0 < Stimulus < 255$, and $Gain = 1, 2, 4, 8$). According to the previous considerations, the tests were repeated on the four available standard references, for reflection coefficients $R = 1, 0.5, 0.25, 0$. Furthermore, some samples of red and white grapes were measured with the same protocol, in order to consider conditions close to the operating ones.

Results

Figures 2 and 3 show graphs regarding two different acquisitions on channel $\lambda = 750 \text{ nm}$, the first using reference $R = 0.25$ and the second with reference $R = 0.5$.

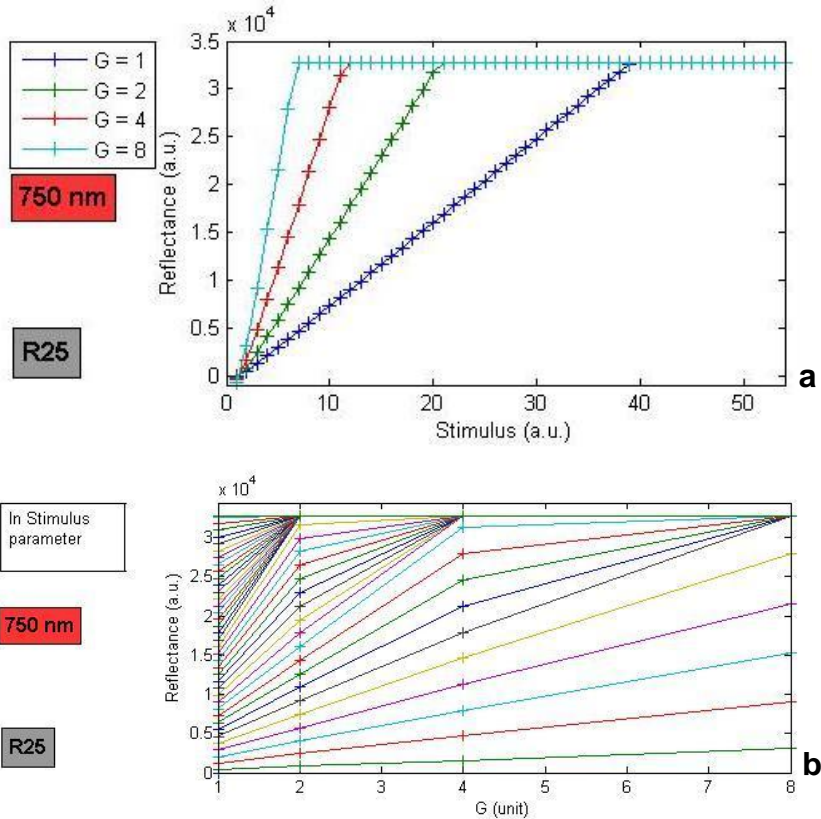


Figure 2. Acquisitions on channel $\lambda = 750 \text{ nm}$, using reference $R = 0.25$. a) absolute reflectance vs *Stimulus*, for the four curves in *Gain (G)* parameter; b) absolute reflectance vs *Gain (G)*, in *Stimulus* parameter

In both figures, left chart represents absolute reflectance value measured in function of *Stimulus* value, for the four curves in *Gain* parameter. For simplicity, was chosen to display only the significant portion of the graphs on the first few *Stimulus* values, beyond which saturation for all curves is notable. For this reason the abscissa scale is therefore different between the two graphs.

Absolute reflectance trend in *Stimulus* function presents an excellent linearity independently from *Gain* value, apart from little predictable boundary effects near zero and near saturation. This trend is a good feature assuring a work range in linearity zone, due to calibration procedure.

Saturation happens very quickly. Actually just a small portion of the available gap $0 < \textit{Stimulus} < 255$ is used. Calibration done on major gradient curves (high *Gain* values) would make saturation chance in acquisition very high; in this way the device would be unusable. The phenomenon is more pronounced in the test on reference $R=0.5$, that is more reflecting respect to $R=0.25$. This is a common behaviour in all the four channels. It is confirmed by tests on other references (data not displayed) and it is caused by optic high efficiency of the system.

