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Setting up of simplified optical tools for the evaluation of fruit and vegetables

Ph.D. Thesis
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To my parents

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Introduction

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. 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.

Some quality evaluation is still performed manually by qualified staff, but this is often tedious, laborious, expensive and unreliable due to its subjective nature. On the same time, classical chemical analyses, common at the moment in the food sector, have several limits: these take time, are wasteful, destructive, and ultimately inadequate to test fruit or vegetables in field.

Increased demands for objectivity, consistency and efficiency moved the focus to non-destructive rapid systems, and in recent years optical techniques have been applied increasingly for food quality evaluation.

The study of non-destructive methods and the design of new devices for monitoring the largest number of samples in short time and give a more comprehensive overview of ripening is an ongoing process.

Currently, visible near infrared (vis/NIR) and near infrared NIR (NIRs) spectroscopy are techniques widely applied in the food sector.

A review of literature reveals that the NIRs techniques (vis/NIR and NIR) were applied to a wide array of agri-food applications. The feasibility of NIRs spectroscopy to measure quality attributes of fruit and vegetables has been shown for many products. 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. 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 only in a limited area of the sample, therefore it is especially suitable for homogeneous products. However these optical technologies are able to reach 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 a suitable technique for this purpose.

Three main types of NIRs devices are available: i) laboratory instruments for applications in research centers or in industry laboratories, ii) sorting and sizing devices designed specifically for the fruit and vegetable industries, e.g. in warehouses, iii) portable devices for the analysis directly in the field. Table 1 shows the main differences between the three groups of NIRs devices.

Table 1. Characteristics of the three main categories of NIRs devices

	Application area	Flexibility of use	Applicability	Measurement accuracy and reproducibility	Cost
Laboratory devices	Research/Industry	Adaptable to different matrices	Fixed system	Optimal	Average/ high
Sorting and sizing	Industry	Specific categories of products	Fixed system	Good	Average/ high
Portable devices	Also in field	Dedicated for individual products	Portable/ handheld	Good	Average

Today, a wide selection of spectroscopic devices is available and there are about 60 NIR spectrometer manufacturers around the globe.

There is certainly a large demand of NIRs portable devices, so more research is required in this area. The availability of low cost miniaturized spectrophotometers has opened up the possibility of portable devices which can be used for monitoring the fruit and vegetables maturity.

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.

Hyperspectral and multispectral imaging combine 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 multi- and hyperspectral imaging 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 these 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 mantaining 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.

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 analyze 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.

Objectives

The fruit and vegetable sector met in recent years an important phase of expansion among consumers due to the high nutritive value and convenience of products. This trend is particularly evident for fresh-cut products (fruit and vegetables minimally processed in ready-to-eat packs) due to: (i) their ease of use, and (ii) nutritional properties.

Monitoring the quality decay of fruits and vegetables is necessary to control quality parameter, e.g. the freshness level or the ripening degree, during the entire production chain and to ensure quality product for the consumer. The non-destructive technologies are currently used only by research institutions or big companies, due to their cost and complexity of use. Therefore, the sector is interested in new simplified systems for rapid analysis that can be performed along the chain and directly at point of sale with a double objective: to use the information from sensors to better manage the product, and to preserve the consumers' expectations providing additional selection criteria.

Objectives of this thesis were the setting up, design and testing of innovative optical simplified devices dedicated to fruit and vegetable products chains. Low-cost and easy-to-use prototypes based on vis/NIR technology and/or multispectral image analysis were presented. In particular, (a) a compact-sized LED technology based device, for the prediction of the main qualitative/ripening parameters of the case studies was realized and tested, and (b) the applicability of multispectral imaging was studied with a view to a simplified optical system for freshness decay assessment.

Different kinds of matrix were investigated (white grapes, salad, and apples), with particular attention to fresh-cut products (*Valerianella locusta* L. salad, *Cripps Pink* apples).

This thesis is structured in autonomous chapters presented in the form of manuscript (some of which have been already published), and is divided into two main parts. Part I is a collection of four chapters that basically deal with vis/NIR spectroscopy, while Part II presents a preliminary study focused on multispectral imaging.

Chapter 1 presents a research that introduces the following works. The aim of this study was to investigate the applicability of non-destructive techniques (i.e. electronic nose and visible–near infrared spectroscopy) in monitoring freshness decay of fresh-cut *Valerianella locusta* L. during storage at different temperatures.

In particular, the applicability of vis/NIR spectroscopy was demonstrated, and this was the starting point for the study discussed in Chapter 2 about the feasibility of a simplified handheld and low-cost optical device. This was focused on identifying a limited number of significant vis/NIR wavelengths able to discriminate freshness levels during shelf-life of fresh-cut *Valerianella locusta* L. Four wavelengths were selected: 520 nm, 680 nm, 710 nm and 720 nm.

The same approach was followed in Chapter 3 to identify the three most significant wavelengths able to discriminate in the field those grapes ready to be harvested. The ripening process of *Vitis vinifera* cv. Nebbiolo grapes was investigated, resulting in the wavelength selection of 670, 730, 780 nm

These studies were the basis for the development of a first prototype of a simplified LED-based optical system, which is presented in Chapter 4. In this case, the device was design taking into account the versatility of use. The measurement on red grapes was considered for the design, i.e. the evaluation of the ripeness directly in field, and the four selected wavelengths proposed were 630, 690, 750 and 780 nm. The development stage of this prototype reached a satisfactory level.

Finally, the aim of Chapter 5 was to test the prototype for the estimation of the ripening parameters of *Chardonnay* white grape for *Franciacorta* wine directly in field. Optical measurements were carried out also with a portable commercial vis/NIR spectrophotometer, as a reference instrument for performance comparison. The overall calibration and prediction results of the chemometric models were satisfactory. Moreover, the design of the prototype with particular attention to modularity (i.e. the possibility to adjust light sources with a specific choice of wavelengths for LEDs) makes it possible to conceive the same simplified optical device for many different applications (i.e. ripeness evaluation, chemicals and physical properties prediction or shelf life analysis) and for different food matrix.

Part II consists of Chapter 6, which presents the use of multispectral imaging technique. The objective of this preliminary study was to test vis/NIR multispectral imaging to assess the freshness decay throughout the day (12 hours) of fresh-cut apple *Cripps Pink* slices. A clear time evolution of the sample was shown and the technique demonstrated to potentially be a rapid and non-destructive approach for monitoring the freshness decay throughout the hours of minimally processed apple slices. This technique could therefore be used to develop a simplified optical system for freshness decay assessment

The conclusions of the overall work are discussed in a final Chapter. This thesis demonstrates how the simplification of traditional optical techniques is possible. The proposed methodologies can be replicated and can be the starting point for new research lines regarding innovative technologies for the agro-food sector.

Part I

VIS/NIR SPECTROSCOPY

1

Monitoring of fresh-cut Valerianella locusta Laterr. shelf life by electronic nose and VIS–NIR spectroscopy


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Monitoring of fresh-cut *Valerianella locusta* Laterr. shelf life by electronic nose and VIS–NIR spectroscopy

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ABSTRACT

The aim of this work was to investigate the applicability of non-destructive techniques in monitoring freshness decay of fresh-cut *Valerianella locusta* L. during storage at different temperature. The sampling was performed for 15 days for *Valerianella* samples preserved at 4 and 10 °C, and for 7 days for samples stored at 20 °C. The quality decay of samples was evaluated by quality parameters (pH, water content, total phenols, chlorophyll *a* fluorescence) and by non-destructive systems (electronic nose and visible–near infrared spectroscopy).

Cluster Analysis (CA) was performed on quality indices and four clusters were identified, namely “fresh”, “acceptable”, “spoiled” and “very spoiled”.

Principal Component Analysis (PCA) was applied on the electronic nose data in order to evaluate the feasibility of this technique as a rapid and non-destructive approach for monitoring the freshness of fresh-cut *Valerianella* during storage.

Linear Discriminant Analysis (LDA) and PLS-discriminant analysis (PLS-DA) models were developed to test the performance of electronic nose and VIS–NIR, respectively, to classify samples in the four classes of freshness. The average value of samples correctly classified using LDA was 95.5% and the cross validation error rate was equal to 8.7%. The results obtained from PLS-DA models, in validation, gave a positive predictive value (PPV) of classification between 74% and 96%.

Finally, predictive models were performed using Partial Least Squares (PLS) regression analysis between quality indices and VIS–NIR data. RPD values <3 were obtained for water content and pH. Excellent results were obtained for total phenols with R_{cv}^2 and RPD equal to 0.89 and 3.19, and for chlorophyll *a* fluorescence with R_{cv}^2 and RPD equal to 0.92 and 3.22, respectively.

Results demonstrated that electronic nose and VIS–NIR are complementary techniques able to support the conventional techniques in the shelf-life assessment of fresh-cut *V. locusta* L. providing information useful for a better management of the product along the distribution chain.

1. Introduction

In recent decades, there has been a substantial increase in the consumption of fresh-cut or minimally processed fruit and vegetables. The international Fresh-cut Produce Association (IFPA) defines, in 1999, 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” [1].

The growth in the ready to use vegetable industry is due to: (i) their ease of use, in fact changes in human life styles have led consumers to move towards ready-to-eat products and (ii) nutritional properties indeed it is known as source of vitamins, minerals, fiber and antioxidants [2].

Flavor (taste and aroma) quality of fruits and vegetables is influenced by genetic, pre-harvest, harvesting, and postharvest factors. The longer the time between harvest and eating, the greater the losses of characteristic flavor and the development of off-flavors in most fruits and vegetables. Postharvest life bas ed on flavor and nutritional quality is shorter than that based on appearance and textural quality [2]. Thus, it is essential that good flavor quality be emphasized in the future by selecting the best-tasting genotypes to produce, by using an integrated crop management system and harvesting at the maturity or ripeness stage that will optimize eating quality at the time of consumption, and by

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using the postharvest handling procedures that will maintain optimal flavor and nutritional quality of fruits and vegetables between harvest and consumption [2].

A critical aspect relevant for this type of products is represented by their actual shelf life since manipulations such as cleaning, washing, trimming, peeling, cutting or slicing and shredding increase the respiration rate and the ethylene production, and cause perishability. These physiological changes may result in degradation of color, texture, flavor and nutritional value [3]. The shelf life of fresh cut products is shorter than that of unprocessed vegetables [4], it is imposed from producer and limited to 5–7 days for most leafy vegetables [5] in order to assuring the good quality of products to consumers. Moreover, temperature is the most important parameter particularly during storage and throughout the distribution chain. In fact, all fresh-cut items should be stored at 0–5 °C to preserving their quality and prolonging the shelf life [6].

Several studies have been carried out to monitor and extend the shelf life of fresh-cut fruit and vegetables. Color, texture, respiration, microbiological indices, pH, sensory and nutritional are the main parameters evaluated [2,3,7,8]. 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.

Among the various non-destructive systems electronic nose (e-nose) stands out its ability to use the information contained in the headspace of food. Torri et al. [9] used an electronic nose in order to monitor the change in the volatile compounds of minimally processed fresh-cut pineapple during storage at different temperatures. Riva el al. used an e-nose equipped with MOSFET and MOS sensors to evaluate the shelf-life of ready to use fresh cut chicory and carrots [10]. Benedetti et al. [11] applied a commercial electronic nose as a non-destructive tool to characterize peach cultivars and to monitor their ripening stage during shelf-life. Gomez et al. [12] monitored tomato storage shelf life during two storage treatments using a commercial electronic nose.

The increasing importance of NIR spectroscopy in postharvest technology is showed by the relevant growth of the number of publications and the use of commercial on-line NIR systems for grading products based on different quality attributes. Nicolai et al. [13] overviewed NIR spectroscopy for measuring quality attributes of fruit and vegetables. Francois et al. [14] predicted sensory attributes of different chicory hybrids using physico-chemical measurements and visible–near infrared (VIS–NIR) spectroscopy. Sánchez et al. [15] proved that NIR spectroscopy, coupled with the use of chemometric techniques, provides a reliable, accurate method of predicting the shelf-life of asparagus under different storage conditions and as a function of post-harvest treatment applied.

The main objective of this preliminary study was to test e-nose and VIS–NIR spectroscopy in order to detect the quality decay of fresh-cut *Valerianella locusta* L. during storage at different temperature. In particular, a commercial e-nose was used to monitor changes in volatile compound during storage, and a VIS–NIR device was applied in order to evaluate diffuse reflectance modifications in visible–near infrared spectral range and correlate the VIS–NIR spectra with the *Valerianella* quality parameters for the elaboration of predictive chemometric models. These techniques can be considered complementary and their combined use could provide rapid information about the appearance, the chemical composition and the aroma profile of *Valerianella*. The availability of a non-destructive instrument that allows to evaluate changes during shelf life or estimate quality parameter may have a wide number of practical applications in the production chain: during the storage period before packaging, during production process for identifying critical point and during distribution chain, the worst critical phase. Furthermore, the possibility to implement the non-destructive technology, for monitoring the freshness at the point of sale, should be a guarantee for consumers.

2. Materials and methods

2.1. Sampling

V. locusta L. was harvested by hand in September 2012, undergone the minimal process [16], packed in sealed plastic (high-density polyethylene) bag (capacity 100 g) and transported to the laboratory the day of packaging (T_0). The commercial expiration date is fixed by the producer at 4 days from packaging.

Three storage temperatures were investigated: 4 °C, 10 °C and 20 °C; the relative humidity was 80%. The temperature of 4 °C simulates the optimal shelf life condition of fresh-cut products [2]. The temperature of 20 °C simulates extreme conditions of storage, at this temperature the physiological activities are accelerated. The temperature of 10 °C can be considered as the most realistic storage condition in the supermarket [17].

The measurements were performed for 16 days for samples preserved at 4 and 10 °C, and for 7 days for samples stored at 20 °C due to the rapid degradation of *Valerianella* at this temperature. The experimental points were 10, 11 and 6 for 4 °C, 10 °C and 20 °C, respectively; a total of 25 samples were collected (Table 1). Each day of sampling *Valerianella* leaves from 3 bags were used for the measurements.

2.2. Quality indices

Three chemical parameters and the chlorophyll *a* fluorescence were considered indices of the quality decay of *V. locusta* L. during shelf-life [18–20].

2.2.1. Chemical parameters

pH: Twenty grams of samples were blended for 2 min in 40 ml of deionized water. The pH was measured using a digital pH meter (Ioncheck 45, Radiometer Analytical SAS, Lyon, France).

Water content: A thermogravimetric analysis was carried out by using a Sartorius MA150 (Bradford, UK) moisture analyzer. Thermogravimetry is the process of determining the loss of mass that occurs when a substance is heated. In this process, the sample is weighed before and after being heated, and the difference between the two weights is calculated. Five grams of samples were directly weighed in the analyzer and heated at 120 °C until

Table 1
Sampling points during shelf life monitoring for 4 °C, 10 °C and 20 °C.

	Days	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Shelf life	**4 °C**	√		√		√				√	√	√	√			√	√	√
	10 °C	√	√		√				√	√	√	√	√			√	√	√
	20 °C	√	√	√	√	√			√									

reaching the constant weight. The water content was calculated as g of water per 100 g of sample.

Total phenols: Ten grams of crushed samples were weighed in a centrifuge tube and added with 15 mL of methanol. The mixture was stirred for 1 h in the dark and centrifuged at 11,200 G for 10 min at 15 °C. The solids were extracted two more times using 15 and 10 mL of the extraction solvent for 15 min under shaking in the dark, and centrifuged in the above-described conditions. Finally, the gathered extracts were made up to 50 mL with the extraction solvent. Total phenols were determined by the Folin–Ciocalteau method [21] and expressed as mg of gallic acid equivalents per 100 g of sample, by comparison with a calibration curve built with the pure standard compound.

At each temperature and storage time all the chemical analysis were carried out in triplicate.

2.2.2. *Chlorophyll a fluorescence*

Chlorophyll *a* fluorescence transients were measured using a portable Handy Plant Efficiency Analyzer (PEA, Hansatech, UK). A quantitative analysis of the O-J-I-P transient has been introduced [22], named as the "JIP-test" after the basic steps of the transient, by which several phenomenological and biophysical parameters quantifying the Photosystem II (PSII) behavior are calculated. Indices derived from JIP analysis were performed on the mean data points, changed during storage for the three temperatures. In *Valerianella* leaf vegetables stored at 4 °C, 10 °C and 20 °C, some key parameters of chlorophyll *a* fluorescence and some derived indices from the JIP test were able to describe the progression of senescence and loss of product quality. Among JIP indices, the PI (Performance Index) is a biophysical parameter useful in revealing differences in the response of PSII to dark stored leafy vegetables. This is the parameter that better highlighted the quality decay of fresh-cut Valerianella samples, during shelf life [19].

The measurements were taken on the sample surface after illumination with a light intensity (LED with maximum emission peak at 650 nm) of 3000 μmol m^{-2} s^{-1} [20]. Every sampling date, 10 leaves were randomly taken from the stored packages and dark adapted with leaf clips for 30 min before of the acquisition. The average of the 10 measurements were used for the statistical analysis. The spectral measurements were performed, for each experimental point, on other 10 leaves taken simultaneously from the same bag.

2.3. *Non-destructive systems*

2.3.1. *Electronic nose*

The e-nose measurements were performed by a commercial portable electronic nose (PEN 2, Win Muster Airsens Analytic Inc., Schwerim, Germany). It consists of a sampling apparatus, a detector unit containing the sensor array and pattern-recognition software (Win Muster v.16) for data recording and elaboration. The sensor array is composed of 10 Metal Oxide Semiconductor (MOS) sensors of different chemical compositions and thicknesses to provide selectivity towards volatile compounds as indicated by the instrument supplier: W1C (aromatic compounds), W5S (broad-range compounds, polar compounds, nitrogen oxides and ozone), W3C (ammonia, aromatic compounds, aldehydes, and ketones),W6S (hydrogen), W5C (alkanes, aromatic compounds, and less polar compounds), W1S (methane and broad-range compounds), W1W (sulfur compounds, terpenes and sulfur organic compounds), W2S (alcohols, partially aromatic compounds, and ketones), W2W (aromatic compounds and sulfur organic compounds) and W3S (methane). The sensor response is expressed as resistivity (Ohm).

Five grams of *V. locusta* L. sample was placed in a 250 mL airtight glass jar fitted with a pierceable Silicon/Teflon disk in the cap. After 1 h equilibration at 20 ± 1 °C, the measurement started. The sample headspace was pumped over the sensor surfaces for 60 s (injection time) at a flow rate of 300 mL min^{-1}, during this time the sensor signals were recorded. After sample analysis the system was purged for 180 s with filtered air prior to the next sample injection, to allow reestablishment of the instrument base line. The sensor drift was evaluated by using a standard solution of 5% ethanol in distilled water included in each measurement cycle. For all the experimental period no sensor drift was experienced. At each temperature and storage time (depending on temperature), three samples were analyzed and the average of the results was used for the statistical analysis.

2.3.2. *VIS–NIR spectroscopy*

Spectral acquisitions were performed on leaves using a VIS–NIR spectrophotometer (Jaz, OceanOptics, USA), which is an optical portable system operating in the wavelength range of 400–1000 nm. The Jaz equipment consists of five components: 1) a VIS–NIR lighting system (halogen lamp), 2) a fiber optic probe for reflection measurement, 3) a spectrophotometer, 4) hardware for data acquisition and instrument control, and 5) a battery as the power supply.

Spectra were acquired in reflectance mode: light radiation was guided from the light source to the sample through a Y-shaped, bidirectional fiber optic probe (OceanOptics, USA). The Y-shaped fiber guided light from the halogen lamp to illuminate the sample while simultaneously collecting the radiation coming from the leaf and guiding it back to the spectrophotometer. The probe consists of a tight bundle of 7 optical fibers in a stainless steel ferrule (6 illumination fibers around 1 read fiber, each one with a diameter of 600 μm). Since the leaf is very thin, a dark surface was placed on the opposite side of the acquisition point. In this manner the light which exceeded the leaf was completely absorbed by the dark surface and only the reflected light was read.

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

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

Every sampling day, for each temperature, spectral measurements on 10 leaves were carried out. Each sample was obtained by averaging 3 spectral acquisitions in three different points of the leaf. Each acquisition represent an average of 5 reflectance spectra. A total of 750 spectra were acquired and 250 leaves (90 for 4 °C, 100 for 10 °C and 60 for 20 °C) were analyzed.

2.4. *Data analysis*

Cluster Analysis (CA) was performed on quality indices using Minitab 16 software package.

CA is an exploratory data analysis tool for solving classification problems. Its aim is to sort cases (people, things, events, etc.) into groups, or clusters, so that the degree of association is strong between members of the same cluster and weak between members of different clusters. The hierarchy of clusters can be represented by a binary tree, called "dendrogram". A final partition, i.e. the cluster assignment of each object, is obtained by cutting the tree at a specified similarity level. There are many subjective choices to make in performing a cluster analysis, the linkage method, the distance measure, the level of resolution or the number of clusters has to be established on the basis of need and circumstances [23]. In this work Ward′s method and the Euclidian distance were used and four classes were identified.

The e-nose data were analyzed by Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) using Minitab 16 software package.

PCA is a linear and unsupervised procedure that permits useful information to be extracted from the data, to explore the data structure and the relationship between objects and the global correlation of the variables [24].

LDA is one of the most widely used classification techniques. The method is a probabilistic parametric classification technique that maximizes the variance between categories and minimizes the variance within categories, by means of a data projection from a high dimensional space to a low dimensional space. In this way, a number of orthogonal linear discriminant functions equal to the number of categories minus one is obtained. The classification model was validated using a leave-one-out procedure [25].

Chemometric analysis on VIS–NIR data was performed using The Unscrambler software package (version 9.8, CAMO ASA, Oslo, Norway).

Spectra (Fig. 1) were pre-processed using the Moving Average smoothing (gap size 15 points corresponding to a window of 4.5 nm) and the first derivative Norris Gap transformation (gap size 21 points). These treatments were applied to improve the signal to noise ratio in order to reduce the effects due to the physiological variability of samples [19].

A classification analysis using the PLS discriminant analysis (PLS-DA) method was applied on the 250 average spectra. The objective of PLS-DA is to find models that allow the maximum separation among classes of objects [26]. PLS-DA accomplishes a rotation of the projection to latent variables focusing on class separation. A matrix of artificial (dummy) variables, assuming a discrete numerical value (zero or one), was used as *Y* data. The *Y* dummy matrix was constructed so that the value of the objects belonging to the class was one, and the value of all other objects was zero [27,28]. In this context, PLS-DA was carried out to assess the evolution of the fresh-cut *Valerianella* during storage. Different models were calibrated for each class obtained by CA performed on the quality indices. Samples were split into calibration and validation sets, assigning randomly 50% of samples for calibration and 50% for validation [29]. In this study PLS-DA regression was performed by using the PLS 2 model regression. The cut-off value for PLS-DA discrimination was fixed at 0.5.

Finally, the VIS–NIR spectra were correlated with indices of quality decay using the partial least square (PLS) regression algorithm. The spectra acquired on 10 leaves for each sampling day were averaged to obtain one mean spectrum for each reference parameter value available. Hence, 25 samples were used for the creation of the chemometric regression model for each parameter considered. PLS is frequently used to understand relationships between two data sets by predicting one data set from the other [30].

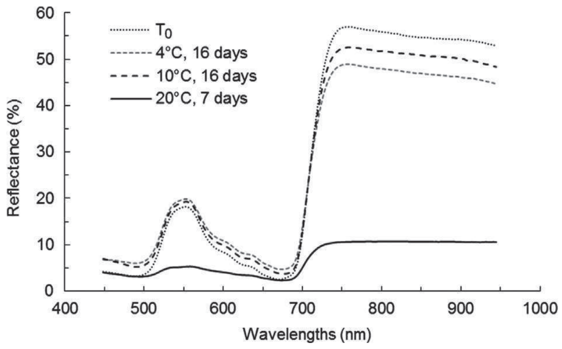

Fig. 1. Average VIS–NIR spectra of *Valerianella* leaves at T_0 and at the end of the shelf life period for each temperature (after 16 days for 4 and 10 °C and after 7 days for 20 °C).

PLS models were constructed using spectral data as predictive variables *X* and the reference parameters (pH, water content, polyphenols and PI) as variables to be predicted *Y*. Cross-validation, an internal validation method usually used with a small number of samples, was performed with five cancellation groups. 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) and the Ratio Performance Deviation (RPD) were applied. RMSECV was calculated as the root of the squared average deviation between predicted and measured *Y*-values in validation [31]. The optimum calibrations were selected based on minimizing the RMSECV. RPD is defined as the ratio between the standard deviation of the response variable and RMSECV [32,33]. The number of latent variables necessary to achieve a minimal RMSECV was selected for the model.

To investigate the feasibility of a low-cost device based on few selected wavelengths, Martens′ Uncertainty Test was applied. This is a significance testing method to assess the stability of regression results and the significance of selected *X*-variables [34,35]. The wavelength selection takes into account the top of the peaks of the *X* regression coefficients plot deriving from the PLS regression [36].

3. Results and discussion

3.1. Quality indices

In Fig. 2 the evolution of chemical parameters investigated during storage of *V. locusta* L. is reported.

Minimally processed vegetables belong to low-acid foods (pH: 5.8–6.0) and in general a pH value in the range of 5–6.5 is considered adequate for quality retention [37]. Fig. 2A shows the pH evolution of *Valerianella* samples stored at 4 °C, 10 °C and 20 °C. At the beginning of shelf life (T_0) the pH value was 6.1; during storage a significant increase was observed after 3, 8 and 9 days at 20 °C, 10 °C and 4 °C, respectively. Moreover, the quality limit (pH=6.5) is exceeded after 3, 9 and 10 days of storage at 20 °C, 10 °C and 4 °C, respectively.

