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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].

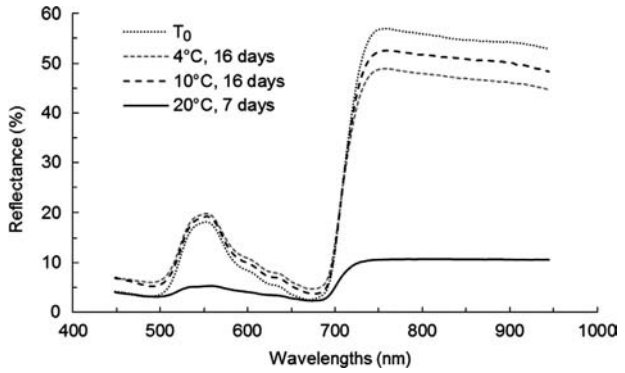

Fig. 1. Average VIS–NIR spectra of *Valerianella* leaves at T_0 and at the end of the shelf life period for each temperature (after 16 days for 4 and 10 °C and after 7 days for 20 °C).

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

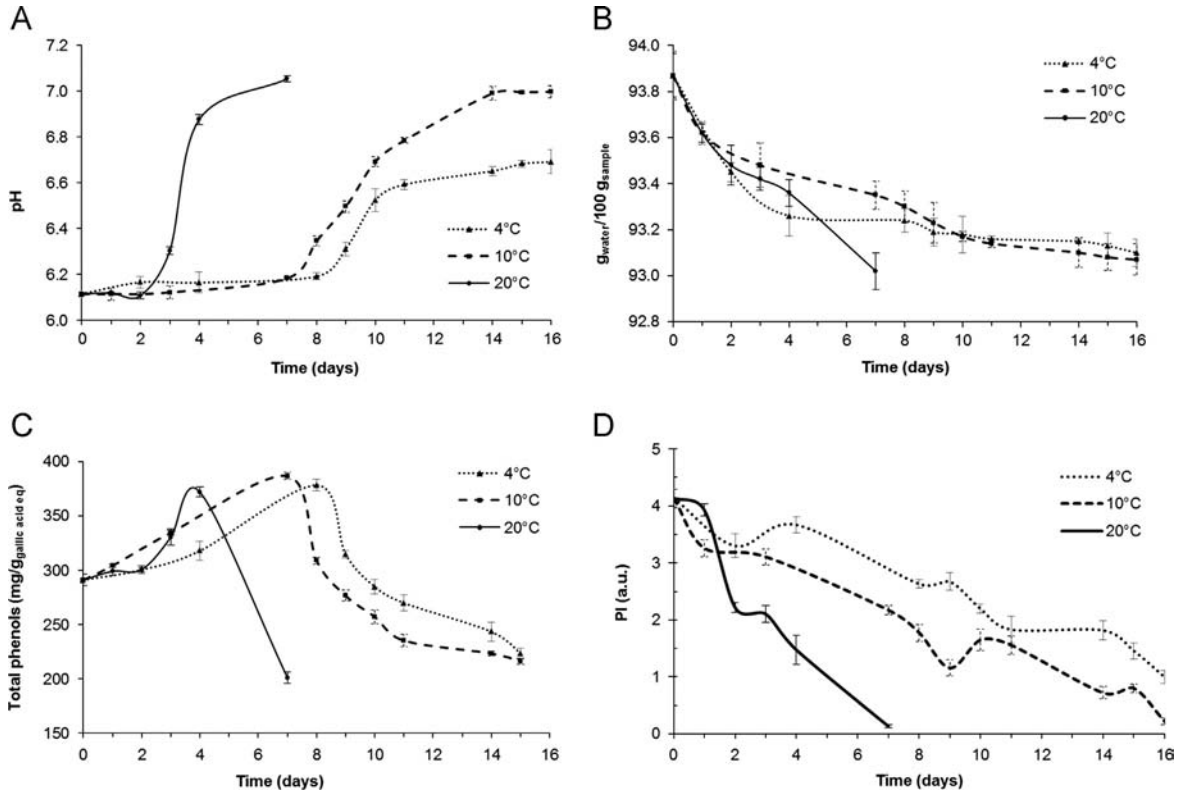

Fig. 2. Evolutions of chemical parameters (A=pH; B=water content; C=total phenols; D=Performance Index, PI), for each sampling date at 4 °C, 10 °C and 20 °C. Bars indicate the standard error within each sampling date (n=3).