Gain value = 1 is the only usable for all the four channels. It is the one corresponding to the less gradient line, developing in the wider gap of *Stimulus* values.

This choice is confirmed by graphs on the right side of Figures 2 and 3, leading to conclusions coherent with the previous ones. They show the same acquisition ($\lambda = 750 \text{ nm}$, reference $R = 0.25$ and $R = 0.5$) representing absolute reflectance measured in function of *Gain*, regarding the 256 parameterized curves in *Stimulus*. In this case, only the curves corresponding to the first values of *Stimulus* parameter are distinguishable. Horizontal reflectance saturation line with full scale value is visible. Every other line collapses on it as *Stimulus* parameter grows.

Linearity in function of *Gain* is very good, clearly for the curves not deformed by saturation effects. The choice, however, of a unique value of minimum *Gain* = 1 for all measurement channels was confirmed by this results, being the one providing the greatest number of significant *Stimulus* values.

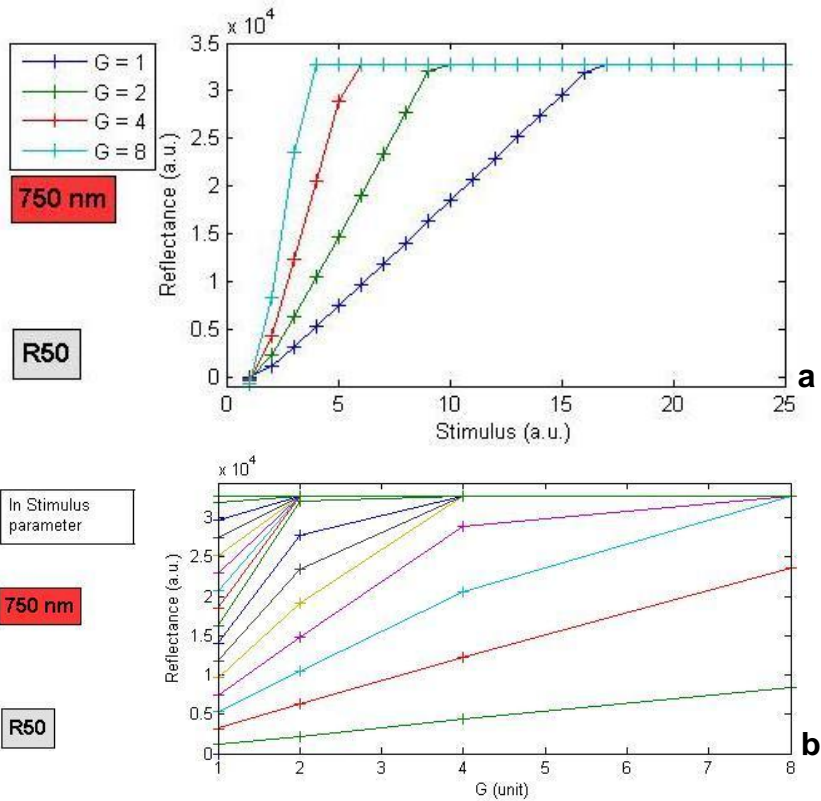


Figure 3. Acquisitions on channel $\lambda = 750$ nm, using reference $R = 0.50$. a) absolute reflectance vs *Stimulus*, for the four curves in *Gain* (G) parameter; b) absolute reflectance vs *Gain* (G), in *Stimulus* parameter

Regarding standard references, also in this case the best behaviour was achieved using a less reflective reference ($R = 0.25$).