Fig. 2B shows the water content measured throughout the storage at the three established temperatures. As reported in literature for minimally processed lettuce [38], the moisture of *Valerianella* samples changed few during storage; the decrease after 7 days at 20 °C, 10 °C and 4 °C was of 0.85%, 0.79% and 0.74%, respectively.

Results from the determination of total phenols are shown in Fig. 2C. At the beginning of shelf life (T_0) total phenolic concentration was of 300 mg 100 g^{-1}. In the samples stored at 20 °C an increment of 28% was observed during the first 4 days of storage. A similar increment was observed after 7 days at 10 °C (32%) and after 8 days at 4 °C (30%), then the decrease of the phenolic content is probably due to their oxidation. Similar results were obtained by Ke and Saltveit [39] and by Kang and Saltveit [40] that observed a marked increment of the phenolic content and antioxidant capacity of iceberg lettuce exposed to several kind of stress (attack of pathogens, ethylene treatment) and after wounding. Babic et al. [41] observed an increase of phenols in ready-to-use carrots as a response to damage.

The evolution of PI is reported in Fig. 2D. The initial (T_0) PI value was about 4.14, a significant decrease to 1.46 (65%) and to 0.8 (81%) was observed after 15 days for samples stored at 4 °C and 10 °C, respectively. At 20 °C the PI value drastically declined, reaching a value close to zero after 7 days. As expected, PI value for samples

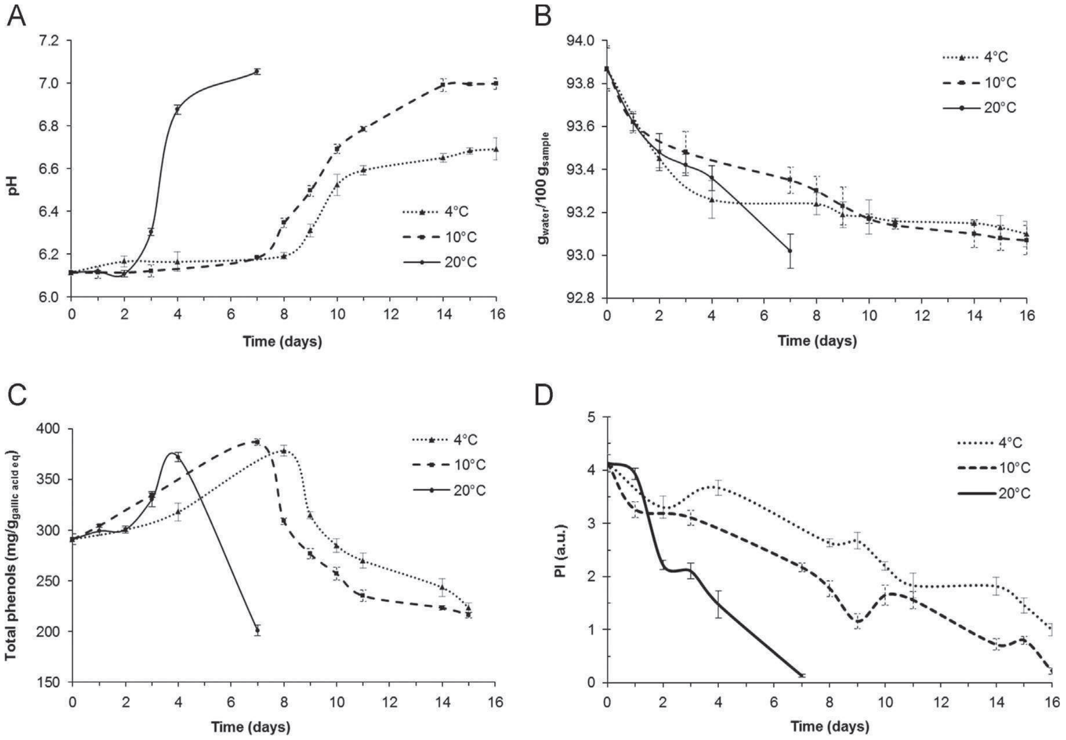

Fig. 2. Evolutions of chemical parameters (A = pH; B = water content; C = total phenols; D = Performance Index, PI), for each sampling date at 4 °C, 10 °C and 20 °C. Bars indicate the standard error within each sampling date ($n = 3$).

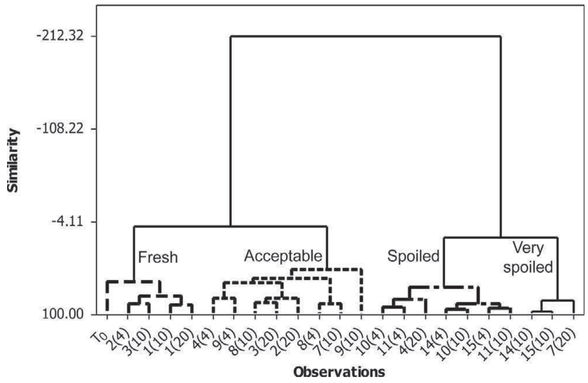

Fig. 3. Dendrogram deriving from elaboration by CA in order to categorize *Valerianella* samples according to their freshness and quality. Different line style was used to identify the clusters.

stored at 20 °C decreased rapidly due to the extreme conditions of storage, indicating a decay in the efficiency of PSII photochemistry. These results were in agreement with those reported by Baldassarre et al. [20].

All data collected by the quality indices were elaborated by CA in order to categorize *Valerianella* samples according to their freshness and quality (Fig. 3). At a similarity level of 14.02 four main groups were identified. The first cluster, classified as “fresh”, included samples similar to T_0 and stored for 1 day at 20 °C and for 2–3 days at 4 °C and 10 °C. The second cluster was classified as “acceptable” and consisted of samples stored for a maximum of 3 days at 20 °C and for a maximum of 9 days at 4 °C and 10 °C. The third and fourth clusters were classified as “spoiled” and “very spoiled”, respectively, and consisted of samples that were no longer acceptable considering the quality indices.

3.2. Non-destructive systems

The e-nose was applied in order to evaluate the evolution of the aroma profile of *Valerianella* during storage. As a first step, in order to evaluate the ability of the e-nose to differentiate samples during shelf life, data were elaborated by PCA performed on covariance matrix. Fig. 4 shows the PCA score plot (A) and loading plot (B) in the plane defined by the first two Principal Components (PC1 and PC2) accounted for 99.8% of the total variance. Examining the PCA score-plot (Fig. 4A) a clear distribution of samples along PC1 and PC2 according to the storage temperature and time was found. In particular, samples stored at 10 °C and 4 °C for up to 3 and 9 days respectively, are located along PC1 at the left of the plot and their aroma profile is similar to that of the fresh product analyzed the day of packaging (T_0). The samples stored for 10–15 days were differentiated along the PC2 and their aroma profile was similar to that of samples stored for 3–4 days at 20 °C and for 10–11 days at 10 °C. A clear evolution of the aromatic profile of samples stored at 20 °C is evident along PC1: after 3–4 days of storage the aromatic fingerprint evolved rapidly, and sample stored for 7 days, located at the right of the plot, was similar to samples stored for 14–15 days at 10 °C. Considering the PCA-loading plot (Fig. 4B), showing the relationship between the e-nose sensors and how they influence the system, the W5S, W2S and W1S sensors, characterized by broad range sensitivity and sensitive to polar compounds, alcohols and ketones, had the highest influence in the pattern file. In particular one sensor (W5S) is relevant in the discrimination of *Valerianella* samples along PC1 on the basis of their storage condition. This result is in accordance with those reported in other studies concerning the applicability of e-nose for evaluating apple, peach, mandarin and tomato maturity. In all these works it was demonstrated that W5S sensor was particularly relevant in monitoring changes in the volatile profile of fruit and vegetables during shelf-life [11,42–44].

LDA was performed on e-nose data, in order to classify *Valerianella* samples into the four clusters identified by CA. LDA was applied considering all the variables and, subsequently, only the three selected variables (W1S; W2S; W5S); the classification matrix is reported in Table 2. LDA applied to the all the e-nose variables gave a calibration error rate of 4.4% and a cross validation error rate of 17.4%. Better classification results in validation were obtained considering only the selected e-nose variables. The average value of samples correctly classified was 95.5% and the cross validation error rate was 8.7%. Although in literature there are few works on e-nose applied to minimally processed vegetables, these results are in agreement with those published which demonstrated that e-nose responses correlate well with classical evaluation of vegetable spoilage and that e-nose is useful tool for monitoring the shelf life of these products [9,10].

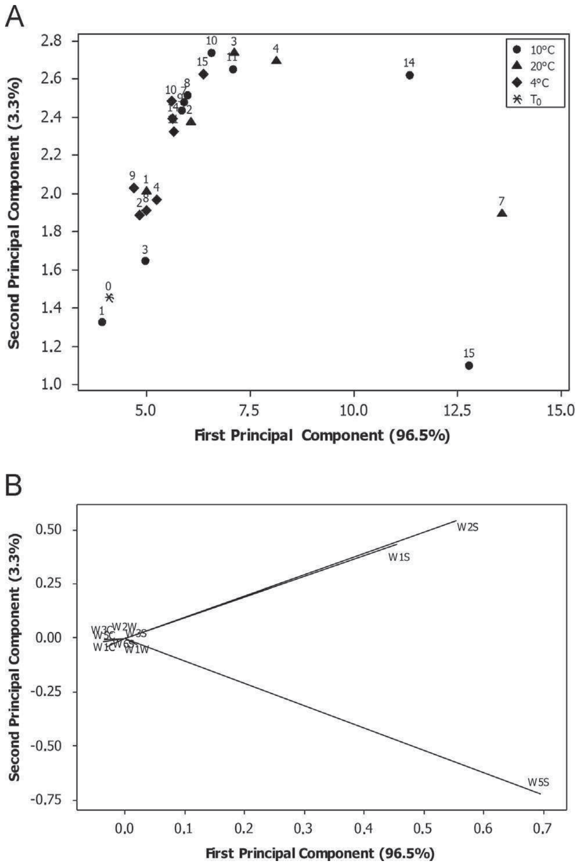

Fig. 4. PCA scores plot (A) and loadings plot (B) deriving from e-nose data.

VIS–NIR spectral data were used for the elaboration of PLS-DA classification models and PLS predictive models.

Results obtained by PLS-DA for classification of *Valerianella* samples into the four clusters identified by CA are shown in Table 3. The PLS-DA models were applied on calibration and on validation sets. The results obtained from validation sets gave a positive predictive value (PPV) of classification between 74% and 96%. In particular, very high PPV were obtained for the class “fresh” and the class “very spoiled” with 94% and 96% of correctly classified samples, respectively.

Table 4 shows descriptive statistics and the estimated PLS regression coefficients for predicting quality indices of *V. locusta* L. The more informative wavebands were selected by Martens′ Uncertainty Test and used for models calibration (Fig. 5). In recent years, there has been a growing interest towards the development of portable systems that could be used in pre- and post-harvest [45–47]. The identification of the most significant bands can be used as starting point for the selection of a few highly informative

Table 3
PLS-DA classification of *Valerianella locusta* L. samples based on the four clusters identified by CA.

Class	Calibration set (n=125)		Validation set (n=125)	
	PPV_{cal}	% PPV_{cal}	PPV_{val}	% PPV_{val}
Fresh	121/125	97	117/125	94
Acceptable	108/125	86	92/125	74
Spoiled	115/125	92	107/125	86
Very spoiled	118/125	94	120/125	96

$PPV_{cal/val}$=Positive predictive value of calibration or validation.

Table 2
LDA classification of *Valerianella locusta* L. samples considering all the e-nose variables and the selected variables (W1S, W2S, W5S).

		Class	Predicted class (%)			
			Fresh	Acceptable	Spoiled	Very spoiled
All e-nose variables	**Calibration**					
		Fresh	**100**	0	0	0
		Acceptable	0	**100**	0	0
		Spoiled	0	14.3	**85.7**	0
		Very spoiled	0	0	0	**100**
	Cross-validation					
		Fresh	**80**	20	0	0
		Acceptable	0	**90**	10	0
		Spoiled	0	40	**60**	0
		Very spoiled	0	0	0	**100**
Selected e-nose variables	**Calibration**					
		Fresh	**100**	0	0	0
		Acceptable	9.1	**90.9**	0	0
		Spoiled	0	0	**100**	0
		Very spoiled	0	0	0	**100**
	Cross-validation					
		Fresh	**100**	0	0	0
		Acceptable	18.2	**81.8**	0	0
		Spoiled	0	0	**100**	0
		Very spoiled	0	0	0	**100**

Table 4
Descriptive statistics and statistics of the PLS models elaborated on VIS–NIR spectra to estimate qualitative decay parameters of *Valerianella locusta* L. and respective wavebands selected.

Quality parameters	No. of samples	Range	Mean	SD	Pretreatment	LV	Calibration model			Validation model			VIS–NIR regions (nm)
							R^2_c	RMSEC	RPD	R^2_{cv}	RMSECV	RPD	
pH	25	6.11–7.06	6.45	0.33	Smoothing Der2	5	0.93	0.09	3.67	0.86	0.13	2.54	510–522; 545–556; 582–598; 660–689; 698–713; 720–737; 752–793; 852–862; 913–917
Total phenols (mg/g $_{\text{gallic acid eq}}$)	25	201.1–386.6	267.0	40.3		5	0.96	7.38	5.46	0.89	12.64	3.19	515–526; 584; 586; 628–638; 647–688; 695–704; 755–768; 773; 774; 790–793
Water content (%)	25	93.02–93.87	93.38	0.27		3	0.85	0.1	2.70	0.84	0.12	2.25	496–519; 533–548; 564–581; 591–623; 638–665; 684–696; 707–753
PI (a.u.)	25	0.13–4.14	2.26	1.16		5	0.94	0.29	4.00	0.92	0.36	3.22	503–528; 543–550; 570; 580–593; 620–641; 656; 680–691; 698–715; 720–741; 757–761; 769–772; 803–805; 818–827; 874; 884–889; 896–912; 919–921; 923

LV = Latent variables.

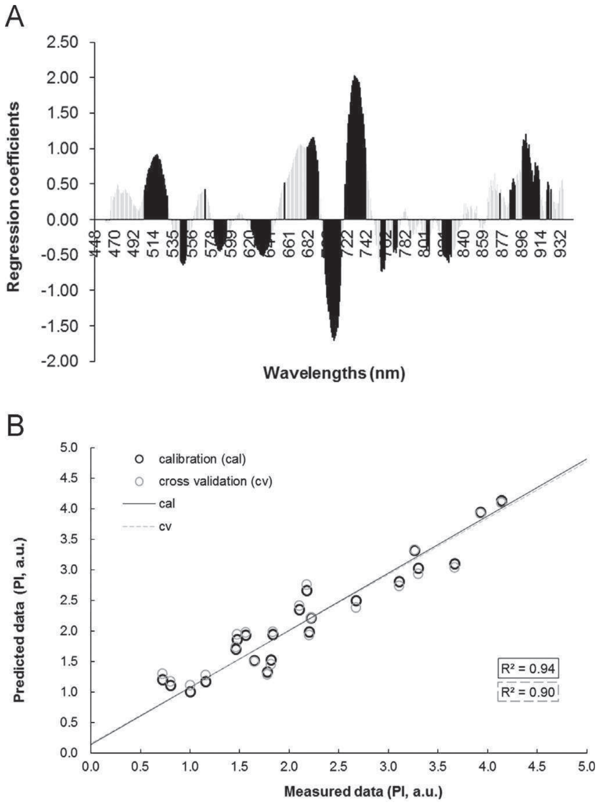

Fig. 5. Loadings plot with highlighted the selected variables by Martens' Uncertainty Test (A) and graph of PLS model for PI prediction (B).

wavelengths. These individual fingerprint wavelengths could be used for the design of a simplified handheld device which would allow real-time assessment of *Valerianella* freshness. Zhang et al. [48] proposed a method to select 25 wavelengths for the estimation of water content in ornamental plant leaves using VIS–NIR spectroscopy. PLS model deriving from the full spectrum (200–1100 nm) showed RPD equal to 3.66 while after the selection PLS model gave an higher RPD value of 4.86. All the quality indices estimated showed good calibration and validation statistics: determination coefficients in validation (R^2_{cv}) ranging between 0.84 and 0.92 and RPD values higher than 2. In particular, RPD values minor than 3 were obtained for water content (2.25) and pH (2.54); a RPD value between 2 and 2.5 indicates that coarse quantitative predictions are possible while a RPD value between 2.5 and 3 or above corresponds to good or excellent prediction accuracy [13,32,33]. The prediction of PI (Fig. 5B) can be considered excellent (R^2_{cv}=0.92, RPD=3.22) and excellent results were also obtained for total phenols (R^2_{cv}=0.89 and RPD=3.19).

4. Conclusions

A portable electronic nose and a portable VIS–NIR spectrophotometer, operating in the range 400–1000 nm, were tested for monitoring freshness decay of fresh-cut *V. locusta* L. during storage at three different temperature (4 °C, 10 °C and 20 °C). CA was performed on quality indices in order to categorize *Valerianella* samples according to their freshness and four main groups were identified. Classification and regression models were performed on e-nose and VIS–NIR data. The e-nose was able to follow the evolution of the aroma profile of *Valerianella* during storage. The PCA-loading plot showed that three sensors, characterized by broad range sensitivity and sensitive to polar compounds, alcohols and ketones, had the highest influence in the pattern file. LDA performed on e-nose data gave 95% of samples correctly classified. PLS-DA classification models and PLS predictive models elaborated on VIS–NIR spectral data gave good results and few selected wavebands were identified to investigate the feasibility of a low-cost device.

Results of the present work demonstrated that these techniques can be proposed as rapid (compared with traditional laboratory analyses) and non-destructive methods to evaluate changes in fresh-cut *Valerianella* during storage and the information provided will be useful for managing the product during production and along the distribution chain. Results are preliminary and a future perspective is the implementation of these devices, equipped with more robust predictive models, directly at the point of sale as a guarantee, for the consumers, of the minimally processed product quality. The instruments proved to be suitable not only for the evaluation of quality parameters, but also for classification according to the storage time. Therefore they could be used as a non-destructive method for classification in homogeneous lots with the purpose of a better management of the destination of lots during the shelf-life in order to avoid fruit wastage. Moreover e-nose and VIS–NIR can be mutually complementary and used in combination. A simplified systems based on few e-nose and VIS–NIR variables can be foreseen providing rapid information about the appearance, the chemical composition and the aroma profile of *Valerianella*.

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References

[1] IFPA (International Fresh-cut Produce Association), PMA (The Produce Marketing Association), Handling guidelines for the fresh-cut produce industry, third ed., Alexandria, 1999.
[2] A.A. Kader, J. Sci. Food Agric. 88 (2008) 1863–1868.
[3] D. Rico, A.B. Martin-Diana, J.M. Barat, C. Barry-Rian, Trends Food Sci. Technol. 18 (2007) 373–386.
[4] J.K. Brecht, M.E. Saltveit, S.T. Talcott, K.R. Schneider, K. Felkey, J.A. Bartz, Hortic. Rev. 30 (2004) 185–246.
[5] R.S. Rolle, G.W. Chism, J. Food Qual. 10 (1987) 157–177.
[6] F. Artés, A. Allende, Eur. J. Hortic. Sci. 70 (2005) 231–245.
[7] R.C. Soliva-Fortuny, O. Martin-Belloso, Trends Food Sci. Technol. 14 (2003) 341–353.
[8] E. Aguayo, A.C. Silveira, Proceedings of the Tropical Fruits in Human Nutrition And Health Conference, 2008, pp. 134–145.
[9] L. Torri, N. Sinelli, S. Limbo, Postharvest Biol. Technol. 56 (2010) 239–245.
[10] M. Riva, S. Benedetti, S. Mannino, Ital. J. Food Sci. 13 (2001) 201–213.
[11] S. Benedetti, S. Buratti, A. Spinardi, S. Mannino, I. Magnani, Postharvest Biol. Technol. 47 (2008) 181–188.
[12] A.H. Gomez, J. Wang, G. Hu, A. Garcıa Pereira, J. Food Eng. 85 (2008) 625–631.
[13] B.M. Nicolai, K. Beullens, E. Bobelyn, A. Peirs, W. Saeys, K.I. Theron, J. Lammertyna, Postharvest Biol. Technol. 46 (2007) 99–118.
[14] I.M. Francois, H. Wins, S. Buysens, C. Godts, E. Van Pee, B. Nicolai, M. De Proft, Postharvest Biol. Technol. 49 (2008) 366–373.
[15] M.T. Sánchez, D. Pérez-Marín, K. Flores-Rojasa, J.E. Guerrero, A. Garrido-Varo, Talanta 78 (2009) 530–536.
[16] M.E. Guerzoni, A. Gianotti, M.R. Corbo, M. Sinigaglia, Postharvest Biol. Technol. 9 (1996) 195–207.
[17] L. Jacxsens, F. Devlieghere, J. Debevere, Postharvest Biol. Technol. 26 (2001) 59–73.
[18] J.F. Brecht, M.E. Saltveit, S.T. Talcott, K.R. Schneider, K. Felkey, J.A. Bartz (Eds.), Horticultural Reviews, vol. 30, John Wiley & Sons, Inc., Hoboken, New Jersey, USA, 2004, p. 185.
[19] A. Ferrante, T. Maggiore, Postharvest Biol. Technol. 45 (2007) 73–80.
[20] V. Baldassarre, G. Cabassi, A. Ferrante, Aust. J. Crop Sci. 5 (2011) 735–741.
[21] V.L. Singleton, J.A. Rossi, Am. J. Enol. Vitic. 16 (1965) 144–158.
[22] B.J. Strasser, R.J. Strasser, in: P. Mathis (Ed.), Photosynthesis: From Light to Biosphere, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1995, pp. 977–980.
[23] J.W. Gardner, P.N. Bartlett (Eds.), Sensors and Sensory Systems for an Electronic Nose, Kluwer Academic Publishers Group, AH Dordrecht, The Netherlands, 1992.
[24] K.R. Beebe, R.J. Pell, M.B. Seasholtz, Chemometrics: A Practical Guide, John Wiley and Sons, New York, USA, 1998.
[25] M. Meloun, J. Militky, M. Forina, Chemometrics for Analytical Chemistry, Ellis Horwood, New York, USA, 1992.
[26] S. Wold, M. Sjöström, L. Eriksson, Chemom. Intell. Lab. Syst. 58 (2001) 109–130.
[27] G. Musumarra, V. Barresi, D.F. Condorelli, C.G. Fortuna, S. Scirè, Comput. Biol. Chem. 29 (3) (2005) 183–195.
[28] F. Liu, Y. He, L. Wang, Anal. Chim. Acta 610 (2008) 196–204.
[29] T. Naes, T. Isaksson, T. Fearn, A.M.C. Davies, A User-Friendly Guide to Multivariate Calibration and Classification, NIR Publication, Chichester, UK, 2000.
[30] S.J. Chung, H. Heymann, I.U. Grun, Food Qual. Prefer. 14 (2003) 485–495.
[31] N. De Belie, D.K. Pedersen, M. Martens, R. Bro, L. Munck, J. De Baerdemaeker., Biosyst. Eng. 85 (2) (2003) 213–225.
[32] P. Williams, in: K.H. Norris (Ed.), Near-Infrared Technology in the Agricultural and Food Industries, American Association of Cereal Chemist, St. Paul, Minnesota, USA, 2001, pp. 145–169.
[33] T. Fearn, NIR News 13 (2002) 12–14.
[34] A. Chudnovsky, E. Ben-Dor, Sci. Total Environ. 393 (2008) 198–213.
[35] K.H. Esbensen, Multivariate Data Analyses, An Introduction to Multivariate Data Analyses and Experimental Design, fifth ed., Aalborg University, Esbjerg, Denmark, 2002.
[36] H.R. Bjørsvik, H. Martens, in: D.A Burns, E.W. Ciurczak (Eds.), Handbook of Near-Infrared Analysis, Taylor and Francis, Boca Raton, FL, USA, 2001, pp. 185–207.
[37] L.R. Beuchat, Dairy Food Environ. Sanit. 12 (1992) 6–9.
[38] D. Rico, A.B. Martin-Diana, C. Barry-Rian, J.M. Frias, G.T.M. Henehan, J.M. Barat, Innov. Food Sci. Emerg. Technol. 9 (2008) 37–48.
[39] D. Ke, M.E. Saltveit, Plant. Physiol. 88 (1988) 1136–1140.
[40] H.M. Kang, M.E. Saltveit, J. Agric. Food Chem. 50 (2002) 7536–7541.
[41] I. Babic, M.J. Amiot, C. Nguyen-The, S. Aubert, J. Food Sci. 58 (2) (1993) 351–356.
[42] S. Benedetti, A. Spinardi, I. Magnani, S. Buratti., Ital. J. Food Sci. 22 (2010) 299–306.
[43] A.H. Gomez, G. Hu, J. Wang, A.G. Pereira, Comput. Electron. Agric. 54 (2006) 44.
[44] A.H. Gomez, J. Wang, G. Hu, A. Garcıa Pereira, Lebensm. Wiss. Technol. 40 (2007) 681.
[45] M. Zude, B. Herold, J.-M. Roger, V. Bellon-Maurel, S. Landahl, J. Food Eng. 77 (2006) 254–260.
[46] T. Temma, K. Hanamatsu, F. Shinoki, J. Near Infrared Spectrosc. 10 (2002) 77–83.
[47] K.B. Walsh, J.A. Guthrie, J.W. Burney, Aust. J. Plant Physiol. 27 (2000) 1175–1186.
[48] Q. Zhang, Q. Li, G. Zhang, Spectrosc. Int. J. 27 (2) (2012) 93–105, http://dx.doi.org/10.1155/2012/276795.

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Setting-up of a simplified handheld optical device for decay detection in fresh-cut Valerianella locusta L.

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Setting-up of a simplified handheld optical device for decay detection in fresh-cut *Valerianella locusta* L.