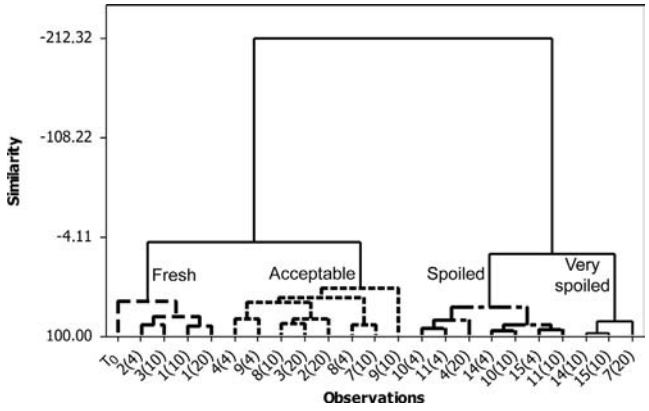

Fig. 3. Dendrogram deriving from elaboration by CA in order to categorize *Valerianella* samples according to their freshness and quality. Different line style was used to identify the clusters.

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].

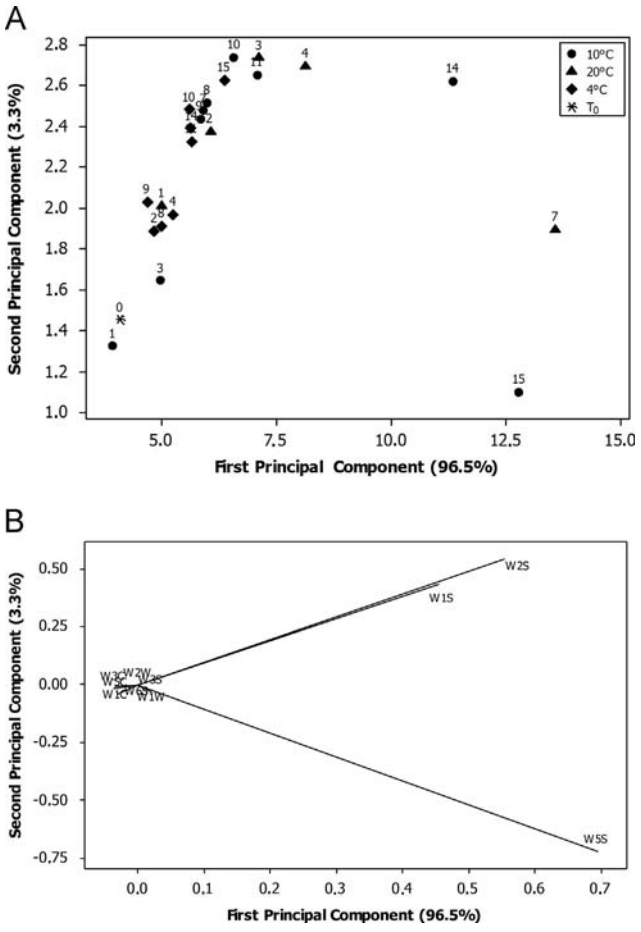

Fig. 4. PCA scores plot (A) and loadings plot (B) deriving from e-nose data.

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.

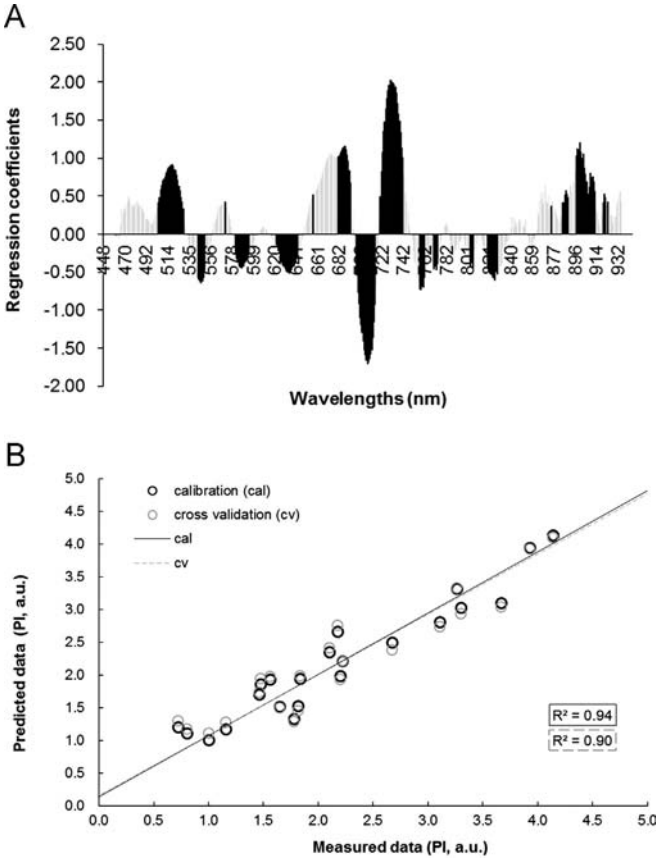

Fig. 5. Loadings plot with highlighted the selected variables by Martens' Uncertainty Test (A) and graph of PLS model for PI prediction (B).

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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