In general, according to the calibration procedure, the more suitable choice is a standard reference which provides spectral reflectance similar to the samples to be measured. Tests carried out on black grapes samples (data not shown) showed the inadequacy of the available standard references: black grapes have in fact a much less reflective spectral profile compared also to reference $R = 0.25$. This phenomenon is particularly evident in correspondence of the first two wavelengths in the visible region. With reference to graphs on the left of Figures 2 and 3, the use of a darker reference and close to sample

spectral characteristics would lead to a further reduction of the slope of the reflectance curve, with the consequent desired enlargement of the usable *Stimulus* range.

For these reasons, a wider and more detailed range of standard reference is needed, in particular for low reflectance values, closer to the spectral characteristics of black grapes. Moreover a broader number of references could allow to analyze in detail response linearity in function of the measured reflectance.

Concerning the comparison among the different measure channels, the system shows the same trends for all the four wavelengths. Under the same answer, parameters values change depending on channel. This is due to LEDs and photodiode working features that changes depending on exercise wavelength, despite they are of the same type, as declared by producing companies. It is then possible to make an independent calibration on every channel. A different standard reference can be used. Direct measures of reflected light on the four wavelengths would not be comparable even in the same acquisition. Comparability remains unchanged as far as relative reflectance measures obtained in post-elaboration.

Conclusions and future works

In this work a prototype of a simplified optical vis/NIR device based on LED technology was designed and tested. A preliminary test focussed on the evaluation of signal linearity gave good results. The prototype is very promising.

A considerable amount of data were collected through laboratory tests in order to evaluate the behaviour of the instrument as a function of the two setting parameters: the *Gain* amplification factor, and the *Stimulus* level for the drive signal of the LEDs. In both cases, an excellent linearity of the response is shown.

The optimal setting value for the amplification parameter has been determined ($Gain = 1$). On the other hand, a semi-automatic calibration procedure has been implemented to allow the user to indirectly define the *Stimulus* level for each LED driving signal. In this way, this can be customized according to the specific experimentation.

Based on this procedure, the tests have shown the need for a broader and more detailed set of standard references to be used in calibration. Standard references with spectral characteristics similar to the samples to be measured should be ideally always available, in order to realize a good calibration. For this purpose, a simple and economic solution could be the definition of a protocol for the realization of handmade references. For example, this could be based on water solutions of standard food dyes at different concentrations.

In this way, and thanks to the modularity given by the LEDs/filters units, it would be potentially possible to use the same device on any food matrix.

The integration of a simple processing algorithm in the microcontroller software would allow to visualize real time values of relative reflectance. Being immediately comparable and interpretable, these would represent a particularly useful information for the user.

Considering results, further tests are desirable in view of the next experimental campaign, in order to use the instrument during ripening directly on grapes in the field or at harvest.

References

- Antonucci, F.; Pallottino, F.; Paglia, G.; Palma, A.; D'Aquino, S. & Menesatti, P., 2011. Non-destructive estimation of Mandarin maturity status through portable VIS-NIR spectrophotometer. *Food and Bioprocess Technology*, 4(5), 809-813
- Beghi, R; Spinardi, A.; Bodria, L.; Mignani, I. & Guidetti, R., 2012. Apples nutraceutic properties evaluation through a visible and near-infrared portable system. *Food and Bioprocess Technology* DOI 10.1007/s11947-012-0824-7
- Bodria, L.; Fiala, M.; Guidetti, R. & Oberti, R., 2004. Optical techniques to estimate the ripeness of red-pigmented fruits. *Trans. of ASAE* 47, 815-820
- Brosnan, T. & Sun, D.W., 2004. Improving quality inspection of food products by computer vision: a review. *Journal of Food Engineering*. 61, 3-16
- Camps, C., & Christen, D., 2009. Non-destructive assessment of apricot fruit quality by portable visible-near infrared spectroscopy. *Food Science and Technology*, 42(6), 1125-1131
- Cen, H. & He, Y., 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. *Trends in Food Science & Technology*, 18, 72-83
- Costa, G.; Bonora, E.; Fiori, G. & Noferini, M., 2011. Innovative non-destructive device for fruit quality assessment. *Acta Hort. (ISHS)* 913, 575-581
- Du, C.J. & Sun, D.W., 2006. Learning techniques used in computer vision for food quality evaluation: a review. *Journal of food engineering*. 72(1), 39-55
- El-Masry, G.; Nassar, A.; Wang, N. & Vigneault, C., 2008. Spectral methods for measuring quality changes of fresh fruits and vegetables. *Stewart Postharvest Review* 4(4), 1-13
- Firtha, F; Fekete, A.; Kaszab, T.; Gillay, B.; Nogula-Nagy, M.; Kovács, Z. & Kantor, D.B., 2008. Methods for improving image quality and