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ABSTRACT

The aim of this work was to study the feasibility of a simplified handheld and low-cost optical device. This study was focused on identifying the most significant vis/NIR wavelengths able to discriminate freshness levels during shelf-life of fresh-cut *Valerianella locusta* L.

The shelf-life of *Valerianella* leaves was monitored using a portable commercial vis/NIR spectrophotometer and by traditional analyses (pH, moisture and total phenols content). The *Valerianella* samples were stored at three temperature: 4 °C, 10 °C, and 20 °C. Through PLS-RCA technique, standardized regression coefficients of PLS models were used to select the relevant variables, representing the most useful information of full spectral region. The four selected wavelengths were 520 nm, 680 nm, 710 nm and 720 nm. Multiple linear regression was applied in order to verify the effectiveness of selected wavelengths. Results demonstrate the feasibility of a simplified device for quickly monitoring the shelf-life of fresh-cut *Valerianella* leaves.

1. Introduction

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". In recent years, a substantial increase in the consumption of fresh-cut, or minimally processed, fruit and vegetables has been occurred. Fresh-cut production raised in Italy and Europe in recent years (Rico et al., 2007). In Italy, the area of fresh-cut cultivation is approximately 6500 ha and the total harvest is 88,000 t y^{-1} (Castoldi et al., 2011). 10% of the economic value of the fruit and vegetable market in Italy is covered by fresh-cut product (Baldi and Casati, 2008).

The technological treatments extend the shelf-life of the most processed foods. Instead, ready-to-eat products are characterized by a shelf-life shorter than that of the original unprocessed raw material (Guerzoni et al., 1996). In fact the sequence of operations necessary to produce a fresh-cut product (i.e. washing, trimming, peeling and/or cutting) promotes the biochemical and microbial instability of the product itself. These foods are often subjected to rapid loss of colour, organic acids, vitamins and other compounds that determine flavour and nutritional value.

Monitoring the quality decay of fresh-cut products is necessary to control the freshness level during the entire production chain and to ensure quality product for the consumer. Hence fresh-cut fruit and vegetable sector could be greatly helped by new analytical methods that are accurate, rapid and could be integrated into the production chain for better managing the shelf-life of minimally processed products and to meet consumer demand.

The non-destructive techniques, and in particular the optical analysis in the region of near-infrared (NIR) and visible–near infrared (vis/NIR), have been developed considerably over the last 20 years (Guidetti et al., 2012; Nicolai et al., 2007). NIR and vis/NIR spectroscopy are based on the study of the interaction of electromagnetic radiation with the structure of the food product. Molecular bonds like OH, CH, CO and NH are subject to vibrational energy changes when irradiated by the radiation. The energy absorption of organic molecules in vis/NIR region occurs when molecules vibrate and this is translated into an absorption spectrum (Cen and He, 2007). These approaches, however, are always related to the analysis of wide spectra (thousands of wavelengths or variables) and, therefore, require multivariate techniques for data processing to build predictive models (Williams and Norris, 2002). In order to explain the chemical information encoded in the spectral data, chemometric analysis is required (Cogdill and Anderson, 2005). For a simplification and greater diffusion of these non-destructive techniques, in recent years, interest has shifted towards the development of portable systems that could be used in

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pre- and post-harvest (Zude et al., 2006; Temma et al., 2002; Walsh et al., 2000).

Chemometrics can be used for the selection of a small number of relevant variables, which represent the most useful information contained in the full spectra (Xiaobo et al., 2010; Sun, 2010). In this way the spectral noise and the variables containing redundant information can be eliminated. Moreover, a reduced cost for potential miniaturized devices, realized to work at only this selected wavelengths, can be foreseen.

Few examples of commercial non-destructive devices based on a small number of wavelengths are already available on the market. These applications are mainly dedicated to fruits. For example, the University of Bologna (Costa et al., 2011) patented innovative and simplified NIRs equipments, namely DA-Meter for apple and Kiwi-Meter for kiwi. These systems are used for the analysis of the ripeness level of the fruit through indices based on differences in absorbance between specific wavelengths. This type of instrument, simple and portable, can be used directly on the fruit on the trees and can help growers in taking decision regarding the best cultural management practices (such as pruning, thinning, and nutrition). In this way the heterogeneity of the product can be reduced and, therefore, can be simplified the management of product lots during post-harvest.

Valerianella locusta, also known as lamb's lettuce, is a member of the family Valerianaceae. It is a diploid, autogamous crop, with the chromosome number $2n = 14$ but otherwise little genetic information (Muminovic et al., 2004). *Valerianella* is actually among the mostly requested baby-leaves commercialised in the Italian market.

The aim of this work was to study the feasibility of a simplified handheld and low-cost optical device, based on a few wavelengths appropriately selected, for quality analysis of *Valerianella* during the production chain or/and to discriminate freshness levels during shelf-life directly at the point of sale.

The main objective of this research was the identification of informative wavelengths using the Partial Least Square Regression Coefficients Analysis (PLS-RCA) method of variable selection, correlating the vis/NIR spectra and the *Valerianella* quality parameters. The prediction performances of partial least square (PLS) models based on the analysis of full vis/NIR spectra were compared with multiple linear regression (MLR) models, created using only the selected wavelengths. Simple equations for the estimation of *Valerianella* freshness were defined. Finally, a possible functional scheme of a compact-sized LED technology based, low-cost, and easy-to-use device was proposed.

2. Materials and methods

The packages of fresh-cut *V. locusta* L. used for the study were provided by a collaborative producer. The *Valerianella* leaves were harvested by hand in September 2012. After a minimal process, the leaves were packed in bags made by high-density polyethylene and sealed. The commercial expiration date was fixed by the producer at 4 days from the packaging date. During their transport to the laboratory, the samples were maintained at the temperature of 4 °C.

2.1. Sampling

Three storage/shelf-life temperature were investigated: 4.0 ± 0.5 °C, 10.0 ± 0.5 °C and 20.0 ± 0.5 °C.

The lower temperature, 4 °C, represents the optimal storage condition for fresh-cut products (Kader, 2008). The storage at 10 °C simulates the realistic supermarket condition (Jacxsens et al., 2001) while 20 °C is the extreme storage situation. At the last temperature the physiological activities of fresh-cut products are accelerated (Toivonen and DeEll, 2002).

The duration of the experimentation was different for the different storage temperature. The *Valerianella* packages preserved at 4 °C and 10 °C were sampled for 16 days, while samples stored at 20 °C were analyzed only for 7 days, due to the rapid degradation of the lettuce at this temperature. The numbers of sampling points during storage monitoring for the fresh-cut leaves were therefore 10, 11 and 6 for 4 °C, 10 °C and 20 °C, respectively (Table 1).

The quality decay of samples was evaluated by chemical parameters (pH, moisture and total polyphenols content) and by a non-destructive optical device (vis/NIR spectroscopy).

2.2. Chemical analyses

At each temperature and sampling time all the chemical analysis were carried out in triplicate. The pH was measured using a digital pH meter (Ioncheck 45, Radiometer Analytical SAS, Lyon, France) on 20 g of sample blended for 2 min in 40 mL of deionized water. To obtain the moisture content (MC) a thermogravimetric analysis (Sartorius MA150, Bradford, UK) was performed. An amount of 5 g of sample was weighed in the analyzer and heated at 120 °C. The difference between the weights of the sample, before and after being heated, represent the MC. The moisture content was calculated as grams of water per 100 g of sample. The sample leaves were crushed and 10 g were weighed in a centrifuge tube and added with 15 mL of methanol. The mixture was stirred for 1 h in the dark and centrifuged at 11,200 rpm for 10 min at 15 °C. The solids were extracted two more times using 15 and 10 mL of the methanol for 15 min under shaking in the dark, and centrifuged in the above-described conditions. The gathered extracts were made up to 50 mL with the extraction solvent. The phenolic compounds quantification was based on the Folin–Ciocalteau method (Singleton and Rossi, 1969) and expressed as mg of gallic acid equivalents per 100 g of sample, by comparison with a calibration curve built with 0.5 mg kg^{-1}, 1 mg kg^{-1}, 2.5 mg kg^{-1}, 5 mg kg^{-1} and 10 mg kg^{-1} standard solution of gallic acid (Sigma–Aldrich, Italia).

2.3. Portable vis/NIR device

The optical analyses were carried out with a commercial vis/NIR spectrophotometer (JAZ, OceanOptics, USA, Fig. 1) operating between 400 and 1000 nm.

The instrument consists of five components (Fig. 2): (1) a vis/NIR halogen lamp, (2) a fiber optic probe for reflection measurement, (3) a spectrophotometer, (4) hardware for data acquisition and instrument control, (5) a battery as the power supply.

Table 1
Sampling points during shelf life monitoring for 4 °C, 10 °C and 20 °C.

	Days	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Shelf life	4 °C	√		√		√				√	√	√	√			√	√	√
	10 °C	√	√		√				√	√	√	√	√			√	√	√
	20 °C	√	√	√	√	√			√									

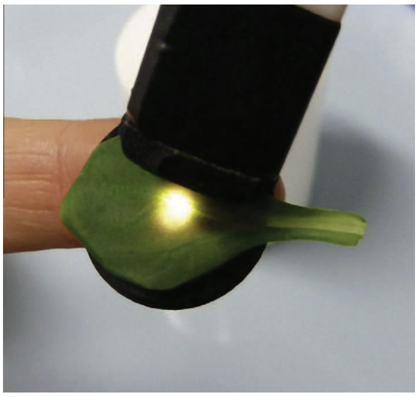

Fig. 1. Particular of spectral acquisitions on *Valerianella* leaf with JAZ vis/NIR system.

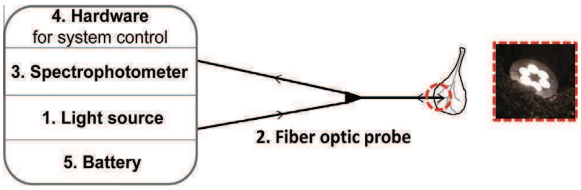

Fig. 2. Scheme of the commercial portable vis/NIR spectrophotometer used for spectral acquisitions and particular of the tip of the fiber optic probe.

The optic probe is a bidirectional *Y*-shaped cable (OceanOptics, USA) in which seven fibers are arranged in a 6-around-1 configuration. The six external fibers guide the light from the light source to the sample, while the single central fiber brings it back from the leaf to the spectrophotometer (Fig. 2).

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

Spectra were acquired in reflectance mode, without any sample preparation. Due to the sample thinness, a dark surface was placed on the opposite side of the leaf, in correspondence to the acquisition point. The dark surface was realized through a generic black rubber material, previously and properly tested in order to guarantee the total light absorption along the whole spectral range. In this way the light exceeding the leaf is completely absorbed by the dark surface, without any interference with the measured light reflected from the leaf. Moreover, the tip of the optical probe was equipped with soft plastic cap to ensure contact with the sample's surface during measurements in order to reduce the interference of environmental light (Fig. 1).

A total number of 270 *Valerianella* leaves were analyzed: 100, 110 and 60 samples for 4 °C, 10 °C and 20 °C, respectively. Measurement of 10 leaves were performed for each sampling time. Each sample was obtained by averaging 3 spectral acquisitions made in three different points of the leaf, for a total of 810 spectra. An averaged spectrum on the 10 leaves, representative of each plastic bag, was finally calculated and used for the data analysis.

2.4. Data processing

The data processing was carried out using The Unscrambler® 9.6 software package (CAMO ASA, Norway). The chemometric approach allows to extract the usable information from the spectral data and to select the most significant wavelengths, in order to develop a simplified device.

The collected spectra were pre-processed using smoothing (moving-average, 15 nm wide window) and reducing techniques. The correlations between spectral data matrix and chemical parameters (pH, MC and TP) were carried out using PLS regression algorithm and the variable selection was performed using regression coefficient analysis (RCA), deriving from PLS analysis (Chong and Jun, 2005; Liu et al., 2008).

The regression coefficients obtained by PLS model were used to calculate the *Y* variable response value (pH, moisture and TP) from the X variables (*Valerianella* leaves spectra). The follow parameters were calculated to evaluate model accuracy (Nicolai et al., 2007; Naes et al., 2002):

- R^2_{cal}, coefficient of determination in calibration.
- R^2_{cv}, coefficient of determination in cross-validation.
- RMSEC, root mean square error of calibration.
- RMSECV, root mean square error of cross-validation.
- RPD, Ratio Performance Deviation.

RPD is defined as the ratio between the standard deviation of the response variable and RMSECV. If RPD is lower than 1.5, the calibration is not useful, on the contrary, if it is higher than 2, the model can perform a quantitative prediction; between 1.5 and 2.0 the algorithm have the possibility to distinguish between high and low values (Williams, 2001; Fearn, 2002).

In order to extract the most useful information from the vis/NIR spectra, RCA was carried out (Xiaobo et al., 2010; Chong and Jun, 2005). This approach was already applied in literature by Cen et al. (2006), on orange juice samples for the estimation of TSS an pH as well as by Liu et al. (2008) on rice vinegars samples. Liu et al. (2009) used RCA to determine the TSS content in beer.

The regression coefficients deriving from the PLS analysis were standardized considering the standard deviation of reflectance and the standard deviation of the reference data (Frank and Todeschini, 1994). The standardized regression coefficients were used for the wavelength selection.

The size of these numerical coefficients gave an indication of the impact of different variables on the response (*Y*). Large absolute values indicate the importance of the corresponding wavelengths on the prediction of *Y* parameter. The final aim was to find which variables were important for predicting the *Y* response. Hence, RCA could be used for essential wavelength selection.

As already used by Liu et al. (2008, 2009), the higher peaks (absolute values) of the regression coefficient plot were chosen. Peaks and valleys, in fact, represent the relevance of the corresponding wavelength in predicting the *Y*-variable.

Finally, another regression method, MLR, was applied to test the effectiveness of the selected wavelengths. The effective wavelengths were employed as the input data matrix for the elaboration of MLR models (Wu et al., 2010; Fernández-Novales et al., 2009; Li et al., 2007). This approach, compared with the traditional PLS method, performs quantitative predictions using only a few important variables. MLR works even when the number of variables is less than the number of samples and is not affected by collinearity (Naes and Mevik, 2001). The MLR models were compared to PLS models, allowing to evaluate the efficiency of variable selection.

Therefore, reflection intensity measured at the selected wavelengths were used to predict quality parameters, in order to determine the different freshness stages during decay.

3. Results and discussion

Francois et al. (2008) investigated sensory attributes of different chicory hybrids using vis/NIR spectroscopy; Ferrante and Maggiore, in 2007, evaluated storage time and temperature of *Valeriana* leafy vegetables using fluorescence technique and Zhang et al. (2012) determined the water content in leaves of potted plants using vis/NIR technique; no specific works are reported in literature regarding vis/NIR spectroscopy on fresh-cut *Valerianella* leaves. Similar portable vis/NIR device was tested by Guidetti et al. for the prediction of ripening indices (total polyphenols) of fresh blueberries (Guidetti et al., 2008) and of fresh grapes (Guidetti et al., 2010), by Beghi et al. (2012) on two apple varieties just before fruit harvest and by Camps and Christen (2009) on fresh apricot under laboratory conditions with similar results to our findings. These results show the possible application of vis/NIR technology for the estimation of many ripening parameters, as widely shown in the literature (Nicolai et al., 2007).

The plots of the standardized regression coefficients (for pH, MC and TP) vs. vis/NIR wavelengths (400–1000 nm) are shown in Fig. 3.

The trend and shape of the regression coefficients plots were different for pH, moisture and TP at specific wavelengths.

The final goal was to select wavelengths showing simultaneously high absolute values for all the three parameters considered. This in order to be all used at the same time for the prediction of each parameter in a hypothesis of a simplified system.

The most relevant wavelengths were selected by choosing the higher absolute regression values (Liu et al., 2008; Cen et al., 2006). Therefore, the candidate effective wavelengths were: 520 nm, reflection wavelength for the green colour; 680 nm, corresponding to chlorophyll maximum absorption peak; 710 nm and 720 nm, corresponding to bands related to the evolution of chlorophyll spectral forms throughout all stages of leaf development, often not completely resolved (Gitelson et al., 1996). Moreover, the authors stated that peaks at 685–706, 710, 725 and 740 nm were dependent on different degree of leaf age and pigment concentration in the leaves.

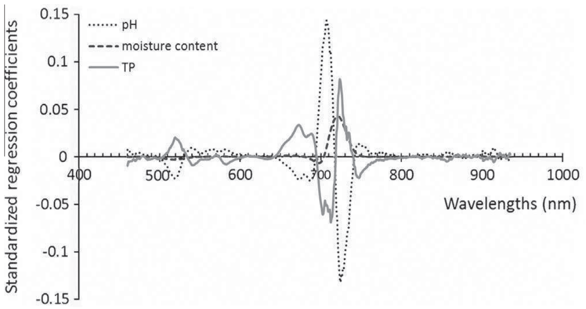

Fig. 3. Standardized regression coefficients for pH, moisture content (MC) and total polyphenols (TP).

For MC, the selected wavelengths of 520 and 680 nm show very low values of standardized regression coefficient and their contribute tends to be cancelled out. The real informative wavelengths for this parameter are only 710 and 720 nm (Fig. 3).

The performance of RCA was confirmed using the visual inspection of average spectral curves (Sun, 2010) and their correspondence to specific absorption peaks. The average spectra of the different shelf-life classes at storage temperature of 10 °C and 20 °C in the considered vis/NIR range are shown in Fig. 4. As expected, the spectra exhibit differences, according to sampling date, more evident for 20 °C storage (Fig. 4B). Average spectra of leaves stored at 4 °C are similar to those stored at 10 °C and are not shown. The changes in the spectra obviously reflect modifications in quality parameters during the shelf-life. In particular, the figure shows relevant differences in the reflectance band centred 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 spectral reflection intensities measured at the selected wavelengths were finally used to predict the quality parameters for the determination of different freshness levels of the leaves. According to the variable selection methods stated above, the selected wavelengths were employed as the inputs for MLR model elaboration for pH, MC and TP. The results are shown in Table 2. The prediction ability of the MLR models was verified to study the efficiency of the selected wavelengths.

For pH, moisture and TP prediction, the statistics of the MLR models were equal to R^2_{cv} = 0.70, 0.75, 0.80 and RPD = 1.83, 2.08, 2.48, respectively. For MC, an alternative MLR model was carried out using only 710 and 720 nm informative wavelengths. Results demonstrated similar determination coefficients, in calibration and validation, compared to the MLR model with the four effective wavelengths (data not shown).

A RPD value between 2 and 2.5 indicates that coarse quantitative predictions are possible (Nicolai et al., 2007). In this work RPD values <3 were obtained for moisture and pH. An RPD >3 is considered adequate for analytical purposes in most NIRs applications for agricultural products (Williams, 2001; Fearn, 2002). Nicolai et al. (2007) wrote a review about the application of NIR spectroscopy for measurement of fruit and vegetable quality and indicated that RPD values between 2.5 and 3 or above corresponds to good and excellent prediction accuracy, respectively.

A comparison between PLS derived from the full vis/NIR spectra (400–1000 nm) and MLR arising only from the four wavelengths was carried out. The overall calibration and prediction results of the MLR models, for all the parameters, were satisfactory, although the performance of the MLR models was slightly worse than the good PLS models. RPD value for pH decreased from 2.54 for PLS to 1.83 for MLR, regarding the MC, RPD showed a slight decrement

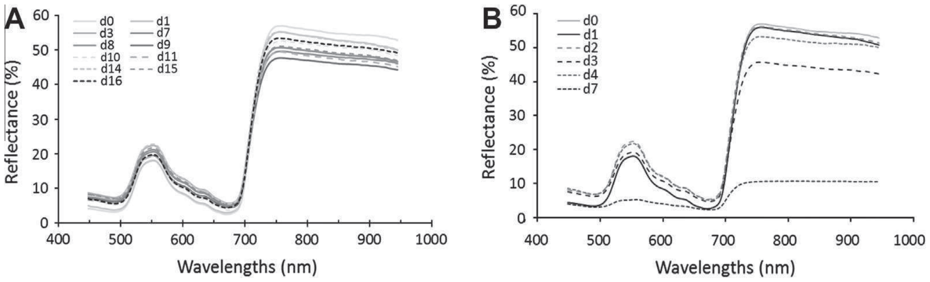

Fig. 4. Example of average spectra of *Valerianella* leaf samples grouped for sampling day (from d0 to d16) for samples stored at 10 °C (A) and from d0 to d7 for samples stored at 20 °C (B).

Table 2
Statistics of the MLR models, based on the four selected wavelengths (520, 680, 710, 720 nm) to predict the freshness level of *Valerianella* leaf samples, and of the PLS models (cross-validation).

Quality parameters	N°	Mean	SD	Calibration MLR			Cross-validation MLR			Cross-validation PLS		
				R^2	RMSEC	RPD	R^2	RMSECV	RPD	R^2	RMSECV	RPD
pH	24	6.45	0.33	0.82	0.13	2.54	0.70	0.18	1.83	0.86	0.13	2.54
TP (mg/100$g_{eq\ gallic\ acid}$)	16	267	40.3	0.88	12.38	3.26	0.80	16.28	2.48	0.89	12.64	3.19
MC (%)	24	93.38	0.27	0.87	0.09	3	0.75	0.13	2.08	0.84	0.12	2.25

SD = standard deviation; LV = latent variables.

from 2.25 to 2.08, and for TP from 3.19 to 2.48 for PLS and MLR respectively (Table 2).

This means that only a small loss of information was noticeable between the PLS models calculated using 2048 wavelengths and the MLR models employing only the four effective variables. Moreover, the samples were distributed closely to the regression line, which shows excellent spectral analysis performance of the PLS-RCA-MLR method.

Wu et al. (2010) applied a similar selection approach on transmittance spectra for the investigation of TSS and pH of grape juice beverages. The authors obtained optimal values for the coefficients of determination, in validation, for both TSS that for pH ranging from 0.89 to 0.97 and 0.91 to 0.96, respectively, relating to the PLS models derived from the full spectra (325–1075 nm). MLR analysis was applied to verify the results of wavelength selection. Regarding TSS, the authors obtained very good results compared with the PLS models with R^2_{cv} = 0.97 and 0.98 for five and nine variables selected, respectively. For pH, they achieved analogous results with coefficients of determination equal to 0.96 and 0.97, respectively. Good results were also obtained by Li et al. (2007) on tea-based soft drinks and by Liu et al. (2009) on beer, using analogous variable selection and validation approaches. Zhang et al. (2012) proposed (Bipls-SPA) a method to select 25 wavelengths for the estimation of water content in ornamental plant leaves using vis/NIR spectroscopy. PLS model deriving from the full spectrum (200–1100 nm) showed RPD equal to 3.66. After the selection PLS model gave an higher RPD value of 4.86.

The selection based on the analysis of the main changes in leaves' optical spectra led to an equation having the general form,

$$Y = b_1{}^{*}I_{520} + b_2{}^{*}I_{680} + b_3{}^{*}I_{710} + b_4{}^{*}I_{720} + b_0 \qquad (1)$$

where the parameters b_1, b_2, b_3, b_4 and b_0 are computed from a multi linear fit of known pairs' values (spectral intensities measured at the four wavelengths, I_{520}, I_{680}, I_{710} and I_{720}, and corresponding chemical data) for the *Valerianella* leaves using MLR analysis.

Equations of MLR models for pH, MC and TP are reported hereafter:

$$\text{Model}_{\text{pH}} \qquad Y_{\text{pH}} = -1.00^{*}I_{520} - 16.76^{*}I_{680} + 9.88^{*}I_{710} - 10.61^{*}I_{720} + 6.66 \qquad (2)$$

$$\text{Model}_{\text{MC}} \qquad Y_{\text{MC}} = 3.74^{*}I_{520} - 4.67^{*}I_{680} - 5.80^{*}I_{710} + 7.37^{*}I_{720} + 92.31 \qquad (3)$$

$$\text{Model}_{\text{TP}} \qquad Y_{\text{TP}} = 665.8^{*}I_{520} + 1835.0^{*}I_{680} - 724.0^{*}I_{710} + 660.8^{*}I_{720} + 242.9 \qquad (4)$$

Once the prediction capabilities using simple MLR equations were evaluated, the designed principle of a compact-sized, low-cost, and easy-to-use device was proposed.

In order to highlight the innovative features of a future device, in Fig. 5 a scheme was proposed. A possible solution consists of a 4 LED illumination source (light emitting diodes) at the specific wavelengths (520, 680, 710 and 720 nm), with filtered photodiodes for the readout signal. The elaboration unit, using equations with a general form like [1], assigns specific coefficients to the signal intensity recorded at the four wavelengths coming from the LEDs photodiodes system. In this way, a prediction of pH, MC and TP is realized. Finally, a display shows the estimation to the user. The foreseen system would support the producers to quickly predict the freshness of fresh-cut *V. locusta* L. and, therefore, take decisions regarding the product management directly at the selling point.

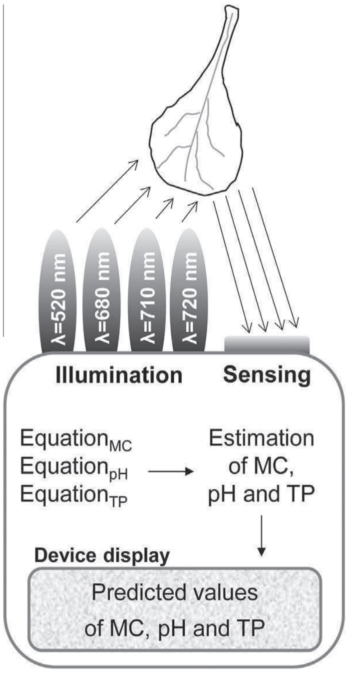

Fig. 5. Functional scheme of a simple, handheld optical device implementing signal intensity measurements at the selected wavebands and equations to estimate pH, moisture content (MC), and total polyphenols (TP) for supporting decisions on *Valerianella* leaf freshness level.

4. Conclusions

In this work, a variable selection methodology has been proposed in order to select a reduced set of wavelengths that are essential in the detection of minimal processed *V. locusta* L. freshness, using vis/NIR spectroscopy.

The four selected candidate wavelengths were 520 nm, 680 nm, 710 nm and 720 nm.

MLR was applied to the wavelengths in order to validate the prediction ability, compared with the PLS models built using the full spectra, and to verify the effectiveness of selected variables. The overall prediction results of the MLR models, for the three analyzed parameters (pH, MC and TP), were satisfactory. The obtained determination coefficients and RPD values were similar for the PLS and MLR models.

These individual fingerprint wavelengths and simply equations, with a general form like [1], could be used for the design of a simplified handheld device which would allow real-time assessment of *Valerianella* freshness. In particular, this device may be based on the measurement and processing of diffuse spectral reflectance at these few appropriately selected wavelengths. A possible functional scheme of a compact-sized LED technology based, low-cost, and easy-to-use device was proposed.

This type of simplified optical tool, avoiding specific chemometric analyses and trained personnel, could support the conventional techniques in the shelf-life assessment of fresh-cut *Valerianella* providing information useful for a better management of the product along the distribution chain. Moreover, the implementation of the device directly at the point of sale should be a guarantee for the consumer.

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References

Baldi, L., Casati, D., 2008. The fresh-cut crops sector and his development in Lombardia Region: the economic aspects (Il settore della IV gamma e il suo sviluppo in Lombardia: gli aspetti economici). In: Società Agraria di Lombardia e Camera di Commercio di Bergamo (Ed.), La IV gamma, Innovazione nell'offerta dei prodotti orticoli. Bergamo, Italy (in Italian).

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. http://dx.doi.org/10.1007/s11947-012-0824-7.

Camps, C., Christen, D., 2009. Non-destructive assessment of apricot fruit quality by portable visible–near infrared spectroscopy. Food Sci. Technol. 42 (6), 1125–1131.

Castoldi, N., Bechini, L., Ferrante, A., 2011. Fossil energy usage for the production of baby leaves. Energy 36 (1), 86–93.

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.

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. 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.

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 Horticulturae 913, 575–581.

Fearn, T., 2002. Assessing calibrations: SEP, RPD, RER and R2. NIR News 13, 12–14.

Fernández-Novales, 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 Res. Int. 42 (2), 285–291.

Ferrante, A., Maggiore, T., 2007. Chlorophyll a fluorescence measurements to evaluate storage time and temperature of Valeriana leafy vegetables. Postharvest Biology and Technology 45 (1), 73–80.

Francois, I.M., Wins, H., Buysens, S., Godts, C., Van Pee, E., Nicolai, B., De Proft, M., 2008. Predicting sensory attributes of different chicory hybrids using physico-chemical measurements and visible/near infrared spectroscopy. Postharvest Biol. Technol. 49, 366–373.

Frank, I.E., Todeschini, R., 1994. The data analysis handbook. Data Handling in Science and Technology. Elsevier, Amsterdam, The Netherland, ISBN 0-444-81659-3.

Gitelson, A.A., Merzlyak, M.N., Lichtenthaler, H.K., 1996. Detection of red edge position and chlorophyll content by reflectance measurements near 700 nm. J. Plant Physiol. 148, 501–508.

Guerzoni, M.E., Gianotti, A., Corbo, M.R., Sinigaglia, M., 1996. Shelf life modelling for fresh-cut vegetables. Postharvest Biol. Technol. 9, 195–207.

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, 877–885.

Guidetti, R., Beghi, R., Bodria, L., 2010. Evaluation of grape quality parameters by a simple vis/NIR system. Trans. ASABE 53 (2), 477–484.

Guidetti, R., Beghi, R., Giovenzana, V., 2012. Chemometrics in food technology. Chemometrics in Practical Applications. InTech, ISBN: 978-953-51-0438-4.

Jacxsens, L., Devlieghere, F., Debevere, J., 2001. Temperature dependence of shelf-life as affected by microbial proliferation and sensory quality of equilibrium modified atmosphere packaged fresh produce. Postharvest Biol. Technol. 26, 59–73.

Kader, A.A., 2008. Perspective flavor quality of fruits and vegetables. J. Sci. Food Agric. 88, 1863–1868.

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. J. Food Eng. 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. Anal. Chim. Acta 635, 45–52.

Muminovic, J., Melchinger, A.E., Lubberstedt, T., 2004. Genetic diversity in cornsalad (*Valerianella locusta*) and related species as determined by AFLP markers. Plant Breeding 123, 460–466.

Naes, T., Mevik, B.H., 2001. Regression models with process variables and parallel blocks of raw material measurements. J. Chemom. 15, 413–426.

Naes, T., Isaksson, T., Fearn, T., Davies, T., 2002. A user-friendly guide to multivariate calibration and classification. NIR Publications, Chichester, UK, 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 Biol. Technol. 46, 99–118.

Rico, D., Martín-Diana, A.B., Barat, J.M., Barry-Ryan, C., 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: a review. Trends Food Sci. Technol. 18 (7), 373–386.

Singleton, V., Rossi, J.A., 1969. Colorymetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. Am. J. Enology Viticulture 16, 144–158.

Sun, D.W., 2010. Hyperspectral imaging for food quality analysis and control. In: Sun, Da-Wen. (Ed.). Elsevier, ISBN 978-0-12-374753-2.

Temma, T., Hanamatsu, K., Shinoki, F., 2002. Development of a portable near infrared sugar-measuring instrument. J. Near Infrared Spectrosc. 10, 77–83.

Toivonen, P.M.A., DeEll, J.E., 2002. Physiology of fresh-cut fruit and vegetables. In: Lamikanra, I. (Ed.), Fresh-cut fruits and vegetables. CRC Press LLC, Boca Raton, Florida, USA, pp. 91–117.

Walsh, K.B., Guthrie, J.A., Burney, J.W., 2000. Application of commercially available, low-cost, miniaturised NIR spectrometers to the assessment of the sugar content of intact fruit. Aust. J. Plant Physiol. 27, 1175–1186.

Williams, P.C., 2001. Implementation of near-infrared technology. In: Williams, P., Norris, K.H. (Eds.), Near-Infrared Technology in the Agricultural and Food Industries. American Association of Cereal Chemist, St. Paul, Minnesota, USA, pp. 145–169.

Williams, P., Norris, K., 2002. Near-Infrared Technology in the Agricultural and Food Industries, 2nd ed. American Association of Cereal Chemist, St. Paul, Minnesota, USA.

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. Anal. Chim. 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. Anal. Chim. Acta 667, 14–32.

Zhang, Q., Li, Q., Zhang, G., 2012. Rapid determination of leaf water content using vis/NIR spectroscopy analysis with wavelength selection. Hindawi Publishing Corporation Spectrosc.: Int. J. 27 (2), 93–105. http://dx.doi.org/10.1155/2012/276795.

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. J. Food Eng. 77, 254–260.

3

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

Wavelength Selection with a View to a Simplified Handheld Optical System to Estimate Grape Ripeness

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Abstract: The aim of this work was to identify the three most significant wavelengths able to discriminate in the field those grapes ready to be harvested using a simplified, handheld, and low-cost optical device. Nondestructive analyses were carried out on a total of 68 samples and 1,360 spectral measurements were made using a portable commercial vis/near-infrared spectrophotometer. Chemometric analyses were performed to extract the maximum useful information from spectral data and to select the most significant wavelengths. Correlations between the spectral data matrix and technological (total soluble solids) and phenolic (polyphenols) parameters were carried out using partial least square (PLS) regression. Standardized regression coefficients of the PLS model were used to select the relevant variables, representing the most useful information of the full spectral region. To support the variable selection, a qualitative evaluation of the average spectra and loading plot, derived from principal component analysis, was considered. The three selected wavelengths were 670 nm, corresponding to the chlorophyll absorption peak, 730 nm, equal to the maximum reflectance peak, and 780 nm, representing the third overtone of OH bond stretching. Principal component analysis and multiple linear regression were applied on the three selected wavelengths in order to verify their effectiveness. Simple equations for total soluble solids and polyphenols prediction were calculated. The results demonstrated the feasibility of a simplified handheld device for ripeness assessment in the field.

Key words: grape, ripeness, vis/NIR spectroscopy, wavelength selection, handheld device, chemometrics

Near-infrared (NIR) spectroscopy has gained wide acceptance in different fields, particularly in postharvest fruit and vegetable production (Wang and Paliwal 2007). The main advantage of NIR technology is its ability to record spectra nondestructively both for solid (using mainly diffuse reflectance acquisition technique) and liquid (using mainly transmittance mode) samples without any pretreatment, allowing for the rapid analysis of products. Cost savings are often achieved for NIR measurements in terms of improved control and product quality and the technique can provide results faster than traditional laboratory analysis.

NIR technology has resulted in a number of publications, including a synthesis of the status of NIR in the agrifood industry (Wang and Paliwal 2007) and a comprehensive overview of NIR spectroscopy for measuring the quality attributes of fruit and vegetables (Nicolai et al. 2007). There have been several NIR applications to estimate the ripeness parameters of different fruit species, especially grapes (Bellincontro et al. 2009, Cozzolino et al. 2006).

In recent years, interest has shifted toward the development of portable vis/NIR systems for use in the field and these systems been tested in controlled laboratory conditions and directly in the field. Laboratory applications included the feasibility of using vis/NIR spectroscopic devices in transmission mode to predict the soluble solids content (SSC) and total acidity of fresh-cut KaoNumpung pomelos (Puangsombut et al. 2012), the ability of a portable single-channel vis/NIR spectrometer to determine the SSC and total acidity of two thick-peel mandarin cultivars (Antonucci et al. 2011), and the use of portable NIR to evaluate apricot quality during postharvest (Camps and Christen 2009). Applications in uncontrolled field conditions have included an optical technique to estimate the ripeness of red-pigmented fruits (Bodria et al. 2004), a portable NIR instrument (640–1300 nm) for determining ripeness in winegrapes (Larrain et al. (2008), a vis/NIR device in reflectance configuration to predict blueberry ripeness (Guidetti et al. 2008), and portable vis/NIR systems to evaluate grape quality parameters (Guidetti et al. 2010) and to assess the nutraceutical properties of apples (Beghi et al. 2003).

All these approaches rely on wide spectral ranges (thousands of wavelengths) and thus require multivariate techniques for data processing to build calibration and prediction models (Cen and He 2007, Williams and Norris 2001). Complex mathematical techniques (chemometrics) are required to explain chemical information encoded in spectral data (Cogdill and Anderson 2005). The most commonly used chemometric techniques are spectral preprocessing, to remove any irrelevant information; principal component analysis (PCA), to perform qualitative data analysis; and partial least squares (PLS) regression, to obtain a quantitative prediction of relevant

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parameters (Wold et al. 2001, Naes et al. 2002, Nicolai et al. 2007, Cen and He 2007).

Chemometrics can be used to select a few relevant variables that represent the most useful information of the full spectral region (Xiaobo et al. 2010). This selection eliminates variables containing mostly redundant information and spectral noise and reduces the cost of the potential miniaturized devices built only with the selected wavelengths. Generally, the selection of these optimal wavelengths reduces the size of the required measurement data while preserving the most important information contained in the data (Sun 2010). Three effective wavelength selection methods combined with vis/NIR spectroscopy have been proposed to determine the SSC of beer, including a successive projections algorithm (SPA), regression coefficient analysis (RCA), and independent component analysis (ICA) (Liu et al. 2009). The maximum number of selected wavelengths, by SPA, ranged from 4 to 21 depending on different pretreatments; 10 essential wavelengths were obtained by both RCA and by ICA.

There are several simplified nondestructive commercial devices based on a few wavelengths available on the market, including an innovative and simplified NIR system (DA-Meter for apples and Kiwi-Meter for kiwifruits) patented by the University of Bologna (Costa et al. 2011). These devices determine the stage of fruit maturity 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 color.

The limited adoption of NIR technology by the enology and viticulture sectors could be attributed to cost, technical limitations, grower resistance, and supply-chain weakness (Magwaza et al. 2012). Thus, the development of simplified handheld devices may encourage adoption. Based on these considerations, the aim of this research was to identify the most significant wavelengths able to discriminate grapes ready to be harvested during the final ripening stages. The specific objectives were (1) to obtain essential wavelength variables based on the PLS-RCA variable selection method; (2) to choose the three most informative wavelengths in a view of a simplified and handheld device; (3) to compare the prediction performance of the calibration models established by PLS on the full vis/NIR spectra and multiple linear regression using the selected wavelengths; and (4) to define simple equations for the estimation of grape ripeness. The possible final application would be a low-cost and user-friendly device that supports small-scale growers in determining optimal harvest date according to ripening degree.

Materials and Methods

Sampling. The experimental plan monitored the grape ripening process just before harvest in the Valtellina viticultural area (Sondrio, northern Italy) using *Vitis vinifera* cv. Nebbiolo (ecotype Chiavennasca), one of the most important red varieties in Italy. Samples were drawn from 17 different zones, throughout the entire viticultural area of the valley, in order to represent environmental variability and monitor the entire production region of DOC (controlled denomination of origin) and DOCG (controlled and guaranteed denomination of origin) wines. A total of 68 samples of grape clusters were collected on four sampling dates (7, 16, and 29 Sept and 12 Oct 2011). For each date, nondestructive analyses were carried out on each sample using a portable commercial vis/NIR spectrophotometer; destructive chemical analyses were then performed.

For each of the 68 samples, the spectral acquisitions of 10 individual berries were carried out, and for each berry two acquisitions were performed in the equatorial region, for a total of 1,360 spectral measurements. Finally, an average spectrum (20 acquisitions) for each sample was calculated and used, coupled with reference chemical data, for the chemometric analysis.

Portable vis/NIR device. Spectral acquisitions were performed on berries directly in the field using a vis/NIR spectrophotometer (Jaz, Ocean Optics, Dunedin, FL), an optical portable system operating in the wavelength range of 400 to 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, and (5) battery power supply.

Spectra were acquired in reflectance mode: light radiation was guided from the light source to the sample through a Y-shaped, bidirectional fiber-optic probe. The Y-shaped fiber guided light from the halogen lamp to illuminate the sample while simultaneously collecting the radiation from the berry and guiding it back to the spectrophotometer. The tip of the optical probe was equipped with a soft plastic cap to ensure contact with the skin of the sample during measurements, while minimizing environmental light interference. The integrated spectrophotometer was equipped with diffractive grating for spectral measurements optimized in the range of 400 to 1000 nm and a charge-coupled device sensor with a 2048 pixel matrix, corresponding to a nominal resolution of 0.3 nm.

Spectral measurements were taken in the field on individual berries after artificial illumination with the probe tip. In order to reduce the possible influence of environmental conditions, especially related to diurnal changes in sunlight, spectral acquisitions were consistently taken a few hours in the morning with the help of the plastic cap, ensuring 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 the berry/probe contact configuration, which were evidently sufficient to cancel out the possible influence of ambient illumination on samples. Air temperature changes during acquisition were limited to a range from 15 to 25°C. The field data set of spectra were assumed to randomly embed possible environment influencing factors.

Chemical analyses. Total soluble solids (TSS) content was measured using a digital pocket refractometer (model DBX-55; ATAGO, Tokyo, Japan) and grape titratable acidity (TA; g tartaric acid dm^{-3}) was measured using an automatic titrator (TitroMatic KF 1S, Crison Instruments, Milan, Italy). Grape phenolic content was estimated according to the Glories method (Glories 1984), in which potential anthocyanins (PA) and extractable anthocyanins (EA), extracted at pH 1 and pH 3.2, respectively (mg anthocyanins dm^{-3}), and total polyphenols (TP) were evaluated. Phenolic compound quantification was based on optical density (OD) measurement at 520 nm and 280 nm for anthocyanins and polyphenols, respectively, using a UV/vis spectrophotometer (model 7800; Jasco, Tokyo, Japan).

Data processing. *Spectral data.* Chemometric analyses were performed using The Unscrambler (ver. 9.6; CAMO Software, Oslo, Norway) to extract the maximum usable information from the spectral data and to select the most significant wavelengths that could be used in a simplified device. Collected spectra were preprocessed using smoothing (moving average, 15-nm-wide window) and reducing techniques. Principal component analysis (PCA) was performed to explore the possible clustering of sample spectra from the same sampling date. PCA identifies the natural clusters in the data set, with the first principal component (PC) expressing the largest amount of variation, followed by the second PC which conveys the second most important factor of the remaining analysis, and so forth (Di et al. 2009). Loading plots obtained with PCA were subsequently analyzed to confirm the variable selection performed using regression coefficient analysis (RCA), deriving from partial least square (PLS) analysis (Chong and Jun 2005).

The correlation between the spectral data matrix and technological (TSS) and phenolic (total polyphenols; TP) parameters were carried out using a PLS regression algorithm, the most widely used regression technique that relates two data matrices, *X* and *Y*, by a linear multivariate model. In this procedure, full cross-validation was used to develop a PLS regression model. To evaluate model accuracy, the statistical parameters used were the coefficient of determination in calibration (R^2_{cal}), the coefficient of determination in cross-validation (R^2_{cv}), the root mean square error of calibration (RMSEC), and the 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/averaged reference values of each parameter (Nicolai et al. 2007, Naes et al. 2002). Moreover, the ratio performance deviation (RPD) value was calculated, which is defined as the ratio between the standard deviation of the response variable and RMSECV (Williams 2001, Fearn 2002). RPD values <1.5 indicate that the calibration is not useful. When the RPD value is >2.0, quantitative predictions are possible.

Wavelength selection. RCA was carried out for relevant variable selection, representing the most useful information of the full vis/NIR spectral region (Xiaobo et al. 2010, Chong and Jun 2005). Standardized regression coefficients of the PLS model were used for the elaboration. The standardization took into account both the standard deviation of reflectance, for each wavelength considered, and the standard deviation of the reference data (TSS and TP) (Frank and Todeschini 1994).

Regression coefficients obtained by the PLS model were used to calculate the Y variable response value (TSS and TP in grape) from the X variables (grape spectra). The size of the numerical coefficients gave an indication of the impact of different variables on the response (Y). The final aim was to find which variables were important for predicting the Y variable. High 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 extreme of the regression coefficient plot, and the three higher peaks (absolute values) were chosen. Finally, qualitative evaluation of the average spectra and loading plot derived from PCA were considered to confirm the effectiveness of RCA variable selection. Spectral reflection intensity measured at selected wavelength ranges were finally used to predict quality parameters (TSS and TP) for the determination of different grape ripening stages.

Therefore, the three wavelengths were used as the input data matrix of PCA and for the elaboration of multiple linear regression (MLR) models (Wu et al. 2010, Fernández-Novales et al. 2009, Li et al. 2007). Compared to PLS, MLR allows for the development of models using only few important variables to predict the outcome of a response. MLR is also well-suited when the number of variables is less than the number of samples and is not affected by collinearity (Næs and Mevik 2001). Verification of the prediction ability of the MLR models was performed to study the efficiency of the selected wavelengths.

Results

Descriptive statistics for berry technological indices (TSS and titratable acidity) and for nutraceutical parameters (potential anthocyanins, extractable anthocyanins, and total polyphenols) were determined and average data are shown, based on all four sampling dates (Table 1). Statistics related to the PLS models obtained by vis/NIR spectroscopy for berry qualitative parameters are shown (Table 2). The model developed for TSS presented a good determination coefficient and a low RMSEC value of 0.78. In validation, R^2_{cv} was lower (0.77); RMSECV% was 3.8%.

Table 1 Descriptive statistics of *V. vinifera* cv. Nebbiolo ripening parameters, including standard deviation (SD) and standard error (SE).

Parameter	Unit	N	Range	Mean	SD	SE
Total soluble solids	Brix	68	15.9–23.8	21.1	1.8	0.2
Titratable acidity	$g_{tartaric\ acid}$ dm^{-3}	68	5.7–16.9	9.1	2.0	0.3
Potential anthocyanins	mg dm^{-3}	68	38.6–911.6	514.5	139.3	19.0
Extractable anthocyanins	mg dm^{-3}	68	199.9–600.8	357.8	93.7	11.8
Total polyphenols	OD_{280} nm	68	22.1–51.5	36.2	7.7	1.0

The titratable acidity data had a high coefficient of determination in calibration and a fair RMSECV value (Table 2). In validation, the determination coefficient was slightly lower and RMSECV was slightly higher. The elaboration of a model for PA and EA displayed, in validation, low values of the determination coefficients and RMSECV% values of 19.1% and 17.4%, respectively.

Ratio performance deviation (RPD) values for technological parameters (TSS and TA) were >2.0 and for PA and EA phenolic indices were ~1.5. The RPD for total polyphenols (TP) was 1.98.

The best correlations were found for TSS and TP. Therefore, the relevant wavelengths describing the features of the spectra for the determination of TSS and TP were selected. RCA was performed, starting from the PLS models for TSS and TP. In particular, the standardized regression coefficients of the PLS model were used to select the relevant variables. The standardization took into account both the standard deviation of reflectance, for each wavelength of the vis/NIR spectra, and the standard deviation of the reference data (TSS and TP). Plots of the standardized regression coefficients, for TSS and TP, versus vis/NIR wavelengths (400–1000 nm) are shown (Figure 1). The trend and shape of the regression coefficient plots were similar for TSS and TP. The three most relevant wavelengths were selected by choosing the higher absolute regression values (Liu et al. 2008, Cen et al. 2006): 670, 730, and 780 nm.

The performance of RCA was confirmed using a loading plot derived from PCA and the visual inspection of average spectral curves (Sun 2010). With close observation, the PCA loading plot (Figure 2) indicated some positive and negative peaks at certain wavelengths. Corresponding to the three wavelengths (670, 730, and 780 nm) sorted by RCA, the loading plot showed (1) maximum differences between the PCs (high positive value for PC1 and high negative value for PC2) at 670 nm, (2) the maximum PC1 peak explaining 97% of the total variance at 730 nm; and (3) the maximum positive value of the PC2 couplet with a fairly high positive value for PC1 at 780 nm.

The average measured spectra of the four sampling dates are shown (Figure 3). The study of spectral evolution over time highlighted which wavelengths were more sensitive to grape variability during ripening: 670 nm, corresponding to the chlorophyll absorption peak (McGlone et al. 2002);

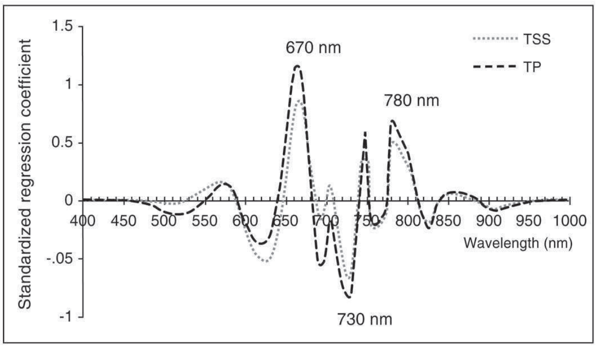

Figure 1 Standardized regression coefficients for total soluble solid content (TSS) and total polyphenols (TP).

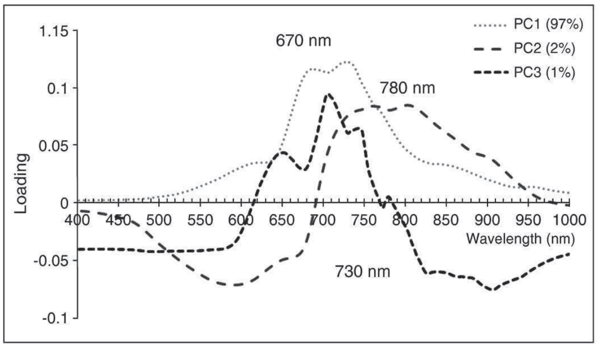

Figure 2 Loading plot of the first three principal components (PC).

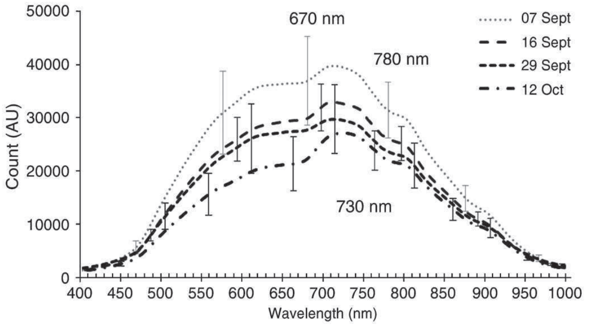

Figure 3 Average raw spectra of 1,360 berries grouped in four sampling dates corresponding to the final stages of ripening. Error bars indicate the standard deviation within each group at different wavelengths.

Table 2 Statistics of the PLS models to predict maturity indices and nutraceutical properties of *V. vinifera* cv. Nebbiolo berries.

		Calibration[a]			Cross-validation[a]					
Parameter[a]	**Unit[a]**	**LV**	**R^2_{cal}**	**RMSEC**	**R^2_{cv}**	**Bias**	**Slope**	**RMSECV**	**RMSECV%**	**RPD**
TSS	Brix	7	0.83	0.66	0.77	0.007	0.80	0.78	3.8%	2.26
TA	$g_{tartaric\ acid}$ dm^{-3}	8	0.76	0.58	0.62	-0.008	0.68	0.75	8.2%	2.66
PA	mg dm^{-3}	7	0.59	76.49	0.41	0.511	0.49	93.48	19.1%	1.61
EA	mg dm^{-3}	7	0.60	49.53	0.39	0.793	0.49	62.18	17.4%	1.51
TP	OD_{280} nm	7	0.81	3.24	0.74	-0.018	0.77	3.88	10.7%	1.98

[a] Abbreviations: TSS, total soluble solids; TA, titratable acidity; PA, potential anthocyanins; EA, extractable anthocyanins; TP, total polyphenols; OD, optical density; LV, latent variables; R^2_{cal}, coefficient of determination in calibration; RMSEC, root mean square error of calibration; R^2_{cv}, coefficient of determination in cross-validation; RMSECV, root mean square error of cross-validation; RMSECV%, percent errors in cross-validation; RPD, ratio performance deviation.