reducing data load of NIR hyperspectral images. *Sensors* 2008, 8, 3287-3298

Guidetti R.; Beghi R. and Giovenzana V., 2012. Book chapter "Chemometrics in Food Technology", book "Chemometrics", ISBN: 978-953-51-0438-4, InTech, Rijeka (2012), pp. 217-252

Guidetti, R.; Beghi, R. & Bodria, L., 2010. Evaluation of Grape Quality Parameters by a Simple Vis/NIR System. *Trans. of the ASABE* 53(2), 477-484

Guidetti, R.; Beghi, R.; Bodria, L.; Spinardi, A.; Mignani, I. & Folini, L., 2008. Prediction of blueberry (*Vaccinium corymbosum*) ripeness by a portable Vis-NIR device. *Acta Horticulturae* 310, ISBN 978-90-66057-41-8, 877-885

Kawano, S., 1998. New application of non-destructive methods for quality evaluation of fruits and vegetables in Japan. *J. Japan. Soc. Hort. Sci.* 67, 1176-1179

Lelievre, J.M.; Latche, A.; Jones, B.; Bouzayen M. & Pech, J.C., 1997. Ethylene and fruit ripening. *Physiologia Plantarum* 101, 727-739

Liu, F.; Jiang, Y. & He Y., 2009. Variable selection in visible/near infrared spectra for linear and nonlinear calibrations: A case study to determine soluble solids content of beer. *Analytica Chimica Acta*, 635 45-52

Lurie, S.; Friedman, H.; Dagar, A.; Weksler, A.; Rot, I.; Kaplunov, T.; Zutahy, Y.; Nyasordzia, J. & Lichter, A., 2012. Non-destructive Tools for Determining Harvest Dates of Deciduous Fruits. CIGR Section VI International Technical Symposium on "Innovating the Food Value Chain" Postharvest Technology and Agri-Food Processing. Stellenbosch, South Africa, 25-28 November, 2012

McClure, W.F. & Tsuchikawa, S., 2007. Near-infrared Spectroscopy in Food Science and Technology, Chapter 4.1, ed. by Y. Ozaki, W. Fred McClure, A.A. Christy (Wiley-Interscience, Hoboken, NJ, 2007) 75-107

- Naes, T.; Isaksson, T.; Fearn, T. & Davies, T., 2002. A user-friendly guide to multivariate calibration and classification. Chichester, UK: NIR Publications ISBN 0-9528666-2-5
- Nicolai, B. M.; Beullens, K.; Bobelyn, E.; Peirs, A.; Saeys, W.; Theron & K. I., Lammertyna J. (2007). Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. *Postharvest Biology and Technology*, 46, 99-118
- Osborne, B.G.; Fearn, T. & Hindle, P.H., 1993. *Practical NIR Spectroscopy: with Applications in Food and Beverage Analysis*, 2nd ed. (Longman Scientific & Technical, Essex, England)
- Saranwong, S.; Sornsrivichai, J. & Kawano, S., 2003. Performance of a portable near infrared instrument for Brix value determination of intact mango fruit. *J. Near Infrared Spectrosc.* 11, 175-181
- Sun, D.W., 2010. *Hyperspectral imaging for food quality analysis and control*. Edited by Da-Wen Sun. Elsevier. ISBN 978-0-12-374753-2
- Wang, W. & Paliwal, J., 2007. Near-infrared spectroscopy and imaging in food quality and safety. *Sens. & Instrumen. Food Qual.* 1, 193-207
- Wold, S.; Sjöström, M. & Eriksson, L., (2001). PLS-regression: a basic tool of chemometrics. *Chemom. Intell. Lab. Syst.* 58, 109-130
- Xiaobo, Z.; Jiewen, Z.; Povey, M.J.W.; Holmes, M. & Hanpin M., 2010. Variables selection methods in near-infrared spectroscopy. *Analytica Chimica Acta* 667, 14-32
- Zheng, C.; Sun, D.W. & Zheng, L., 2006. Recent developments and applications of image features for food quality evaluation and inspection: a review. *Trends in food Science & Technology.* 17, 642-655