730 nm, equal to the maximum reflectance peak; and 780 nm, representing the third overtone of OH bond stretching (Clement et al. 2008, Bertrand 2000).

Spectral reflection intensity measured at selected wavelengths was finally used to predict the quality parameters (TSS and TP) for the determination of different berry ripening stages. Therefore, the three essential wavelengths were used as the input data matrix of PCA and for the elaboration of simple MLR models. Verification of the prediction ability of the MLR models was done to study the efficiency of the selected wavelengths.

The PCA derived from the initial full vis/NIR spectra (Figure 4) and the PCA derived from only the three selected variables (Figure 5) were compared. For the PCA score plot of the full spectra, the ripening process was well fitted by PCA: spectral data corresponding to different sampling dates were sorted from negative PC1 and positive PC2 values to positive PC1 and negative PC2 values. The same behavior was displayed in the PCA score plot of the PCA arising from the effective wavelengths. In both cases, PC1 accounted for separating berries in terms of ripening stages.

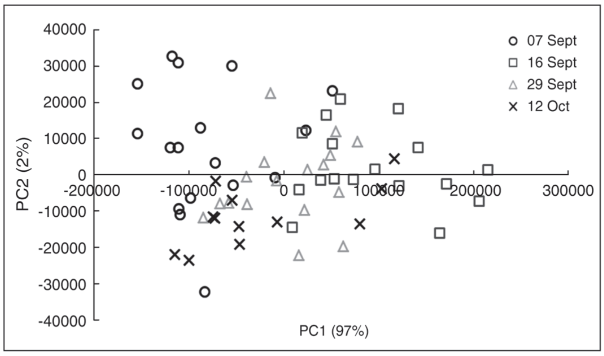

Figure 4 Principal component analysis deriving from the initial full vis/NIR spectra.

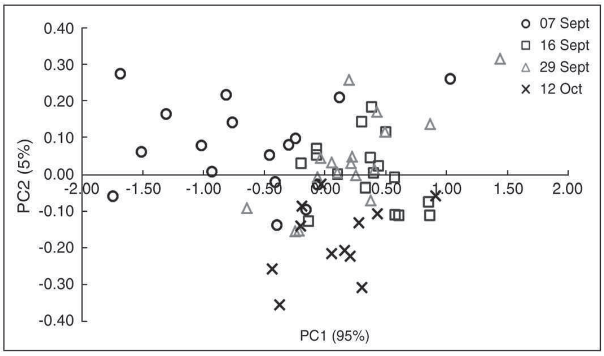

Figure 5 Principal component analysis deriving from the only three normalized spectral ratios.

According to the variable selection methods stated above, the selected wavelengths were used as the inputs for MLR model elaboration for TSS and TP (Table 3). The selection based on an analysis of the main changes the optical spectra of the berries measured during ripening led to the equation,

$$Y = b_1{*}I670 + b_2{*}I730 + b_3{*}I780 + b_0 \qquad \text{Eq. 1}$$

where the parameters b_1, b_2, b_3, and b_0 are computed from a multilinear fit of known pair values (spectral intensities measured at the three wavelengths, I670, I730, and I780 nm, and corresponding chemical data) for the berries using MLR analysis.

Equations of MLR models for TSS and TP are reported as:

$$\text{Model}_{TSS} \qquad Y_{TSS} = 13.15{*}I670 - 38.55{*}I730 + 37.81{*}I780 + 15.69 \qquad \text{Eq. 2}$$

$$\text{Model}_{TP} \qquad Y_{TP} = 60.75{*}I670 - 254.93{*}I730 + 382.73{*}I780 - 162.20 \qquad \text{Eq. 3}$$

Discussion

Similar results for PLS models were obtained using a portable vis/NIR system (450–980 nm) showing for fresh Nebbiolo berries (same conditions as this study) $R^2_{cv} = 0.72$ and RMSECV = 0.79 Brix for TSS, $R^2_{cv} = 0.66$ and RMSECV = 1.48 g TA dm^{-3} for titratable acidity, and $R_{cv}^2 = 0.50$ and 0.46 for the phenolic parameters PA and EA, respectively (Guidetti et al. 2010). Similar results were also found using a portable NIR device (640–1300 nm) on winegrapes (RMSECV from 1.01 to 1.27 Brix) under field conditions (Larrain et al. 2008). Portable vis/NIR devices were also tested for the prediction of ripening indices (TSS and TP) of fresh blueberries (Guidetti et al. 2008), of two apple varieties just before fruit harvest (Beghi et al. 2012), and of fresh apricot under laboratory conditions (Camps and Christen 2009), with similar results to our findings reported here.

These results show the possible application of vis/NIR technology for the estimation of many ripening parameters (Nicolai et al. 2007). The application of portable devices under field conditions is certainly more complex than laboratory experimentation, given the uncontrolled environmental

Table 3 Statistics of the MLR models based on the three selected wavelengths (670, 730, 780 nm) to predict the ripening parameters of *V. vinifera* cv. Nebbiolo berries.

		Calibration[a]		Cross-validation[a]					
Parameter[a]	**Unit[a]**	**R^2_{cal}**	**RMSEC**	**R^2_{cv}**	**Bias**	**Slope**	**RMSECV**	**RMSECV%**	**RPD**
TSS	Brix	0.75	0.77	0.71	-0.006	0.74	0.83	3.9%	2.13
TP	OD_{280} nm	0.74	3.95	0.70	-0.034	0.73	4.30	11.9%	1.79

[a] Abbreviations: TSS, total soluble solids; TP, total polyphenols; OD, optical density; R^2_{cal}, coefficient of determination in calibration; RMSEC, root mean square error of calibration; R^2_{cv}, coefficient of determination in cross-validation; RMSECV, root mean square error of cross-validation; RMSECV%, percent errors in cross-validation; RPD, ratio performance deviation.

conditions such as ambient light and fluctuating temperatures, as highlighted in several studies (Nicolai et al. 2007, Wang and Paliwal 2007).

The explorative PCA conducted on the full vis/NIR spectra resulted in three most significant PCs explaining 100% of the total data variance (PC1, 97%; PC2, 2%; and PC3, 1%). The PCA score plot (Figure 4) shows the evolution of sample ripening as a function of sampling date on PC1. PC loadings (Figure 2) were analyzed in a search for the main wavelength bands contributing to PCs as candidate discriminators for the final stages of the ripening process. As expected, the spectra exhibit significant differences according to sampling date, corresponding to the final stages of ripening. Changes in the spectra obviously reflect modifications in quality parameters during ripening. The observed changes in the visible region spectra between 500 and 700 nm are due to changes in the amount of pigment, especially linked to anthocyanin accumulation, during ripening. This leads to a decrease in reflectance in the visible band associated with the anthocyanin absorption peak centered around 540 nm (Tamura and Yamagami 1994).

For TSS and TP prediction, the statistics of the MLR models were equal to $R^2_{cv} = 0.71$ and 0.70, RMSECV = 0.83 Brix and 4.3 OD_{280}, respectively. A similar selection approach was tested on transmittance spectra (325–1075 nm) for the investigation of TSS and pH of grape juice beverages, and RMSECV values, in validation, were 0.360 and 0.054, respectively (Wu et al. 2010). These results are better than our findings, but the experimental settings under controlled laboratory conditions and the homogeneous matrix of the juice helped provide these excellent outcomes. Good results were also obtained on tea-based soft drinks (Li et al. 2007) and on beer (Liu et al. 2009), using analogous variable selection and validation approaches. The RCA method has been also applied to select the most important wavelengths to determine the TSS and pH of rice vinegars (Liu et al. 2008) and of orange juice (Cen et al. 2006).

A comparison of the PLS derived from the full vis/NIR spectra and the MLR arising only from the three wavelengths was carried out for TSS and TP. The overall calibration and prediction results of the MLR models were satisfactory, although the performance of the MLR models was slightly less accurate than the PLS models. The obtained RMSECV values were similar for PLS (0.81 Brix, 3.9 OD_{280}) and MLR (0.71 Brix, 4.3 OD_{280}) models. Similarly, the RPD value for TSS decreased from 2.26 for PLS to 2.13 for MLR and the value for TP decreased from 1.98 for PLS to 1.76 for MLR. PLS and MLR models for both the quality parameters showed very low bias values and almost the same slope (~0.8). Thus, only a small loss of information was noticeable between the PLS model calculated using 2048 wavelengths and the MLR model using three effective variables. Moreover, the samples were distributed closely to the regression line, indicating excellent spectral analysis performance of the PLS–RCA–MLR method.

In the similar selection approach on transmittance spectra for the investigation of TSS and pH of grape juice beverages (Wu et al. 2010), the authors obtained optimal determination coefficients. PLS models derived from the full spectra (325–1075 nm) achieved, for TSS and pH, R^2 values in validation ranging from 0.89 to 0.97 and from 0.91 to 0.96, respectively. MLR analysis was applied to verify the results of wavelength selection. For TSS, the authors obtained very good results compared with the PLS models with $R^2_{cv} = 0.97$ and 0.98 for five and nine variables selected, respectively. For pH, they achieved analogous results with determination coefficients equal to 0.96 and 0.97, respectively.

In a study on reducing sugar content in red grape must, there was a slight difference between the PLS model calculated using the full spectra (800–1050 nm) and the MLR model based on four sensitive wavelengths ($RMSEP_{PLS} = 12.20$ g dm^{-3}, $RMSEP_{MLR} = 20.51$ g dm^{-3}) (Fernández-Novales et al. 2009).

Once the prediction capabilities using simple MLR equations were evaluated, the designed principle of a compact-sized, low-cost, and easy-to-use device was studied in this work. A possible functional scheme of this device can be envisioned using LED (light-emitting diodes) technology for sample illumination at the specified wavelengths (670, 730, and 780 nm) and filtered photodiodes for the read-out signal. From the intensity signals sensed at the three wavebands, a microcontroller can compute the TSS predicted value by equation 2 and the TP value by equation 3 and display it to the user. Such a system would support the grapegrower in rapidly estimating the ripening grape parameters and making a decision on harvest time.

Conclusion

A variable selection methodology was proposed to determine a reduced set of features that are effective in the detection of berry ripening using vis/NIR spectroscopy. The aim was to identify, using the PLS-RCA variable selection method, the effective wavelengths able to discriminate berries ready to be harvested with a simplified handheld and low-cost optical device.

The three selected candidate wavelengths were 670, 730, and 780 nm. PCA and MLR were applied to the wavelengths to validate the prediction ability, compared with the PCA and PLS models using the full spectra, and to verify the effectiveness of selected variables. The overall prediction results of the MLR models, for both TSS and TP, were satisfactory. The obtained determination coefficients and RPD values were similar for PLS ($R^2 = 0.77$ and RPD = 2.26 for TSS, $R^2 = 0.74$ and RPD = 1.98 for TP) and MLR ($R^2 = 0.71$ and RPD = 2.13 for TSS, $R^2 = 0.70$ and RPD = 1.76 for TP).

Both qualitative (PCA) and quantitative (MLR) analyses highlighted the results, showing good sample separation during ripening and confirming the choice of wavelengths. The potential of vis/NIR to predict TSS and TP was ascertained, and the essential wavelengths, which were strongly related to these indices, were obtained through multivariable analysis. These individual fingerprint wavelengths and the equations for TSS and TP could be used for the design of a simplified, low-cost handheld device which would allow for real-time assessment of berry ripeness in the field. In particular, this device may be based on the measurement and processing of diffuse spectral reflectance at these few appropriately selected wavelengths.

Literature Cited

Antonucci, F., F. Pallottino, G. Paglia, A. Palma, S. D'Aquino, and P. Menesatti. 2011. Non-destructive estimation of Mandarin maturity status through portable vis-NIR spectrophotometer. Food Bioprocess Technol. 4(5):809-813.

Beghi, R., A. Spinardi, L. Bodria, I. Mignani, and R. Guidetti. 2013. Apples nutraceutic properties evaluation through a visible and near-infrared portable system. Food Bioprocess Technol. 6(9):2547-2554.

Bellincontro, A., I. Nicoletti, M. Valentini, A. Tomas, D. De Santis, D. Corradini, and F. Mencarelli. 2009. Integration of nondestructive techniques with destructive analyses to study postharvest water stress of winegrapes. Am. J. Enol. Vitic. 60:57-65.

Bertrand, D. 2000. Spectroscopie de l'eau. *In* La Spectroscopie Infrarouge et ses Applications Analytiques. D. Bertrand and E. Dufour (eds.), pp. 93-105. Lavoisier Tec et Doc, Paris.

Bodria, L., M. Fiala, R. Guidetti, and R. Oberti. 2004. Optical techniques to estimate the ripeness of red-pigmented fruits. Trans. ASAE 47(3):815-820.

Camps, C., and D. Christen. 2009. Non-destructive assessment of apricot fruit quality by portable visible-near infrared spectroscopy. Food Sci. Technol. 42(6):1125-1131.

Cen, H., and Y. He. 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. Trends Food Sci. Technol. 18:72-83.

Cen, H., Y. He, and M. Huang. 2006. Measurement of soluble solids contents and pH in orange juice using chemometrics and vis-NIRS. J. Agric. Food Chem. 54:7437-7443.

Chong, I.G., and C.H. Jun. 2005. Performance of some variable selection methods when multicollinearity is present. Chemometr. Intell. Lab. 78:103-112.

Clement, A., M. Dorais, and M. Vernon. 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., and C.A. Anderson. 2005. Efficient spectroscopic calibration using net analyte signal and pure component projection methods. J. Near Infrared Spec. 13:119-132.

Costa, G., E. Bonora, G. Fiori, and M. Noferini. 2011. Innovative non-destructive device for fruit quality assessment. Acta Hort. 913:575-581.

Cozzolino, D., R.G. Dambergs, L. Janik, W.U. Cynkar, and M. Gishen. 2006. Analysis of grapes and wine by near infrared spectroscopy. J. Near Infrared Spec. 14:279-289.

Di, C.Z., C.M. Crainiceanu, B.S. Caffo, and N.M. Punjabi. 2009. Multilevel functional principal component analysis. Ann. Appl. Stat. 3:458-888.

Fearn, T. 2002. Assessing calibrations: SEP, RPD, RER and R2. NIR News 13:12-14.

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

Frank, I.E., and R. Todeschini. 1994. The Data Analysis Handbook. Elsevier, Amsterdam.

Glories, Y. 1984. The colour of red wines. Conn. Vigne Vin 18:195-217.

Guidetti, R., R. Beghi, and L. Bodria. 2010. Evaluation of grape quality parameters by a simple vis/NIR system. Trans. ASABE 53(2):477-484.

Guidetti, R., R. Beghi, L. Bodria, A. Spinardi, I. Mignani, and L. Folini. 2008. Prediction of blueberry (*Vaccinium corymbosum*) ripeness by a portable vis-NIR device. Acta Hort. 310:877-885.

Larrain, M., A.R. Guesalaga, and E. Agosin. 2008. A multipurpose portable instrument for determining ripeness in wine grapes using NIR spectroscopy. IEEE T. Instrum. Meas. 57(2):294-302.

Li, X., Y. He, C. Wu, and D.W. Sun. 2007. Nondestructive measurement and fingerprint analysis of soluble solid content of tea soft drink based on vis/NIR spectroscopy. J. Food Eng. 82:316-323.

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

Magwaza, L.S., U.L. Opara, H. Nieuwoudt, P.J.R. Cronje, W. Saeys, and B. Nicolaï. 2012. NIR spectroscopy applications for internal and external quality analysis of citrus fruit—A review. Food Bioprocess Technol. 5:425-444.

McGlone, V.A., R.B. Jordan, and P.J. Martinsen. 2002. Vis/NIR estimation at harvest of pre- and post-storage quality indices for 'Royal Gala' apple. Postharvest Biol. Tech. 25:135-144.

Naes, T., and B.H. Mevik. 2001. Regression models with process variables and parallel blocks of raw material measurements. J. Chemometr. 15:413-426.

Naes, T., T. Isaksson, T. Fearn, and T. Davies. 2002. A User-Friendly Guide to Multivariate Calibration and Classification. NIR Publications, Chichester, UK.

Nicolai, B.M., K. Beullens, E. Bobelyn, A. Peirs, W. Saeys, K.I. Theron, and J. Lammertyna. 2007. Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biol. Tech. 46:99-118.

Puangsombut, A., S. Pathaveerat, A. Terdwongworakul, and K. Puangsombut. 2012. Evaluation of internal quality of fresh-cut pomelo using vis/NIR transmittance. J. Texture Stud. 43:445-452.

Sun, D.W. 2010. Hyperspectral Imaging for Food Quality Analysis and Control. Academic Press/Elsevier, San Diego.

Tamura, H., and A, Yamagami. 1994. Antioxidative activity of monoacylated anthocyanins isolated from Muscat Bailey A grape. J. Agric. Food Chem. 42:1612-1615.

Wang, W., and J. Paliwal. 2007. Near-infrared spectroscopy and imaging in food quality and safety. Sens. Instrumen. Food Qual. 1:193-207.

Williams, P.C. 2001. Implementation of near-infrared technology. *In* Near-Infrared Technology in the Agricultural and Food Industries. P. Williams and K.H Norris (eds.), pp. 145-169. Am. Association of Cereal Chemists, St. Paul, MN.

Williams, P., and K. Norris (eds.). 2001. Near-Infrared Technology in the Agricultural and Food Industries. 2d ed. Am. Association of Cereal Chemists, St. Paul, MN.

Wold, S., M. Sjöström, and L. Eriksson. 2001. PLS-regression: A basic tool of chemometrics. Chemometr. Intell. Lab. 58:109-130.

Wu, D., Y. He, P. Nie, F. Cao, and Y. Bao. 2010. Hybrid variable selection in visible and near-infrared spectral analysis for non-invasive quality determination of grape juice. Anal. Chim. Acta 659:229-237.

Xiaobo, Z., Z. Jiewen, M.J.W. Povey, M. Holmes, and M. Hanpin. 2010. Variables selection methods in near-infrared spectroscopy. Anal. Chim. Acta 667:14-32.

4

Development of a Light Emitting Diodes (LED) based simplified system for rapid evaluation of grape ripeness

Development of a Light Emitting Diodes (LED) based simplified system for rapid evaluation of grape ripeness

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Abstract

NIR spectroscopy has proved to be one of the most efficient and advanced tools to monitor product quality. Portable vis/NIR instruments are particularly versatile and suitable to be directly used in field to monitor the ripening process or quality parameters.

The aim of this work is to develop a simplified optical system for the measurement of grape ripeness directly in field. It was developed according to results arising from a wavelengths selection. Light Emitting Diodes (LEDs) were chosen as light sources in order to design the optical device. Spectral acquisition based on reflectance at four selected wavelengths (630, 690, 750 and 780 nm) was proposed.

The use of a small number of variables allows the integration of simple processing algorithms in the microcontroller software of the instrument. The prototype design is realized with 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 sample matrix.

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 an handheld, inexpensive and easy to use device. Farmers could employ it directly in field for a quick evaluation of grapes ripening level.

Keywords: portable optical device, non-destructive analysis, grape, reflectance, ripening.

1 Introduction

The study of non-destructive methods and the design of new devices for monitoring the largest number of samples in short time and give a more comprehensive overview of ripening is an ongoing process [1-3].

Currently, visible near infrared (vis/NIR) and near infrared NIR (NIRs) spectroscopy are techniques widely applied in the food sector [4].

A review of literature reveals that the NIRs techniques (vis/NIR and NIR) were applied to a wide array of agro-food applications. The feasibility of NIRs spectroscopy to measure quality attributes of fruit and vegetables has been shown for many products [2]. 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. 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 only in a limited area of the sample, therefore it is especially suitable for homogeneous products. However these optical technologies are able to reach 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 a suitable technique for this purpose.

Three main types of NIRs devices are available: i) laboratory instruments for applications in research centers or in industry laboratories, ii) sorting and sizing devices designed specifically for the fruit and vegetable industries, e.g. in warehouses, iii) portable devices for the analysis directly in the field. Table 1 shows the main differences between the three groups of NIRs devices.

Table 1. Characteristics of the three main categories of NIRs devices

	Application area	Flexibility of use	Applicability	Measurement accuracy and reproducibility	Cost
Laboratory devices	Research/Industry	Adaptable to different matrices	Fixed system	Optimal	Average/high
Sorting and sizing	Industry	Specific categories of products	Fixed system	Good	Average/high
Portable devices	Also in field	Dedicated for individual products	Portable/handheld	Good	Average

Today, a wide selection of spectroscopic devices is available and there are about 60 NIR spectrometer manufacturers around the globe [5].

For every device categories calibration models to be used in practice should be based on large datasets, encompassing several orchards, climate conditions, seasons and operational conditions (e.g. temperature, environmental lighting) and optimized towards robustness by incorporating appropriate preprocessing methods [2-3]. Sample presentation is also a crucial step in NIRs analyses. 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) [3, 6].

There is certainly a large demand of NIRs portable devices, so more research is required in this area [2]. The availability of low cost miniaturized 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. 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 minimized by appropriate data processing [2].

Portable vis/NIR instruments were tested in controlled laboratory conditions by Antonucci et al. [7] for the evaluation of mandarin maturity status and by Camps and Christen [8] for assessing apricot quality. In uncontrolled field conditions, instead, a portable vis/NIR device was tested in order to estimate apples nutraceutic properties [9], to evaluate grape quality parameters [10], to assess the ripeness of red-pigmented fruits [11] and to predict blueberry ripeness [12].

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 [3, 5].

Few portable devices are commercially already available, for examples FANTEC Fruit Tester 20 was used [13], Jaz Modular Optical Sendsing Suite, Ocean Optics and QS_300, UNITEC spa.

For each device category (Table 1) 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 [2, 14-16].

Also for the use of portable NIRs devices are required skilled operator able to process complex data in order to extract useful information and build ripening prediction models [4, 16]. Therefore, In order to support the small-scale producers, simplified, easy to use, low-cost devices for real-time measurements 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 few variables which contain useful information and the elimination of variables containing mostly noise [17]. In a view of simplified optical systems, different variables selection approaches were applied in previous studies on fresh-cut Valerianella [18], on wine grapes [19] and on blueberries [20].

Only a few examples of simplified optical commercial systems are already available on the market. For example, the University of Bologna [21] 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 among specific wavelengths. Based on these considerations the aim of this work is to develop and test a simplified optical system, based on LEDs technology for samples illumination at four selected wavelengths for the rapid monitoring of grape ripeness degree directly in field. In order to evaluate the performance of the prototype, characteristic curve and operating point were established and tests on standard solutions were finally performed.

2 Material and methods

Light Emitting Diodes (LED) technology was chosen as light source in order to design a prototype of a simplified optical device [3]. The system is based on reflectance spectral acquisition at the wavelengths of interest. Wavelength variables selected in the previous study [19] were 670, 730 and 780 nm.

The LEDs actually used to develop the device were chosen at the wavelengths that most closely matched the theoretical choice, according to the availability on the market. Therefore, the wavelengths used are: 630 and 690 nm, near to the chlorophyll absorption peak, 750 and 780 nm near to the third overtone of OH bond stretching.

2.1 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 PIC^{TM} 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. This is realized by a specific application written in C# language using the Microsoft® Visual Studio development environment.

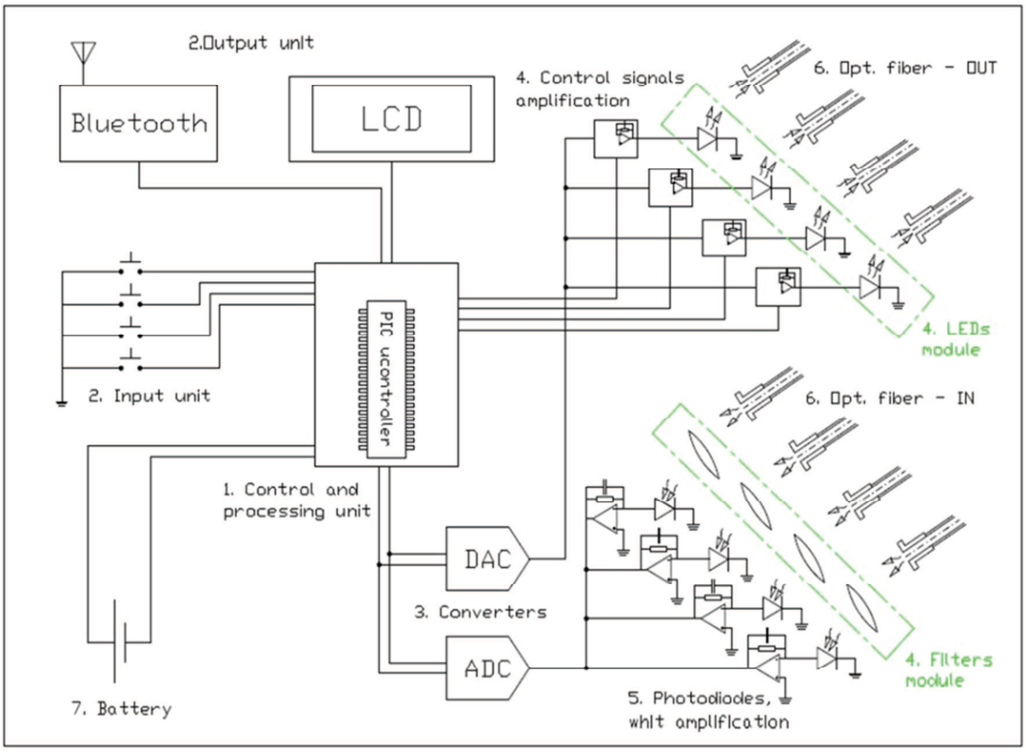

Figure 1. Block diagram of the main components of the prototype

3. 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.

4. 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 (λ = 630, 690, 750, 780 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 [2].

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).

5. 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).

6. 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.

7. The battery

The prototype is powered by a single internal battery of 12 V. This makes the instrument portable and suitable for field use.

2.2 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 a second, after which the device is ready for a new acquisition.

2.3 Modularity

The design of the prototype of the simplified optical device was realized 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 sample matrix. Interesting applications for instruments of this type would be, for example, the estimation of the main technological and phenolic parameters of grapes for wine [10] or early detection of infection (i.e. *Botrytis cinerea*) on leaves [22] or berries.

2.4 Characteristic curve and operating point

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. The setting parameters should be chosen in order to establish a proper operating point along the characteristic curve of the system, both by ensuring the linearity of the response, and by expressing an adequate sensitivity with respect to the specific application. On the one hand, comparisons between measurements become immediate with a linear response. On the other, an appropriate sensitivity which exploit the totality of the available measurement scale should make possible to describe optical phenomena characterized by small reflective dynamics.

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 of the LED drive signal, henceforth called Stimulus, which modulates the intensity of the incident light and, consequently, of the reflected light, too;
- the amplification factor of the signal detected by the photodiode, henceforth called Gain, 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, i.e. the setting of an operating point along the characteristic curve which is appropriate with respect to a specific experimentation, in fact, is in general not immediate.

The optimal value of the amplification Gain for each channel must therefore be determined *una tantum* and preset 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 smallest level of Stimulus that induces a detected light at least equal to an appropriate threshold value, i.e. corresponding to the half value of the total range 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.

According to the previous considerations, the behavior of the device as a function of the Stimulus and Gain parameters, that is the operation characteristic of every channel of the system, and the identification of an appropriate operating point were investigated. 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 $0 < \text{Stimulus} < 255$, and Gain = 1, 2, 4, 8). These tests were repeated on the four available standard references, for reflection coefficients $R = 1, 0.5, 0.25, 0$.

2.5 Tests on standard solutions

Finally, the same configuration of the device was tested on three handmade standard samples consisting in distilled water solutions of blue food dye (Brilliant Blue R - CAS Number 6104-59-2, Sigma-Aldrich Co. LLC., United States) at concentration of 0.5% (w/v), 1% (w/v), and 2% (w/v), respectively. These tests aimed to assess the measuring capability of the device, by simulating acquisitions on samples similar to dark fruits, e.g. a berry of red grape or a blueberry, known to have a low and difficult to detect reflectance response in the visible range, that is in this case for the channels at 630 and 690 nm.

3 Results

3.1 Characteristic curve and operating point

Each of the four channels has proved to have a similar response with the change of the Stimulus and Gain parameters. Only the results coming from the tests related to a single channel are therefore presented, knowing that these are valid for the other channels too. As an example, Figures 2 and 3 show graphs regarding two different acquisitions for the channel at λ = 750 nm, the first one using reference R = 0.25 and the second one with reference R = 0.5.

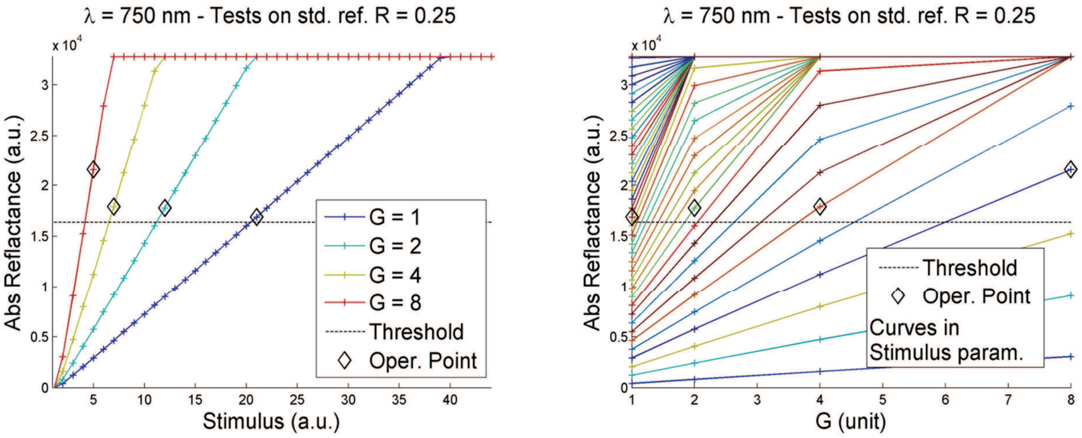

Figure 2. Acquisitions on channel λ = 750 nm, using reference R = 0.25. a) absolute reflectance vs Stimulus, for the four curves in Gain (G) parameter; b) absolute reflectance vs Gain (G), in Stimulus parameter

In both the figures, the left chart represents the absolute reflectance value measured in function of Stimulus variable, for the four curves in Gain parameter. Only the significant portion of the graphs is displayed, that is for the first few Stimulus values, as long as all the curves do not reach saturation. For this reason the abscissa scale is different between the corresponding graphs in the two figures.

The characteristic curve of the system, in terms of absolute reflectance as a function of Stimulus variable, clearly presents an excellent linearity independently from the Gain value. It is almost a straight line apart from little predictable boundary effects (near zero and near saturation). As expected, a higher value of Gain parameter corresponds to an increase in the slope of the line. The same effect can be obtained by acquiring more reflective standard references, as shown in Figure 3 with reference R = 0.5.

The linearity of the response with the change of the Stimulus variable is then assured for every choice of the Gain parameter and, at least in theory, of the operating point position along the curves between zero and saturation.

Nevertheless, saturation is reached very quickly and actually just a small portion of the available range 0 < Stimulus < 255 is used. The highest slope curves (high Gain values), combined with highly reflective standard references, could lead to the risk of reaching saturation even during the calibration procedure, with just the first Stimulus increments. The phenomenon is more pronounced in the test on reference R = 0.5, that is more reflective respect to R = 0.25, and it is confirmed by tests on other references (data not displayed). This is a common behavior in all the four channels due to the optic high efficiency of the system.

In practice, Gain = 1 is the only acceptable value for all the four channels and is therefore preset as a fixed parameter. It is the one corresponding to the smallest slope curve, developing in the wider range of Stimulus values before saturation is reached. With a preset value for the Gain parameter, the user can still affect the slope of the characteristic curve by changing the standard reference to adopt in calibration. He can therefore indirectly identify the operating point at the intersection between the curve itself and the threshold horizontal line, without taking care in its actual position in the parameter space and, more in general, in how the device works. The only thing the operator has to pay attention to is the choice of the standard reference in calibration which, according to this procedure, should provide spectral reflectance as similar as possible to the samples to be measured, ensuring a proper positioning of the operation point for the specific application. The identification of the preset value for the Gain parameter (G = 1) is confirmed by graphs on the right side of Figures 2 and 3, which lead to conclusions consistent with the previous ones. They show the same acquisition (λ = 750 nm, reference R = 0.25 and R = 0.5, respectively) representing absolute reflectance measured in function of Gain variable, for the 256 curves parameterized in Stimulus. In this case, only the curves corresponding to the first values of Stimulus parameter are distinguishable. All other curves collapse on the horizontal reflectance saturation line (Reflectance = 32767 a.u.) as Stimulus parameter grows, and are therefore coincident.

The linearity of the response with the change of Gain variable is very good, clearly for the curves not deformed by saturation effects (for low value of Stimulus). The choice, however, of a single value of minimum Gain for all the measurement channels is confirmed by this results, being the one providing the greatest number of significant Stimulus values.

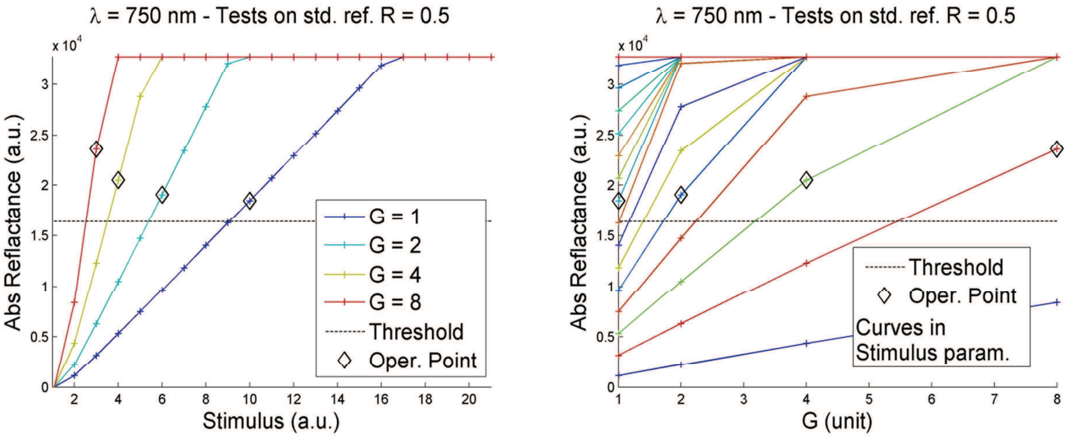

Figure 3. Acquisitions on channel λ = 750 nm, using reference R = 0.50. a) absolute reflectance vs Stimulus, for the four curves in Gain (G) parameter; b) absolute reflectance vs Gain (G), in Stimulus parameter

Concerning the comparison among the different measure channels the system shows the same behavior for all the four wavelengths. However, parameters values change in general depending on the channel. This is due to LEDs and photodiode working features that changes depending on the exercise wavelength, despite these components are of the same type, as declared by the manufacturers. This can be taken into account, by simply making an independent calibration on every channel using different standard references. This optimizes the behavior of each channel also depending on the spectral characteristics of the sample to be measured. While absolute measures of reflected light on the four wavelengths are not comparable even into the same acquisition, comparability remains unchanged with regard to the relative reflectance measures obtained in post-processing.

3.2 Tests on standard solutions

Figures 4 presents the results coming from the tests on the aqueous solutions of blue dye at concentration of 0.5% (w/v), 1% (w/v), and 2% (w/v), respectively. The curves of the absolute reflectance values measured in function of Stimulus variable for the three samples are shown together in the same graphs (the Gain parameter is now set at the fixed value Gain = 1 previously determined). Only the results for the two visible channels are presented (λ = 630 nm at left, and λ =

690 nm at right), since these are the most critical wavelengths for dark samples, and the ones which wanted to be investigated.

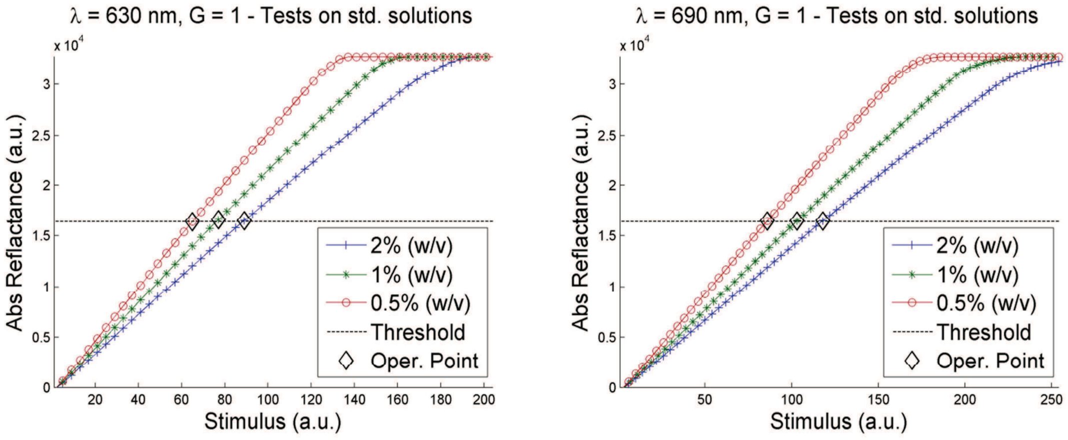

Figure 4. Absolute reflectance vs Stimulus (Gain G = 1) for acquisitions on aqueous solutions of blue dye at different concentrations. a) channel at λ = 630 nm; b) channel at λ = 690 nm.

As expected from the previous results, the curves obtained show an excellent linearity and have a slope that decreases with the increasing concentration of the dye. The slope is therefore consistent with the degree of the darkness of the sample. This proves the capability of the device to discriminate between dark samples which are apparently similar in a visible sense, by detecting the correspondent differences in spectral reflectance. This is the typical situation of dark fruits, like berries of red grape or blueberries.

On the basis of this and previous considerations, the solutions at different concentrations of dye could be used as handmade and economic references to calibrate the instrument for measurements on dark samples. A wider and more detailed range of references could be realized according to a simple protocol and then replicated when needed, depending on the specific application.

4 Conclusions

In this work a prototype of a simplified optical vis/NIR device based on LED technology was designed and tested. A preliminary test focused on the evaluation of the characteristic curve gave good results. The prototype is very promising.

A considerable amount of data were collected through laboratory tests in order to evaluate the behavior 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, and therefore the operating point. 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 references to be used in calibration. 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.

References

01 El-Masry, G.; Nassar, A.; Wang, N.; Vigneault, C. Spectral methods for measuring quality changes of fresh fruits and vegetables. *Stewart Postharvest Review* **2008**, *4(4)*, 1 - 13.

02 Nicolai, B. M.; Beullens, K.; Bobelyn, E.; Peirs, A.; Saeys, W.; Theron, K. I.; Lammertyna J. Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. *Postharvest Biology and Technology*, **2007**, *46*, 99-118.

03 Wang, W.; Paliwal, J. Near-infrared spectroscopy and imaging in food quality and safety. *Sens. & Instrumen. Food Qual.* **2007**, *1*, 193-207.

04 Guidetti, R.; Beghi, R.; Giovenzana, V. Chemometrics in Food Technology. In *Chemometrics*, ISBN: 978-953-51-0438-4, InTech, Rijeka, 2012; pp. 217-252.

05 McClure, W.F.; Tsuchikawa, S. 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; pp. 75-107.

06 Osborne, B.G.; Fearn, T.; Hindle, P.H. Practical NIR Spectroscopy with Applications in Food and Beverage Analysis, 2nd ed., Longman Scientific & Technical, 1993, Essex, England.

07 Antonucci, F.; Pallottino, F.; Paglia, G.; Palma, A.; D'Aquino, S.; Menesatti, P. Non-destructive estimation of Mandarin maturity status through portable VIS-NIR spectrophotometer. *Food and Bioprocess Technology* **2011**, *4(5)*, 809-813.

08 Camps, C.; Christen, D. Non-destructive assessment of apricot fruit quality by portable visible-near infrared spectroscopy. *Food Science and Technology* **2009**, *42(6)*, 1125-1131.

09 Beghi, R; Spinardi, A.; Bodria, L.; Mignani, I.; Guidetti, R. Apples nutraceutic properties evaluation through a visible and near-infrared portable system. *Food and Bioprocess Technology* **2013**, *6,* 2547-2554.

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

11 Bodria, L.; Fiala, M.; Guidetti, R.; Oberti, R. Optical techniques to estimate the ripeness of red-pigmented fruits. *Trans. of the ASABE* **2004**, *47*, 815-820.

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

13 Saranwong, S.; Sornsrivichai, J.; Kawano, S. Performance of a portable near infrared instrument for Brix value determination of intact mango fruit. *J. Near Infrared Spectrosc.* **2003**, *11*, 175-181.

14 Naes, T.; Isaksson, T.; Fearn, T.; Davies, T. A user-friendly guide to multivariate calibration and classification. Chichester, UK: NIR Publications, 2002, ISBN 0-9528666-2-5,

15 Wold, S.; Sjöström, M.; Eriksson, L. PLS-regression: a basic tool of chemometrics. *Chemom. Intell. Lab. Syst.* **2001**, *58*, 109-130.

16 Cen, H.; He, Y. Theory and application of near infrared reflectance spectroscopy in determination of food quality. *Trends in Food Science & Technology*, **2007**,*18*, 72-83.

17 Xiaobo, Z.; Jiewen, Z.; Povey, M.J.W.; Holmes, M.; Hanpin M. Variables selection methods in near-infrared spectroscopy. *Analytica Chimica Acta* **2010**, *667*, 14-32.

18 Beghi, R.; Giovenzana, V.; Civelli, R.; Malegori, C.; Buratti, S.; Guidetti, R. Setting-up of a simplified handheld optical device for decay detection in fresh-cut Valerianella locusta L. *Journal of Food Engineering* **2014**,*127*, 10-15.

19 Giovenzana, V.; Beghi, R.; Malegori, C.; Civelli, R.; Guidetti, R. Wavelength selection with a view to a simplified handheld optical system to estimate grape ripeness. *Am. J. Enol. Vitic.* **2014,** *65(1)*, 117-123.

20 Beghi, R.; Giovenzana, V.; Spinardi, A.; Bodria, L.; Guidetti, R.; Oberti, R. Derivation of a blueberry ripeness index with a view to a low-cost, handheld optical sensing device for supporting harvest decisions. *Trans. of the ASABE* **2013**, *56(4)*, 1551-1559.

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

22 Wu, D.; Feng, L.; Zhang, C.; He, Y. Early detection of botrytis cinerea on eggplant leaves based on visible and near - infrared spectroscopy. *Trans. of the ASABE* **2008**, *51(3)*, 1133-1139.

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Testing of a simplified LED based vis/NIR system for rapid ripeness evaluation of white grape for Franciacorta wine

Testing of a simplified LED based vis/NIR system for rapid ripeness evaluation of white grape for *Franciacorta* wine

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Abstract

The aim of this work was to test a simplified optical device for a rapid estimation of the ripening parameters of white grape for *Franciacorta* wine directly in field.

Spectral acquisition based on reflectance at four wavelengths (630, 690, 750 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.

Non-destructive analyses were carried out on 95 grape bunches for a total of 475 berries. Measurements were realized by weekly samplings during the last ripening stages. Optical measurements were carried out both using the simplified system and a portable commercial vis/NIR spectrophotometer, as reference instrument for performance comparison. Chemometric analyses were performed in order to extract the maximum useful information from optical data. Principal component analysis (PCA) was performed for a preliminary evaluation of the data. Correlations between the optical data matrix and ripening parameters (total soluble solids content, SSC; titratable acidity, TA) were carried out using partial least square (PLS) regression for spectra and using multiple linear regression (MLR) for data from the simplified device. Classification analysis were also performed with the aim of discriminate ripe and unripe samples. PCA, MLR and classification analyses show the effectiveness of the simplified system in separating samples among different sampling dates and in discriminating ripe from unripe samples. Finally, simple equations for SSC and TA prediction were calculated.

Keywords: simplified optical device, non-destructive analysis, vis/NIR spectroscopy, white grape, ripening, chemometrics.

1 Introduction

Establishing ripeness at harvest is a crucial issue since fruit quality are closely related to it. Grape ripeness monitoring in the orchard may provide valuable data to control quality of wine.

Ripening is a complex process, biochemical changes occur at skin and pulp level. To monitor these changes, routinely measurements of ripening parameters, essential for fruit and vegetables (e.g. soluble solids content, acidity, phenols, anthocyanins, firmness, etc.) are needed.

The search for non-destructive methods which could explore a larger sample and give a rapid and comprehensive overview of ripening would be helpful.

NIRs analyses are based on the use of spectral information arising from the interaction between food matrix and light for the simultaneous detection of all parameters necessary to monitor ripening. The light reflected from the product contains information about constituents in the inner layers and at the surface of the sample.

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). Arana, et al., (2005) studied NIR spectroscopy (800-1500 nm) in order to determine soluble solids content and to identify different varieties and origins of Chardonnay grapes. Thereafter, Kemps et al, (2010) evaluated the concentration of extractable anthocyanins, polyphenols, sugars and the density of 4 red grape varieties using vis/NIR spectrophotometer.

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).

For this reason, in order to support the small-scale producers, simplified, easy to use, low-cost systems 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. The authors already tested different variables selection approaches in previous studies in order to identity effective wavelengths to monitor quality decay in fresh-cut Valerianella (Beghi et al., 2014), to estimate ripening parameters on wine grapes (Giovenzana et al., 2013) and blueberries (Beghi et al., 2013), in a view of simplified optical systems.

Few examples of simplified non-destructive commercial system are already available on the market, based on absorbance differences between specific wavelengths to monitor ripening evolution of apples and kiwifruits (Costa et al., 2011) or on fluorescence to follow changes during grape ripening (Lurie et al., 2012).

The aim of this work was to design and test a LED based simplified optical device operating at only four wavelengths in the vis/NIR region for a rapid estimation of ripening parameters of white grape for *Franciacorta* wine directly in field.

2 Materials and methods

2.1 Sampling

The experimental plan monitored the grape ripening in the *Franciacorta* wines (DOCG, controlled and guaranteed denomination of origin) viticultural area (Adro, Brescia, northern Italy) during the last period of ripening just before harvest. The experimentation was carried out on white grape *Chardonnay* cultivar, one of the most important varieties in Italy. A total of 95 bunches of white grapes were collected in 2013 on five sampling dates (August 7th, 14th, 19th, 28th and September 4th). For every date, non-destructive analyses were carried out on each sample using both a LED based simplified system operating at four vis/NIR wavelengths and a portable commercial vis/NIR spectrophotometer. Destructive chemical analyses were also performed.

For each bunch the optical acquisition was carried out on 5 individual berries for a total of 475 berries. For each berry two acquisitions were performed in the equatorial region and then averaged (950 optical measurements for each device).

An average data for each bunch was also calculated and used coupled with reference chemical data for the chemometric analysis.

2.2 The simplified system

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). Wavelengths used for the development of the simplified optical system are: 630 and 690, near chlorophyll absorption peak (McGlone et al., 2002); 750, close to the third overtone of OH stretching (Clement et al., 2008, Bertrand, 2000); 850, close to the third combination overtone of sugar OH stretching at 840 nm (Golic et al., 2003).

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.

2.2.1 The control/processing unit

The control/processing unit 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.

2.2.2 The input/output unit

The user can control the instrument through a four buttons keyboard, which is connected to the main board (point 2 in Figure 1) and represents the input unit of the system. Using the keyboard, the operator can perform various tasks, i.e. the operator 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 (point 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 (point 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.

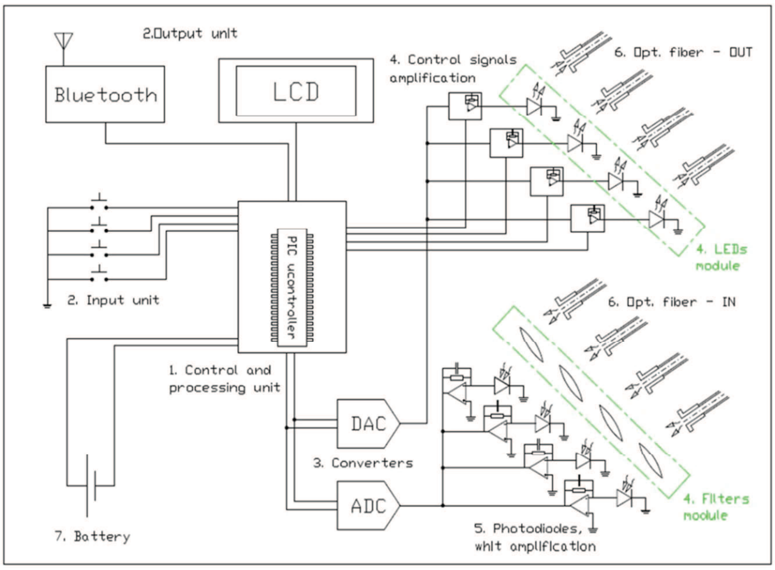

Figure 1. Block scheme of the main components of the simplified LED based system

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 (point 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.

2.2.3 The LEDs, optical filters modules and photodiodes

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 (λ = 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 (point 4 in Figure 1) that can be change according to the specific application.

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).

2.2.4 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.

The prototype is powered by a single internal battery of 12 V. This makes the instrument portable and suitable for field use.

When the user presses the acquisition button (point 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 laboratory tests focused on the evaluation of signal linearity, in terms of gain amplification factor and stimulus level for the drive signal of LEDs, were conducted (Giovenzana, 2013).

2.3 Commercial portable vis/NIR device

At the same time, spectral measurements usable for data comparison were realized with a commercial spectrophotometer. Spectral acquisitions were performed on grape berries directly in the field using a vis/NIR spectrophotometer (Jaz, OceanOptics, USA), which is an optical portable system operating in the wavelength range of 400-1000 nm. The equipment consists of five components: 1) a vis/NIR lighting system (halogen lamp), 2) a fiber optic probe for reflection measurement, 3) a spectrophotometer, 4) hardware for data acquisition and instrument control, 5) a battery as the power supply.

Spectra were acquired in reflectance mode: light radiation was guided from the light source to the sample through a Y-shaped, bidirectional fiber optic probe (OceanOptics, USA). The Y-shaped fiber guided light from the halogen lamp to illuminate the sample while simultaneously collecting the radiation coming from the fruit and guiding it back to the spectrophotometer. The tip of the optical probe was equipped with a soft plastic cap to ensure contact with the sample's skin during measurements, while minimizing environmental light interference. The integrated spectrophotometer was equipped with diffractive grating for spectral measurements optimized in the range of 400-1000 nm and a CCD sensor with a 2048 pixel matrix, corresponding to a nominal resolution of 0.3 nm.

For both the instruments, spectral measurements were taken in the field on individual berries after artificial illumination with the probe tip. In order to reduce the possible influence of environmental conditions, especially related to diurnal changes in sunlight, spectral acquisitions were always done during a few hours in the morning with the help of the plastic cap, ensuring contact between the probe tip and the measured berry.