PART 3

General conclusions and future works

The conventional methods for determination of fruits quality parameters (i.e. sugar content, soluble solid content, acidity, phenolic compounds) are time consuming, require preparation of samples, are often expensive, and generally highlight only one or a few aspects of fruits quality. However, the difficult preparation of samples for this analysis requires a well-equipped laboratory, as well as waiting 8 or 10 h for results. A limited number of laboratories and lack of quick information mean that producers could not take the best decision in the best moment about process control and management. For example, wineries must begin the process of winemaking without having such data available, reducing their chances to diversify production and achieve high-quality products.

Therefore, there is a strong need in the modern industry for a simple, rapid, and easy-to-use method for objectively evaluating the quality of agro-food products. This kind of tools would enable real-time analysis at the receiving station and allow preliminary decision-making about fruits by the rapid analysis of various parameters simultaneously.

The solution can be found in non-destructive optical analysis.

This work shows the principal non-destructive applications for analysis in food sector: vis/NIR and NIR spectroscopy and image analysis (traditional, multispectral and hyperspectral). They are rapid techniques used in combination with chemometrics analysis for qualitative and quantitative analysis.

Experimental works collected in this thesis were focused on the application of vis/NIR spectroscopy, the most adequate technique for its objectives.

Vis/NIR spectroscopy measures the spectral components of light emerging from sample after penetrating its surface.

Reflectance measurement technique in vis/NIR is well suited for investigating the main components of first representative layers of fruit pulp and not only on its surface. Radiation is partly absorbed and partly backscattered by the first internal layers. Measuring the spectral modifications of the radiation after interaction with the fruit is possible to

establish a relationship between spectral reflectance and specific components concentration in the sample.

Moreover vis/NIR spectroscopy is the technique that has been developed further in recent years. This success is because spectral measurement for one sample could be done in a few seconds. Numerous samples could be analysed and multi-indexes analysis can be carried out.

The vis/NIR datasets are often very large and highly complex, often involving thousands of variables. In recent years, developments in both chemometrics and optical instrumentation have resulted in rapid methods for predicting the concentration of specific chemical constituents. Vis/NIR spectroscopy coupled with chemometric methods appears to be one of the most powerful analytical tools for studying agro-food products.

Chemometrics is applied to solve both descriptive and predictive problems in chemical, pharmaceutical, and food sectors (Beebe et al., 1998).

Many works are focused on the study of chemometrics. This is because an important challenge is to build robust calibration models. It is important, in fact, to apply chemometric methods able to select useful information from big size matrix of spectral data. Moreover food researchers and analysts are looking for the sensitive wavelength in vis/NIR region representing the characteristics of food products, with the aim of develop some simple and low-cost instruments (Cen & He, 2007).

Portable vis/NIR instruments are often used in controlled laboratory conditions (Xing et al., 2005; De Belie, 2000). However, this acquisition technique is particularly versatile and is suitable for designing compact, portable, handheld devices, to be directly used in field to monitor the ripening process or quality parameters (Bodria et al., 2004; Costa, et al., 2011).