2.4 Chemical Analyses

The total soluble solids content (SSC, °Brix) was measured using a digital pocket refractometer (DBX-55 ATAGO, Tokyo, Japan) while white grape titratable acidity (TA, $g_{tartaric\ acid}\ dm^{-3}$) was measured using an automatic titrator (TitroMatic KF 1S, Crison Instruments, Milan, Italy).

2.5 Data Processing

Mean, standard deviation (SD), and confidence intervals ($\alpha = 0.05$) were calculated on SSC and TA.

Chemometric analyses were performed using The Unscrambler® 9.6 software package (CAMO ASA, Norway) in order to extract the maximum usable information from the spectral data.

Collected spectra from the commercial device were pre-processed using smoothing (moving average, 15 nm wide window) and reducing techniques (reducing factor 5). Principal components analysis (PCA) was performed to explore the possible grouping of sample spectra from the same sampling date. In fact PCA identifies the natural clusters in the data set with the first principal component (PC), expressing the largest amount of variation, followed by the second PC which conveys the second most important factor of the remaining analysis, and so forth (Di et al., 2009).

The correlation between the spectral data matrix from the commercial device and the reference parameters (SSC and TA) were carried out using the PLS regression algorithm. It is the mostly used regression technique that relates two data matrices, X and Y, by a linear multivariate model. In this procedure, cross-validation with 7 cancellation groups was used to develop a PLS regression model. The data registered by the simplified system, at the four wavelengths, were employed as the input data matrix for the elaboration of MLR models (Wu et al., 2010, Fernández-Novales et al., 2009, Li et al., 2007). MLR is a regression method that, compared with PLS, allows to develop models using only few important variables, to predict the outcome of a response. This method is also well-suited when the number of variables is less than the number of samples and is not affected by collinearity (Næs and Mevik, 2001). The performances of the MLR models were evaluated to verify the efficiency of the simplified device for white grape ripeness assessment.

To evaluate model accuracy, PLS or MLR, the statistical parameters used were the coefficient of determination in calibration (R^2_{cal}), the coefficient of determination in cross-validation (R^2_{cv}), the root mean square error of calibration (RMSEC) and the root mean square error of cross-validation (RMSECV). The best calibrations were selected based on minimizing the RMSECV. Moreover the Ratio Performance Deviation (RPD) value was calculated. RPD is defined as the ratio between the standard deviation of the response variable and RMSECV. RPD values below 1.5 indicate that the calibration is not useful. When the value for the RPD is higher than 2, quantitative predictions are possible. Between 1.5 and 2.0 the algorithm have the possibility to distinguish between high and low values (Williams, 2001; Fearn, 2002).

Ratio error range (RER) value was also calculated. The RER is the ratio of the range in reference values of the validation samples divided by the RMSECV. Williams and Sobering (1996) suggest that the RER value should be 10 or higher.

Finally, a classification analysis was performed using PLS models and MLR models elaborated for data deriving from the commercial vis/NIR device and from the simplified system, respectively. The percentages of correctly and misclassified grape samples were determined according to ripeness thresholds for the analyzed parameters (ripe > 17 °Brix for SSC and < 7 g dm^{-3} for TA) suggested by the *Franciacorta* Consortium. Total PPV (Positive Predictive Value) indicating the total percentage of correctly classified samples was also computed.

For correlation and classification analyses, separated models for SSC deriving from data collected on berries and from bunches (mean of 5 single berries) were considered.

3 Results and discussion

The evolution of chemical indices analyzed during ripening is presented in Figure 2. The results showed, as expected, significant changes in SSC and TA during the last ripening phases. The ready to harvest values for the analyzed parameters suggested by the *Franciacorta* Consortium (SSC > 17 °Brix and TA < 7 g dm^{-3}) were achieved at the last sampling date (t5, September 4th).

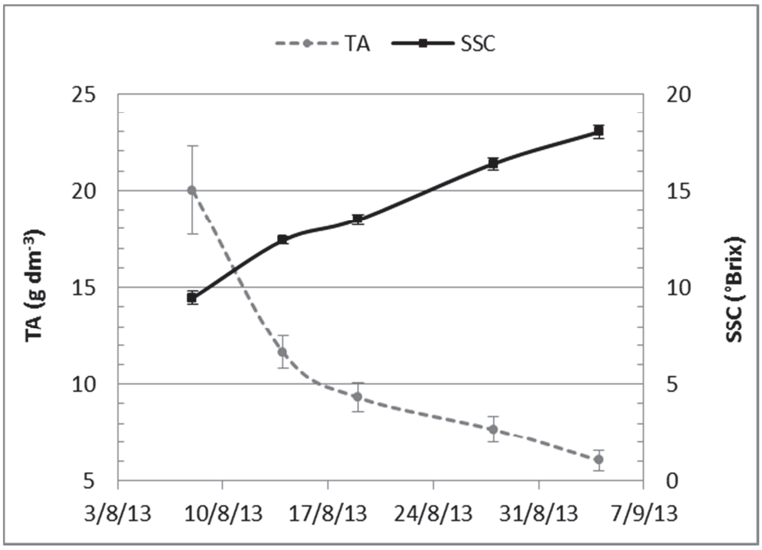

Figure 2. Evolution of SSC and TA during the last weeks of the ripening period. Bars indicate the confidence interval (α= 0.05) for each sampling time.

Regarding PCA, the scores plots deriving from the full vis/NIR spectra registered with the commercial spectrophotometer and from the only four wavelengths with the simplified LED based system are shown in Figure 3, grouped according to sampling dates. Ninety-nine percent of the total data variance is explained by the first two principal components (PCs) in both cases. In general, the PCAs show the ripening trend in grapes from high positive values to high negative values of PC1.

The scores plot of PC1 vs. PC2 for spectral data from the commercial device (Fig. 3a) reveals that PC1 greatly accounts for separating grades t1 (grape totally unripe) from the other grades (unripe, t2-t3; close to ripeness, t4; or fully ripe, t5) which are characterized by negative values of PC1. Grades from t3 to t5 are almost completely overlapping along the PC1 axis, whereas PC2 shows only a partial discriminating capability between these ripening stages. Concerning the PCA based on optical measurements of the simplified system (Fig. 3b), a fairly good sample separation in the PCs plan was obtained. Samples close to ripeness and fully ripe (grades t4 and t5) show both negative values of PC1 and, between them, they are partially discriminated by PC2 (10% of total variance explained) with mostly negative values for t4 and positive values for t5.

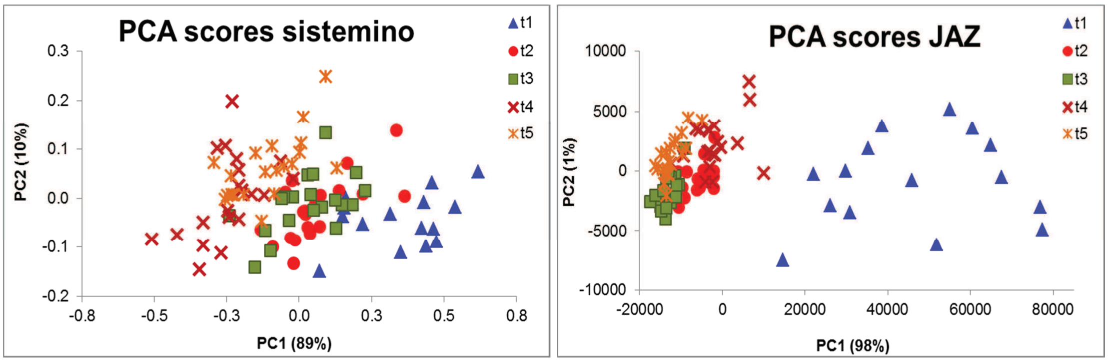

Figure 3. PCA deriving from the full vis/NIR spectra registered with the commercial spectrophotometer (a) and PCA from the only three normalized spectral ratios deriving from optical measurements with the simplified LED based system (b). Grape samples are graded according to sampling dates.

In Table 1 are summarized the descriptive statistics and the statistics related to the PLS models obtained by vis/NIR spectroscopy for grape ripening parameters (SSC and TA).

The model developed for SSC on bunches and for TA presented a fairly good results with determination coefficients, in validation, equal to 0.8-0.81, RPD values > 2 and RER close to 9 for SSC on bunches and > 10 for TA. Slightly worse results were obtained for the prediction of SSC on berries with R^2_{cv} =0.66-0.85, RPD value close to 2 and RER close to 9. Similar results were achieved by Arana et al., (2005) for SSC on Chardonnay grapes, showing regression models with determination coefficients for calibration and validation of 0.75 and 0.70 using NIR spectrometer (800-1500 nm). Larrain et al. (2008) also obtained R^2 = 0.87 in validation for SSC using a portable NIR device (640-1300 nm) on Chardonnay grapes under field conditions.

Regarding TA, Guidetti et al. (2010), using a portable vis/NIR system (450-980 nm), showed for fresh berries of red Nebbiolo grapes R^2_{cv} = 0.66 and RMSECV = 1.48 g dm^{-3}.

Table 1. Descriptive statistics and statistics of the PLS models elaborated on Vis/NIR spectra to estimate ripeness parameters of white grapes for *Franciacorta* wine.

				Calibration PLS			**Cross-validation PLS**		
Ripeness parameters	N°	Mean	SD	LV	R^2	RMSEC	R^2	RMSECV	RPD
SSC (°Brix) *on berries*	475	14.1	3.5	7	0.73	1.8	0.71	1.8	1.94
SSC (°Brix) *on bunches*	95	14.2	3.2	7	0.85	1.3	0.80	1.5	2.13
Titratable acidity (g dm^{-3})	95	10.5	5.1	5	0.86	1.9	0.81	2.2	2.32

SD= Standard deviation; LV=latent variables

Spectral reflection intensity measured at the four wavelengths on the same samples was used to predict the ripening parameters using MLR analysis (Table 2).

This analysis leads to an equation having the general form:

$$Y = b1*I630 + b2*I690 + b3*I750 + b4*I850 + b0 \quad [1]$$

where the parameters b1, b2, b3, b4 and b0 are computed from a multi linear fit of known pairs' values (spectral intensities measured at the four wavelengths, I630, I690, I750, I850 nm, and corresponding chemical data) for the white grape samples using MLR analysis.

Equations of MLR models for SSC_{berry}, SSC_{bunch} and TA are reported hereafter:

$$Model_{SSCberry}\ Y_{SSC} = 0.29*I630 + 0.11*I690 - 0.08*I750 - 0.13*I850 + 18.71 \quad [2]$$

$$Model_{SSCbunch}\ Y_{SSC} = 0.27*I630 + 0.15*I690 - 0.10*I750 - 0.10*I850 + 18.86 \quad [3]$$

$$Model_{TA}\ Y_{TA} = 0.11*I630 - 0.56*I690 + 0.13*I750 + 0.11*I850 + 4.96 \quad [4]$$

For SSC prediction, the statistics of the MLR models were equal to R^2_{cv} = 0.66 and 0.65, RPD =1.74 and 1.67, and RER = 8.26 and 7.31 for berry and bunch, respectively. The best MLR model was obtained for TA estimation with R^2_{cv} = 0.85, RPD = 2.5 and RER = 12.4.

A comparison between PLS derived from the full vis/NIR spectra (commercial device) and MLR arising only from four wavelengths (simplified device) was carried out on SSC and TA. Figure 4 shows examples of PLS and MLR models for SSC on bunches and TA prediction, respectively. The overall calibration and prediction results of the MLR models were satisfactory, although the performance of the MLR models was slightly worse than the PLS models for SSC prediction. RPD value for SSC on berries decreased from 1.94 for PLS to 1.74 for MLR and, regarding SSC on bunches, RPD showed a decrement from 2.13 to 1.67, respectively.

The best correlations were found for TA both for PLS and MLR regression models. The elaboration of the MLR model for TA displayed, in validation, higher values of the determination coefficient (0.85), RPD (2.50) and RER (12.40) than values carried out by PLS (R^2_{cv} = 0.81, RPD = 2.32, RER = 10.14).

This means that only a small loss of information was noticeable between the PLS model calculated using 2048 wavelengths and the MLR models employing only the four channels of the simplified device, especially for the TA estimation.

The authors in a previous study (Giovenzana et al., 2013) tested a variable selection methodology in order to select a reduced set of effective variables for the monitoring of red grape ripening using vis/NIR spectroscopy. The obtained determination coefficients and RPD values showed a similar trend to those of the present work: for the PLS and MLR, R^2 = 0.77, RPD = 2.26 and R^2 = 0.71, RPD = 2.13 were obtained, respectively, for SSC prediction. Wu et al. (2010) applied a similar selection approach on transmittance spectra for the investigation of TSS and pH of grape juice beverages. The authors obtained optimal determination coefficients, in validation, for both TSS that for pH ranging from 0.89 to 0.97 and 0.91 to 0.96, respectively, relating PLS models derived from the full spectra (325-1075 nm). MLR analysis was applied to verify the results of wavelength selection. Regarding TSS, the authors obtained very good results compared with the PLS models with $R^2_{cv} = 0.97$ and 0.98 for five and nine variables selected, respectively.

Fernández-Novales et al., (2009) compared PLS model calculated using the full spectra (800-1050 nm) with the MLR based on four sensitive wavelengths for the estimation of the reducing sugar content in grape must. The four-wavelength model produced equations slightly less accurate than those obtained with the full range (RPD_{PLS}=12.20, RER_{PLS} = 21.21; RPD_{MLR}=5.12, RER_{MLR} =16.53), corresponding to a reduction of model performance close to 20%.

Table 2. Descriptive statistics and statistics of the MLR models, based on data of the simplified device (four wavelengths 630, 690, 750, 850 nm) to predict the ripeness parameters of white grapes for *Franciacorta* wine.

				Calibration MLR		**Cross-validation MLR**		
Ripeness parameters	N°	Mean	SD	R^2	RMSEC	R^2	RMSECV	RPD
SSC (°Brix) *on berries*	433	14.4	3.3	0.67	1.9	0.66	1.9	1.74
SSC (°Brix) *on bunches*	89	14.1	3.0	0.69	1.7	0.65	1.8	1.67
Titratable acidity (g dm^{-3})	89	9.9	4.5	0.87	1.6	0.85	1.8	2.50

SD= Standard deviation

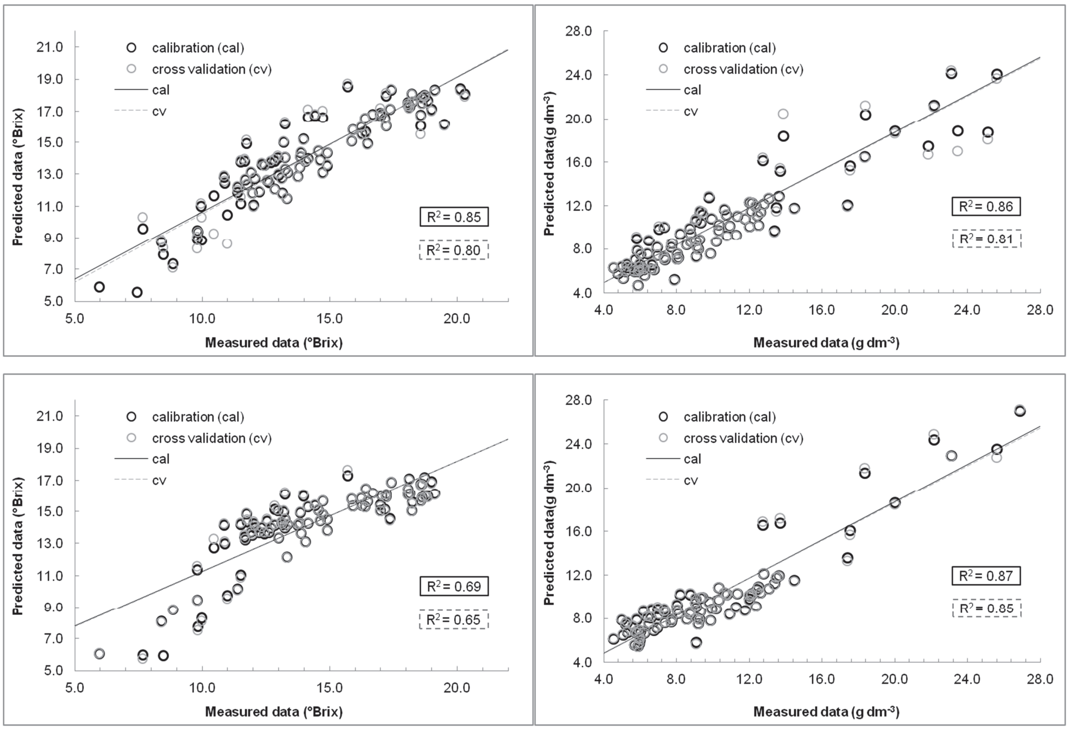

Figure 4. Example of PLS models for SSC on bunches (a) and TA (b) prediction and example of MLR models for SSC (c) and TA (d) prediction.

Table 3. Percentage of correctly and misclassified grape samples according to ripeness thresholds for the analysed parameters (17 °Brix for SSC and 7 g dm^{-3} for TA). Total PPV (Positive Predictive Value) indicates the total percentage of correctly classified samples.

Commercial device							
		SSC on berry(°Brix)		**SSC on bunch (°Brix)**		**TA (g dm^{-3})**	
		Measured					
	(%)	**Ripe**	**Unripe**	**Ripe**	**Unripe**	**Ripe**	**Unripe**
Predicted	**Ripe**	77.1	22.9	81.8	18.2	95	5
	Unripe	13.9	86.1	8.2	91.8	10.7	89.3
	Total PPV	84.6		89.5		90.5	

Simplified device							
		SSC on berry(°Brix)		**SSC on bunch (°Brix)**		**TA (g dm^{-3})**	
		Measured					
	(%)	**Ripe**	**Unripe**	**Ripe**	**Unripe**	**Ripe**	**Unripe**
Predicted	**Ripe**	75.0	25.0	66.7	33.3	93.3	6.7
	Unripe	20.6	79.4	22.1	77.9	17.6	82.4
	Total PPV	79.0		77.5		84.3	

Finally, a comparison between classification performances of the two systems was carried out. In Table 3 percentage of correctly and misclassified grape samples according to ripeness thresholds for the analysed parameters (17 °Brix for SSC and 7 g dm^{-3} for TA) are shown.

Regarding the vis/NIR commercial device, total PPVs of 84.6%, 89.5% and 90.5% were obtained for SSC on berry, SSC on bunch and TA, respectively. The best classification model was calculated for TA with a percentage of correctly classified ripe samples equal to 95.0%. Similarly, for the simplified system, the best classification model was obtained for TA (correctly classified ripe samples equal to 93.3%). Overall PPV results deriving from the simplified system showed a slightly low classification capabilities (SSC on berry = 79.0%, SSC on bunch = 77.5%, TA = 84.3%) compared to the commercial device. The classification performances for SSC on berry, SSC on bunch and TA decreased of 5.6, 12.0 and 6.2 percentage points, respectively.

For both optical instrumentations, the amount of the unripe samples misclassified as ripe for SSC parameters is higher than for TA index.

Classification performances related to the two optical instrumentations reflect the trend of the quantitative PLS models confirming the small loss of information using only four wavelengths and, consequently, the applicability the of the simplified LED system.

4 Conclusions

In this work a prototype of a simplified optical vis/NIR device based on LED technology was tested for a rapid estimation of the ripening parameters of white grape for *Franciacorta* wine directly in field. Spectral acquisition based on reflectance at four wavelengths (630, 690, 750 and 850 nm) was proposed. Optical measurements were carried out also with a portable commercial vis/NIR spectrophotometer, as reference instrument for performance comparison.

Correlations between the optical data matrix and ripening parameters were carried out using partial least square (PLS) regression for spectra and using multiple linear regression (MLR) for data from the simplified device.

The overall calibration and prediction results of the MLR models were satisfactory, although the performance of the MLR models was slightly worse than the PLS models for SSC prediction. Classification performances related to the two optical instrumentations reflect the trend of the quantitative PLS models confirming the small loss of information using only four wavelengths and consequently the applicability of the simplified LED system in order to monitor the grape ripening for *Franciacorta* wine.

The integration of a simple processing algorithms derived from the MLR models in the microcontroller software would visualize real time values of the predicted parameters. Farmers could employ it directly in field for a quick evaluation of grapes ripening levels.

Moreover, the design of the prototype of the simplified optical device was realized 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 (i.e. ripeness evaluation, chemicals and physical properties prediction or shelf life analysis) and for different food matrix.

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.

References

1. 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
2. Arana, I., Jaren, C., & Arazuri, S. (2005). Maturity, variety and origin determination in white grapes (Vitis Vinifera L.) using NIRS. Journal of Near Infrared Spectroscopy, 13(6), 349–357
3. B. Kemps, L. Leon, S. Best, J. De Baerdemaeker, B. De Ketelaere, Assessment of the quality parameters in grapes using VIS/NIR spectroscopy, Biosystems Engineering, Volume 105, 4, 2010, Pages 507-513
4. Beghi R., Giovenzana V., Civelli R., Malegori C., Buratti S., Guidetti R., 2014. Setting-up of a simplified handheld optical device for decay detection in fresh-cut Valerianella locusta L. Journal of Food Engineering 127: 10-15.
5. Beghi R., Giovenzana V., Spinardi A., Bodria L., Guidetti R. and Oberti R., 2013. Derivation of a blueberry ripeness index with a view to a low-cost, handheld optical sensing device for supporting harvest decisions. Transactions of the ASABE, 56(4): 1551-1559.
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
7. Bertrand, D. 2000. Spectroscopie de l'eau. In La Spectroscopie Infrarouge et ses Applications Analytiques, Bertrand, D., Dufour, E., Eds., TEC and DOC: Paris, France, 93-105.
8. 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

9. 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

10. 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

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

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

13. Di, C.Z., Crainiceanu, C.M., Caffo, B.S., Punjabi, N.M., 2009. Multilevel functional principal component analysis. Annals of Applied Statistics 3: 458-88.

14. Fearn, T., 2002. Assessing calibrations: SEP, RPD, RER and R2. NIR News 13, 12–14.

15. Fernández-Novales, J., M.I. López, M.T. Sánchez, J. Morales, and V. González-Caballero, 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.

16. Giovenzana V., (2013). Application of vis/NIR spectroscopy for ripeness evaluation and postharvest quality analysis of agro-food products. PhD thesis in “Technical Innovation for Agricultural, Food an Environmental Sciences”, Università degli Studi di Milano, Chapter 9, pp. 235-259.

17. Giovenzana V., Beghi R., Malegori C., Civelli R., Guidetti R., (2013). Wavelength selection with a view to a simplified handheld optical system to estimate grape ripeness. Am. J. Enol. Vitic. 65(1): 117-123.

18. Golic, M., Walsh, K.B., Lawson, P., 2003. Short-wavelength near-infrared spectra of sucrose, glucose, and fructose with respect to sugar concentration and temperature. Appl. Spectrosc. 57, 139–145.

19. 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

20. 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

21. 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

22. Li, X., Y. He, C. Wu and D.W. Sun, 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.

23. 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

24. 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

25. 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

26. McGlone, V.A., R.B. Jordan, and P.J. Martinsen. 2002. Vis/NIR estimation at harvest of pre- 456 and post-storage quality indices for 'Royal Gala' apple. Postharvest Biology and 457 Technology, 25(2), 135-144.

27. Naes, T., and B.H. Mevik, 2001. Regression models with process variables and parallel blocks of raw material measurements. J. Chemometr. 15 413-426.

28. 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

29. 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

30. Wang, W. & Paliwal, J., 2007. Near-infrared spectroscopy and imaging in food quality and safety. Sens. & Instrumen. Food Qual. 1, 193-207

31. Williams P.C., Sobering D.C., 1996. How do we do it: A brief summary of the methods we use in developing near infrared calibrations A.M.C. Davies, P. Williams (Eds.), Near infrared spectroscopy: The future waves, NIR Publications, Chichester (1996), pp. 185–188.

32. Williams, P.C., 2001. Implementation of near-infrared technology. In: Williams, P., Norris, K.H. (Eds.), Near-Infrared Technology in the Agricultural and Food Industries. American Association of Cereal Chemist, St. Paul, Minnesota, USA, pp. 145–169.

33. Wold, S.; Sjöström, M. & Eriksson, L., (2001). PLS-regression: a basic tool of chemometrics. Chemom. Intell. Lab. Syst. 58, 109-130

34. Wu, D., Y. He, P. Nie, F. Cao, and Y. Bao. 2010. Hybrid variable selection in visible and near-infrared spectral analysis for non-invasive quality determination of grape juice, Analytica Chimica Acta, (1-2), 229-237.

35. 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

Part II

MULTISPECTRAL IMAGING

6

Daily freshness decay of minimally processed apples using vis/nir multispectral imaging: preliminary tests

DAILY FRESHNESS DECAY OF MINIMALLY PROCESSED APPLES USING VIS/NIR MULTISPECTRAL IMAGING: PRELIMINARY TESTS

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Abstract

In recent years, a substantial increase in the consumption of fresh-cut, or minimally processed, fruit and vegetables has been occurred. Changes in human life styles in fact often lead the consumers towards ease of use and healthy products. Monitoring the quality decay of fresh-cut products is necessary to control the freshness level during the entire production chain. Hence fresh-cut fruit and vegetable sector could be greatly helped by new analytical methods that are accurate, rapid and could be integrated into the production chain.

The objective of this study was to test vis/NIR multispectral imaging to assess the freshness decay throughout the day (12 hours) of fresh-cut apple slices.

Twelve *Cripps Pink* apples were bought at the same moment at a large retail supermarket, intending to replicate the same experiment every seven days for four weeks on three different apples. The variability of the samples was in this way increased thanks to the natural processes of senescence during the weeks. At every experiment replication, three samples were obtained by cutting a slice from an apple, transversally with respect to the stem-calyx axis. Only half slice portion was treated to simulate a ready-to-eat product, by dipping it for 5 minutes into an aqueous solution of 2% (w/v) L-ascorbic acid, while the other half was left unprocessed as a control portion. The apples were monitored every sixty minutes for twelve consecutive hours through the use of an 18-channel imaging system (430-970 nm, VideometerLab, by Videometer A/S, Denmark).

A color correction was performed on each acquisition through a normalization with respect to a multicolor reference acquired together with each sample, in order to take into account the stability of measuring conditions. Each image was segmented by using the k-means algorithm and the apple flesh, i.e. the only part of interest, was separated from the background, the peel and the fruit core. After these pretreatments, all the acquisitions of the day for each slice were put together in a single dataset, unfolded in a two dimensional matrix, and the Principal Component Analysis (PCA) was applied. False color scores images were finally recomposed in order to highlight significant differences developed in a single apple slice. The same unfolding and refolding procedure was followed to apply the k-means clustering analysis with 4 clusters.

The results are encouraging. For all the samples the analysis distinguished between the treated portion of the slice and the unprocessed one. Furthermore a clear time evolution along hours is shown by almost all the apple slices for both the processed and unprocessed portions. This preliminary study demonstrated the applicability of multispectral imaging as a rapid and non-destructive approach for monitoring the freshness decay throughout the hours of minimally processed apple slices.

Keywords: vis/NIR multispectral imaging, fresh-cut apples, freshness decay, optical device, non-destructive analysis, chemometrics.

1 Introduction

In recent decades, there has been a substantial increase in the consumption of fresh-cut or minimally processed fruit and vegetables. The international Fresh-cut Produce Association (IFPA) defines, in 1999, 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” [1]. The growth in the ready to use vegetable industry is due to: (i) their ease of use, in fact changes in human life styles have led consumers to move towards ready-to-eat products and (ii) nutritional properties indeed it is known as source of vitamins, minerals, fiber and antioxidants [2].

The technological treatments extend the shelf-life of the most processed foods. Instead, ready-to-eat products are characterized by a shelf-life shorter than that of the original unprocessed raw material [3]. In fact the sequence of operations necessary to produce a fresh-cut product (i.e. washing, trimming, peeling and/or cutting) promotes the biochemical and microbial instability of the product itself. These foods are often subjected to rapid loss of color, organic acids, vitamins and other compounds that determine flavor and nutritional value.

Monitoring the quality decay of fresh-cut products is necessary to control the freshness level during the entire production chain. Hence fresh-cut fruit and vegetable sector could be greatly helped by new analytical methods that are accurate, rapid and could be integrated into the production chain [4]. The applicability of optical methods, i.e. vis/NIR and NIR spectroscopy, in agro-food sector is widely be proven. Hyperspectral and multispectral imaging system represent now new possibilities to be adopting as the starting point for the development of simplified system.

The research presented in this manuscript represents a preliminary study on minimally-processed *Cripps Pink* apples. The objective was to test vis/NIR multispectral imaging to assess the freshness decay throughout the day (12 hours) of fresh-cut apple slices, with a view to with a view to a simplified optical system for freshness decay assessment

2 Materials and methods

A package box with twelve fresh *Cripps Pink* apples was bought at a large retail supermarket at the first experimental day. The apples were considered to be at that moment a homogeneous set of samples with regard to their history, in the sense that the cultivation practices, the harvest period and the storage conditions were assumed to have been similar. This original a priori hypothesis about the apples history is a reasonable assumption, considering that they all came from the same producer and were packed all together into the same box. Nowadays, in fact, the fruit producers typically adopt industrial approaches and are used to manage batches of products according to standardized procedures. Furthermore, regarding post-harvest techniques, Beghi et al. in [5] demonstrated that the quality parameters of apples are maintained during long-term storage in controlled atmosphere warehouses, meaning that fresh apples features likely remain stable along all the production chain, till the refrigerator bench at the supermarket.

2.1 The sampling

Four replications of the same experiment were carried out on three different apples every seven days, along three consecutive weeks starting from the purchase date, until the fruits lasted.

During this 21-day period, the apples were stored at room temperature of 20° C under natural atmosphere, in order to promote the natural processes of senescence. The variability of the samples was in this way increased by introducing different starting point conditions in terms of the product freshness.

At each measuring day, three samples, i.e. three minimally-processed apple slices, were prepared according to the following procedure.

- Three apples were arbitrarily chosen from the package box.
- A sample was obtained from each apple by cutting a 18 mm thick slice from the middle part of the fruit, transversally with respect to the stem-calyx axis. The slices were cut by using an automatic slicer in order to get parallel-faces slices and standardize the operation.
- For each sample, only half slice portion was treated to simulate a ready-to-eat product, by dipping it for 5 minutes into an aqueous solution of 2% (w/v) L-ascorbic acid, while the other half was left unprocessed as a control portion [6, 7 and 8] (see the RGB image on the left part of Figure 1).
- As removed from the dipping, the slices were first drained for three minutes, any excess liquid was then gently absorbed with paper towels, and a final drying air flow at room temperature (20° C) was applied for other three minutes. After that, the samples were ready

to be measured and the experiment began, approximately 15 minutes after the cutting operation.

The experiment consisted in monitoring the apple slices by taking a picture with a multispectral imaging system every sixty minutes for twelve consecutive hours. At each measuring day, 36 multispectral images were therefore acquired on the three slices, for a final dataset of 144 multispectral images after the last experimental replication.

2.2 The multispectral imaging system

The multispectral camera used is the commercial imaging system VideometerLab, by Videometer A/S (Denmark), that acquires multispectral images in up to 18 different wavelengths ranging from 430 to 970 nm, i.e. 430, 450, 470, 505, 565, 590, 630,645, 660, 700, 850, 870, 890, 910, 920, 940, 950, 970 nm.

The VideometerLab system is characterized by a standardized easy-to-use acquisition setup, realized thanks to the interposition of an integrating sphere with a diameter of 420 mm between the camera and the sample, for light diffusion purposes. The camera is located on the top of the sphere looking downwards through it, while the sample has to be placed at the bottom inside the sphere, resulting exactly aligned under the camera focus. When performing an acquisition, a sequence of radiant powers at the 18 different wavelengths is obtained by strobing successively among the high power LED light sources, located into the sphere. At the same time, the monochrome high resolution CCD camera takes an image of the sample at each wavelength. In this way, an acquisition finally results in a multispectral image of dimensionality $W \times H \times \lambda = 1280 \times 960 \times 18$ (where W and H refer to the spatial width and height, and λ to the spectral wavelength).

This system ensures reproducibility of acquired images with regard to the spatial focus and the spectral calibration. The diffuse illumination conditions into the sphere are in fact standardized. Furthermore, the spectral calibration is automatically optimized for each individual channel with simple adjustments of the light intensity for each wavelength band, resulting in an improved signal-to-noise ratio.

2.3 Data Processing

The data processing was carried out using the MATLAB software, in the R2014a release equipped with the Image Processing Toolbox and the Statistics Toolbox (The Mathworks Inc., Natick, USA). All the software developed is freely available upon request.

2.2.1 Regions of interest (ROI) and removal of undesired pixels

At a first sight, at least four different regions are visible in an image, corresponding to four objects (see the RGB picture in Figure 1): the sample (i.e. the apple slice), a multicolored reference (the use of which will be discussed later), the background and the integrating sphere borders. Furthermore, with regard to the sample, only the apple flesh represent the real region of interest (ROI), which is influenced by the freshness decay of the product.

In addition to all this, the possible presence of problematic pixels must be considered. These can be dead pixels due to malfunction of the camera CCD sensor, or saturated pixels that could be caused by any specular reflections related to wet points in the field of view.

All the pixels in an image that refer to areas that have nothing to do with the investigated phenomena, including the peel and the fruit core areas, can significantly affect the results and must therefore be removed [9].

This task, commonly known as a problem of image segmentation, was realized starting from the RGB images, by defining a series of masks to identify the different regions. A mask related to a certain object is intended to be a logical index matrix of the same size of a single-wavelength image, containing a logical 1 (true) where the object is present, and a logical 0 (false) otherwise. These masks could then be applied successively up to identify the apple flesh data into each of the multispectral cube images, this data reshaped in 2-dimensional matrices, and chemometric processing could be finally carried out.

Specific algorithms were developed to automatically segment all the acquired images.

First of all, considering an acquisition, any possible presence of dead or saturated pixels in each wavelength image was taken into account by isolating them with a first sparse mask.

The integrating sphere borders and the multicolored reference were then simply masked using built-in MATLAB functionalities, since these regions have regular geometric shapes (circular and rectangular shapes) and since they always have the same fixed position for all the acquisitions.

Ones the sphere bordered and the color reference were removed, it was possible to separate the apple flesh from the background and the peel by applying the k-means algorithm on the RGB image data with a segmentation with 2 clusters. Apple flesh in fact is very different from both the background and the peel in terms of color, and this difference is clearly identified by the k-means algorithm.

Finally, for each acquisition a circular disk with a fixed diameter was placed in the center of the slice in order to remove the fruit core area from the apple flesh. This wanted to simulate the action of the automatic machine that mechanically removes the core when producing ready-to-eat apples.

The final result of the masking procedures is shown in Figure 1, on the right.

Figure 1. RGB picture of an apple slice (on the left) with labels indicating the L-ascorbic acid treatment. Furthermore, all the visible regions are visible: the apple slice (composed of the flesh, the core and the peel), the multicolored reference, the background and the integrating sphere borders. Final identification of the region of interest (on the right), after the masking procedures.

2.2.2 Stability of measuring conditions and color correction

It is known that the lighting conditions are a critical aspect in multispectral imaging [10]. In general, there is a need to assure that the changes in the images do come from the samples modifications caused by the investigated phenomena, and not from any changes in the illumination, in the instrument response or, in general, in the measuring conditions.

As explained above, the used camera system intends to reduce the negative effects related to these issues, by adopting an integrating sphere and giving a preset acquisition setup. To further take into accounts this aspects, at each acquisition (i) a dark zero-light image was taken, and (ii) a home-made color standard reference was inserted into the camera field of view and acquired together with the sample.

First of all, each acquisition was referred to its zero level, by subtracting the corresponding dark image to every of the 18 wavelengths images. This wanted to reduce any possible *bias* due to the environmental noise, i.e. any light draughts entering the integrating sphere, and the electronic noise of the instrument.

A color correction was then applied to each acquisition, as described in detail in [11 and 12]. This is based on the home-made standard realized with 8 colored plastic patches: gray, dark, yellow, red, orange, green, blue and white. The scale of colors was expressly defined to be varied, in order to possibly have a broad spectral response. In practice, the correction procedure consists of two steps. In the first step, only the color standards data images (identified with the dedicated masks) are considered. Starting from one of these taken as reference, an intensity linear transformation is determined for each acquisition, in order to make the correspondent color standard as similar as possible to the reference one. The linear operator is calculated through a least squares minimization of the residuals, and in general it is specific for each acquisition. In the second step, the determined corrective transformations are applied to the flesh data images, so that they can be compered

2.2.3 Principal Component Analysis

The PCA (Principal Component Analysis) is one of the most useful and widespread exploratory techniques within the framework of Food Science. Its ability to extract the main sources of variability and qualitatively study distribution of elements on an image makes PCA a versatile and essential tool before applying any other multivariate model. The adaptation of the bilinear PCA model to multi- and hyper-cubes comes with a previous step of unfolding the 3-dimension matrix into a 2-dimiension matrix. This procedure is widely explained in [11 , 13 and 14].

In order to investigate all the acquisitions at the same time, all the multispectral images were put together in a single dataset, data auto-scaled, and a single PCA model was built. Due to computational issues, this was possible only after a reduction to 50% of the spatial sizes of the images, by applying a bicubic interpolation algorithm together with an anti-aliasing filtering, to limit the impact of aliasing on the reduced images. After the application of PCA and the determination of the scores, the final step is the re-folding of the scores to obtain the so-called score images. The score surfaces explain the variability of the pixels, making PCA an ideal technique for exploratory analysis with no need for any other external information.

2.2.4 Cluster analysis

The main feature of clustering methods is that they classify objects (in vis/NIR multispectral images, the objects are the pixels) into groups without involving a previous learning set [15].

The k-means algorithm assigns each pixel of the image to the k_{th} cluster, whose center is nearest, by minimizing the sum of the squared distances of each pixel to its corresponding center [14]. The authors explain how to apply this technique to multispectral image dataset. The main advantage of this algorithm is the simplicity that allows it to run with larger datasets. Nevertheless, the main drawbacks of the algorithm are: there is a risk of converging to a local minimum in the iterations; and, it forces to each pixel to belong exclusively to one cluster.

Following the same procedure about the unfolding the 3-dimension image matrix into a 2-dimiension matrix, the k-means algorithm was applied with the arbitrary initialization to 4 clusters. The classified data were re-folded for visual purpose, resulting in a false color image with clusters highlighted.

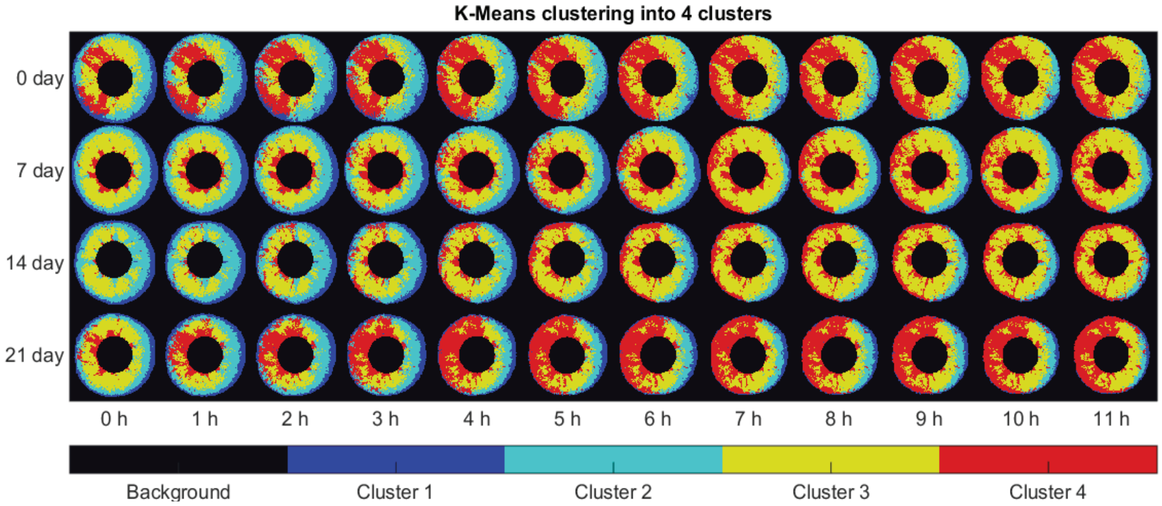

Figure 2. Cluster analysis with k-means algorithm using 4 clusters. In the column-wise order are presented 4 slices coming from 4 different measuring days. The row-wise order shows the evolution of these sample along the hours of the day.

3 Results and discussion

The score surfaces resulting from the PCA highlighted the variability of the pixels in a qualitative way. An evolution of the sample in the hours was shown in particular by PC1 score surface, explaining the 63.83% of the variance (results not shown). Similar considerations apply for PC2, which explained the 28.45% of the variance. In this case, a clear difference between the two halves of the samples was highlighted, with reference to the treated and no-treated portions.

The results of clustering analysis are shown for 4 apple slices in Figure 2. The samples refer to 4 different days of measurement, i.e. 4 different dates from the apple purchase, as it is visible in the vertical axis labels. The evolution of these samples along the hours of the day is shown in the row-wise order (horizontal labels).

The evolution along the hours is sharply shown for all the slices. The cold colors clusters (blue and light blue) can be associated with a good levels of freshness of the products, while hot color clusters (yellow and red) refer to an increased degree of apples senescence, probably related with the fluid loss. The figure show how cold colors are progressively replaced by the hot colors, meaning that the freshness degree is decreasing.

Furthermore, it is well shown the difference between the right half of the sample which were treated with the L-ascorbic acid, and the no-treated left part. The effect of the treatment consists in slowing down the process of browning of the samples, at least from an optical point of view. This is an important aspect for the opinion of the consumer.

The evolution of the samples along the days is not so clear. Probably this is related to the both the facts that (i) apples are stable fruits, and that (ii) the samples shown in different days are obviously different, meaning that issues about the individuality of the sample are to be considered. In any case, older-day samples seems to evolve more quickly during the hours one the slice is cut, and the peel protective effect removed.

The percentages of the cluster extension with respect to the total surface of each slice are shown in Figure 3. The qualitative evolution trends described above are now quantified, appear sill more clearly. The behavior of the lines are the same for all the clusters and for all the slices. Furthermore, it is possible to notice how the hot clusters, i.e. the freshness degradation, effectively extend with a higher rate for older-day samples, as it is shown by the slope of the curves.

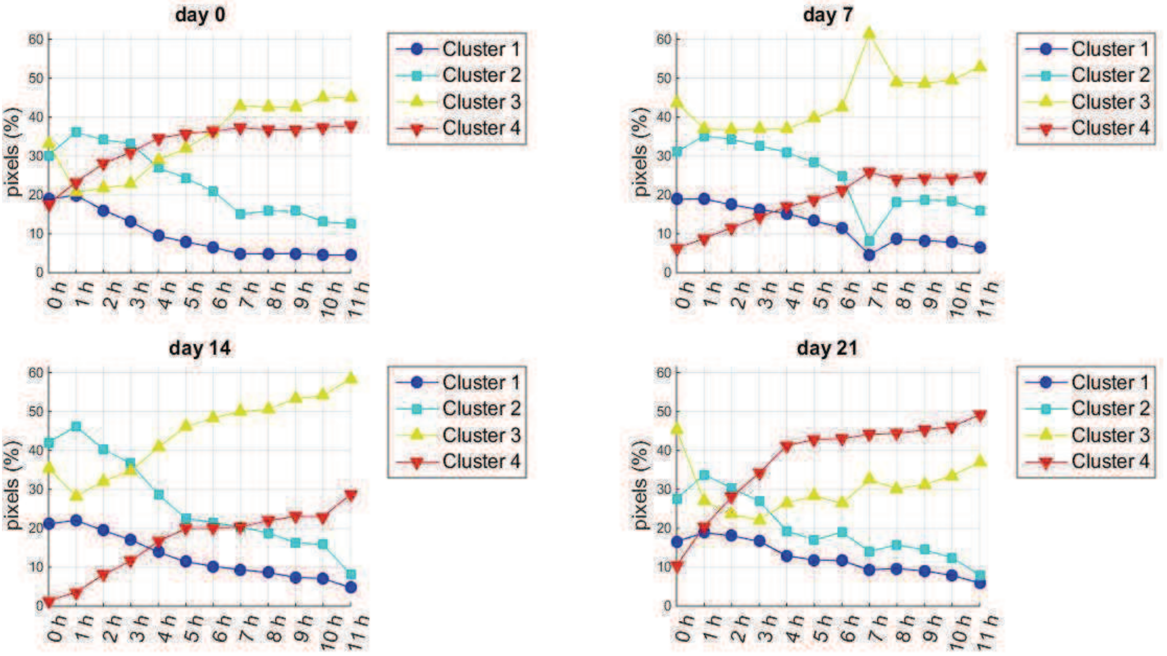

Figure 3. Relative extensions of the clusters and their evolution along the hours for each of the 4 slices shown in Figure 2.

4 Conclusions

This preliminary study demonstrated the applicability of multispectral imaging as a rapid and non-destructive approach for monitoring the freshness decay throughout the hours of minimally processed apple slices.

In particular, k-means cluster analysis seems to be an effective technique to evaluate the freshness decay along the time of ready-to-eat apples. In association with multispectral low-cost imaging, this processing analysis could be the starting point for the development of a simplified optical system for the support of fresh-cut sector.

Further research is necessary, first of all to increase the number of samples. The study should them be applied to industrial minimally processed apple slices and the presence of plastic package considered, in order to test this technology directly in every step of the production chain, until refrigerator bench at the supermarket

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References

[1] IFPA (International Fresh-cut Produce Association), PMA (The Produce Marketing Association), Handling guidelines for the fresh-cut produce industry, third ed., Alexandria, (1999).

[2] A.A. Kader, *Flavor quality of fruits and vegetables*, J. Sci. Food Agric. 88, 1863 (2008).

[3] M.E. Guerzoni, A. Gianotti, M.R. Corbo, M. Sinigaglia,. Shelf life modelling for fresh-cut vegetables. Postharvest Biol. Technol. 9, 195–207 (1996)

[4] R. Beghi, V. Giovenzana, R. Civelli, C. Malegori, S. Buratti, R. Guidetti, *Setting-up of a simplified handheld optical device for decay detection in freshcut Valerianella locusta L*, Journal of Food Engineering, 127, 10 (2013).

[5] R. Beghi, G. Giovanelli, C. Malegori, V. Giovenzana, R. Guidetti, *Testing of a VIS-NIR System for the Monitoring of Long-Term Apple Storage*, Food Bioprocess Technol, 7, 2134-2143 (2014)

[6] Y. Luo, G.B. Barbosa-Cánovas, *Preservation of apple slices using ascorbic acid and 4-hexylresorcinol*, Food Scince and Technology International, 2, 315 (1996).

[7] M.I. Gil, J.R. Gorny, A.A. Kader, *Responses of 'Fuji' Apple Slices to Ascorbic Acid Treatments and Low-oxygen Atmospheres*, HortScience, 33, 305 (1998).

[8] R.C. Soliva-Fortuny, G. Oms-Oliu, O. Martín-Belloso, *Effects of Ripeness Stages on the Storage Atmosphere, Color, and Textural Properties of Minimally Processed Apple Slices*, Journal of Food Science, 67, 5, 1958-1963 (2002)

[9] M. Vidal, J.M. Amigo, *Pre-processing of hyperspectral images. Essential steps before image analysis*, Chemometrics and Intelligent Laboratory Systems, 117, 138–148, (2012)

[10] J.M. Amigo, *Practical issues of hyperspectral imaging analysis of solid dosage forms*, Anal Bioanal Chem, 398, 1, 93-109, (2010)

[11] M. Vidal, J.M. Amigo, R. Bro, M. Ostra, C. Ubide, J. Zuriarrain, *Flatbed scanners as a source of imaging. Brightness assessment and additives determination in a nickel electroplating bath*, Anal. Chim. Acta, 694, 38 (2011).

[12] G.Y. Tian, D. Gledhill, D. Taylor, D. Clarke, Proceedings of the Sixth International Conference on Information Visualization IV'02, 483–488, (2002), doi:10.1109/IV.2002.1028817

[13] J.M. Amigo, I. Martí, A. Gowen, *Hyperspectral Imaging and Chemometrics: A Perfect Combination for the Analysis of Food Structure*, Composition and Quality. In Federico Marini, editor: Data Handling in Science and Technology, 28, 343-370 (2013)

[14] J.M. Amigo, J. Cruz, M. Bautista, S. Maspoch, J. Coello, M. Blanco, *Study of pharmaceutical samples by NIR chemical-image and multivariate analysis*, Trends in Analytical Chemistry, 27, 8, 696-713 (2008)

[15] L. Zhang, M.J. Henson, S.S. Sekulic, Anal. Chim. Acta, 545, 262 (2005)

Conclusions and future works

The study of non-destructive methods to support the agro-food sector with a comprehensive overview of ripening and quality parameters is an ongoing process. The applicability of NIRs spectroscopy to measure quality attributes of fruit and vegetables has been shown for many products. In the same way, computer vision employing image processing techniques has been quickly developed, in order to characterize complex properties of foods like size, shape, color and texture. Hyperspectral and multispectral imaging combining spectroscopy and traditional imaging have recently become interesting techniques to be studied.

However, the non-destructive technologies are currently used only by research institutions or big companies, due to their cost and complexity of use. Therefore, the sector is interested in new simplified systems for rapid analysis that can be performed along the chain and directly at point of sale, with a double objective: to use the information from sensors to better manage the product, and to preserve the consumers' expectations providing additional selection criteria.

Starting from this considerations, in this thesis (a) a compact-sized LED technology based device, for the prediction of the main qualitative/ripening parameters of the case studies was realized and tested, and (b) the applicability of multispectral imaging was studied with a view to a simplified optical system for freshness decay assessment.

The development stage of the developed simplified has reached an operative prototype level. An evolution and an engineering of the instrument is still desirable in order to obtain a handheld, user friendly, non-destructive and inexpensive device for farmers utilization in the field. Particular attention must be paid to the modularity concept (i.e. the possibility to adjust light sources with a specific choice of wavelengths for LEDs). This makes it possible to conceive the same simplified optical device for many different applications (i.e. ripeness evaluation, chemicals and physical properties prediction or shelf life analysis) and for different food matrix. Furthermore, an optimization in the calibration for each individual measuring channel is the other significant, since it ensures the reduction of signal to noise ratio for all the measured data.

The multispectral imaging study still is at a preliminary level, but sufficient to demonstrate the applicability of this principle as a rapid and non-destructive approach to develop a simplified low-cost optical system for freshness decay assessment. Further work in necessary to develop the research based on this.

All the proposed methodologies can be replicated and can be the starting point for new research lines regarding innovative technologies for the agro-food sector. Other optical and nondestructive techniques are to be investigated. For example, in parallel with the studies presented, a research not reported in this publication wants to investigate laser light backscattering imaging as an innovative technique to monitor freshness decay of ready-to-eat products. The results obtained are consistent with what achieved with the multispectral imaging, indicating also this technique as a useful technology to design a simplified laser-based system to support sector.

In conclusion, this thesis demonstrates how a real simplification of traditional optical techniques is possible, and how the universities and the research institutions can make research in order to approach the industrial world and give an answer to its problems. Optical techniques are today mature technologies, but still a lot of research is to be carry out in order to develop innovative optical simplified devices dedicated to fruit and vegetable products chains. The future challenge is how to combine cheap and robust sensors together with advanced data analysis to provide an immediate response of quality.

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Raffaele Civelli