According to results arising from this thesis, an experimental device based on LED (light emitting diodes) technology was designed. At the moment the level of development of the system has reached a satisfactory level. The simplified optical device undergoes a first testing

phase employing laboratory optical standards with the aim of identifying the LED setting which can provide the best performance.

This first prototype of the simplified optical device was equipped with four LEDs emitting at the selected wavelengths. The LED technology for samples illumination was chosen thanks to simplification advantages and cost reduction.

Research activities for the selection of most significant wavelengths was carried out on Nebbiolo grape (red grape).

The design of the prototype of the simplified optical device was realised with particular attention to versatility and modularity. The possibility to adjust the light source with a specific choice of wavelengths for LEDs, makes it possible to use the same simplified optical device for many different applications. This modular design allows an easy adjustment for different objective (i.e. ripeness evaluation, chemicals and physical properties prediction or shelf life analysis), and for different kind of sample matrix.

The future aims will be to test the instrument equipped with appropriate selected wavelengths on different studied agro-food products, in order to achieve the research objectives. These experimentation will be carry out in view to develop a system equipped with modular optical tools. This dynamic approach will allow a quick interchange of optical tool, in order to make available the same simplified LED based system to the different products.

Moreover, this approach of variable selection could be extend to other non destructive techniques. For example, hyperspectral imaging, combined with chemometric technique, is a powerful method to identify key wavelengths in order to develop of multispectral system, for on-line applications.

This kind of device would be a compact, pocket-size, inexpensive and easy to use system. Farmers could employ it directly in field for a quick evaluation of fruits ripening level or consumers could employ it directly on department store to check freshness of fresh-cut products.

This work demonstrate good results regarding the prototype step. With the possible help of industrial partners an evolution and an engineering of the system will be desirable. This would consolidate results, create a userfriendly interface and reduce dimension of the device to pocket size.

References

- Beebe, K.R., Pell, R.J. & Seasholtz M.B., 1998. *Chemometrics: A Practical Guide*. New York, N.Y.: John Wiley and Sons
- Bodria, L., Fiala, M., Guidetti, R., Oberti, R., 2004. Optical techniques to estimate the ripeness of red-pigmented fruits. *Trans. of ASAE*, 47, 815-820
- Burger, J. & Gowen, A., 2011. Data handling in hyperspectral image analysis. *Chemometrics and intelligent Laboratory Systems*, 108, 13-22
- Cen, H. & He, Y., 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. *Trends in Food Science & Technology*, 18, 72-83
- Cheng X.; Chen Y.R.; Tao Y.; Wang C.Y.; Kim M.S. & Lefcourt A.M., 2004. A novel integrated PCA and FLD method on hyperspectral image feature extraction for cucumber chilling damage inspection. *Transactions of the ASAE, American Society of Agricultural Engineers* ISSN 0001-2351. 47(4), 1313-1320
- Costa, G.; Bonora, E.; Fiori, G. & Noferini, M., 2011. Innovative non-destructive device for fruit quality assessment. *Acta Hort. (ISHS)*, 913, 575-581
- De Belie, N.; Schotte, S.; Coucke, P. & De Baerdemaeker, J., 2000. Development of an automated monitoring device to quantify changes in firmness of apples during storage. *Postharvest Biology and Technology*, 18(1), 1-8

Acknowledgments

I ringraziamenti vengono dal cuore, in italiano mi sembrano più sentiti e data la padronanza della lingua inglese non renderei l'idea, già in italiano ho i miei dubbi!

...tre anni sono passati...quanta fatica...ma quanto è stato bello!

Purtroppo nella vita non ci sono molte occasioni per comunicare alle persone che ti stanno accanto quanto sono importanti per te, e per ringraziarle di per quello che hanno fatto, che fanno e che faranno. Penso che il capitolo "ringraziamenti" della tesi di dottorato, sia una bella occasione.

La prima persona che voglio ringraziare è Roby che ha partecipato fattivamente alla stesura di questo lavoro. Grazie per ciò che mi hai insegnato, ti devo molto. Grazie per la pazienza, per le chiacchierate, grazie di avermi ascoltato, supportato, sopportato e consolato. La tua calma e razionalità sono fondamentali. Sono affascinata dal tuo modo di ragionare ma soprattutto di fare di ogni situazione un momento UCAS. Ti stimo molto, sei un riferimento.

Grazie al prof Guid. Arrivare in ufficio tutti i giorni ed essere accolta con un sorriso, il buon umore, l'entusiasmo, per me è fondamentale. Grazie per le opportunità che mi ha dato, delle esperienze che mi ha permesso di vivere. Grazie delle risate. Grazie per la fiducia, per gli stimoli, grazie di avermi permesso di affrontare cose grandi, anche più di me, penso di avere imparato molto.

Grazie al prof Luigi Bodria per aver creato il team di lavoro...e che team! Lo trovo di fondamentale importanza per lavorare bene. Grazie di aver fatto in modo che io potessi farne parte. Grazie per la fiducia.

Grazie al prof RobOb. E' entusiasmante vedere la passione, la ricerca attenta e precisa del linguaggio appropriato che ha quando lavora. Ma accanto a una mente così scientifica trovo commovente l'amore che ha nelle "piccole cose".

Ezio, credo che i comportamenti delle persone siano sempre dettati da un motivo, non ti ho sempre capito completamente, ma in molti casi sì. Grazie per quello che hai fatto per me.

Grazie a Ester, Anna, Sergio, Roberto, Giorgio e Barbara per i fondamentali supporti tecnici.

Grazie al prof Cini. Grazie del tempo che ha dedicato alla lettura del lavoro, grazie delle belle parole, sono state molto importanti per me. Grazie della sua voglia di lavorare ancora bene e con umiltà.

Gracias a el team chileno: Stanley, Fabiola, Hernán, Lorenzo, Rodrigo, Claudio y Valeska. Gracias por su tiempo, para hacerme sentir como a casa, gracias por las risas, por el tiempo que pasamos juntos, gracias por la diversión, gracias por lo que me has enseñado, gracias por los asados y por las fiestas. Grazie anche ad Aira, Raffa e Roby per i momenti cileni.

Grazie all'UCAS: Alby, Ale, Aira, Cri, Jacopo, Martina e Margherita, Pao, Raffa, Roby, Simo e Nicolò, Vale e Marino. L'Università dovrebbe ringraziarvi di lavorare per lei...per il momento dovete accontentarvi solo del mio grazie. Sono ancora molto arrabbiata con l'Università per aver permesso ad alcuni di voi di andarsene...che grande perdita! Sono convinta che insieme potremo farcela a lavorare bene, in onestà, per cambiare l'Università italiana, siamo noi il futuro. Anche se le persone a cui esprimo questo mio desiderio mi ridono in faccia, finchè avrò energia, voglia ed entusiasmo ci proverò. Conto su di voi!. Grazie per i fantastici momenti passati insieme, per i pranzi quotidiani, mi fate sentire a casa, con la mia famiglia. Grazie per le risate, per le chiacchierate, per i confronti, per i consigli, per gli sfoghi, per le parole di conforto, per la gioia. Mi diverto sempre! Grazie di esserci! Un grazie particolare a Raffa e Cri per la collaborazione alla tesi.

Grazie mami, grazie papi, anche sta volta ce l'abbiamo fatta!!!

“Le persone arrivano nella tua vita per una ragione, per una stagione o per tutta la vita. Quando qualcuno entra a far parte della tua vita, di solito, è per soddisfare un bisogno, per assisterti di fronte a una difficoltà, per aiutarti fisicamente, emotivamente o spiritualmente. Le persone entrano a far parte della tua vita perché è arrivato il momento di condividere, crescere e imparare con loro. Ti portano un'esperienza di vita, ti fanno ridere, ti danno gioia.”

Grazie a tutti per essere parte della mia vita, che sia per una ragione, per una stagione o per tutta la vita!