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Hyperspectral imaging and multivariate accelerated shelf life testing (MASLT) approach for determining shelf life of rocket leaves

Muahmmad M.A. Chaudhry[a], Maria L. Amodio[a,*], Farahmand Babellahi[a], Maria L.V. de Chiara[a], José M. Amigo Rubio[b], Giancarlo Colelli[a]

[a] Dip.to di Scienze Agrarie, degli Alimenti e dell'Ambiente, Università di Foggia, Via Napoli 25, 71122 Foggia Italy
[b] Dept. of Food Science (KU-FOOD), Chemometrics and Analytical Technologies CAT. Faculty of Sciences, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

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ABSTRACT

The feasibility of using spectral profiles for the estimation of the shelf life of the rocket leaves was evaluated using a multivariate accelerated shelf life testing (MASLT) approach. Spectral changes over time were modeled by using principal component analysis (PCA) and as variation to the conventional method, partial least squares (PLS) method. Kinetic charts were built fitting the first principle component (PC1) and the first latent variable (LV1) scores versus time. In both cases, the kinetics were described by a first order reaction, obtaining R^2 values of 0.73, 0.94 and 0.95 for samples stored at 5, 10 and 15 °C, respectively. The spectra of samples judged unacceptable were used for the calculation of the cut-off value, estimated to be 3.955, leading to shelf life estimations of 9.8, 4.3 and 3.1 days for PCA based MASLT at the three temperatures, respectively. For PLS based MASLT the shelf life was 9.4, 4.5 and 3.3 days for samples stored at the three respective temperatures. Conclusively, shelf-life was correctly estimated by conventional MASLT using PCA and also with the newly proposed technique using PLS.

1. Introduction

A significant increase has been observed in the consumption of minimally processed ready-to-eat foods in the last decades (Artés et al., 2009). This rapid rise in the consumption is a result of consumer preference for healthy, fresh, convenient, highly nutritive and appetizing food products (Ma et al., 2017; Oliveira et al., 2015).

Rocket leaves (*Diplotaxis tenuifolia*) are popular leafy vegetables especially in the Mediterranean countries, mostly preferred by consumers because of their pungent smell and strong flavour. Moreover, they are a rich source of health-promoting phytonutrients such as flavonoids, fiber, vitamin C and glucosinolates (Martínez-Sánchez et al., 2006; Cavaiuolo and Ferrante, 2014; Nurzyńska-Wierdak, 2015; Amodio et al., 2016). Normally rocket leaves are sold in packages after minimal processing operations including washing and drying due to which they are also prone to rapid degradation. Particularly, yellowing caused by chlorophyll degradation, wilting, and the production of off-odors are the main source of deterioration (Koukounaras et al., 2006, 2007; 2009; Nielsen et al., 2008). The shelf life of rocket leaves ranges between 7 and 14 days depending upon the raw material, handling, processing and especially the temperature of storage (Toivonen and Brummell, 2008).

At the market shelves, the consumer criteria for the selection of the leafy vegetables as rocket is the fresh appearance and green color (Løkke et al., 2012) and repurchase of the product depends on the quality at the consumption stage often evaluated by color, texture and flavor (Barrett et al., 2010). Freshness and green color are quick indicators of the fact that the product can sustain under prescribed conditions for a certain time.

The shelf life determination of any food product is usually conducted by monitoring the quality parameters most associated with time by developing kinetic models for deterioration under market and extreme conditions using accelerated shelf life testing methods (ASLT) (Labuza, 1982; Hough et al., 2006). In case of ASLT approach, the samples are subjected to severe storage conditions other than the market storage conditions and shelf life charts also known as kinetic charts are developed (Hough et al., 2006). Various studies have proved that ASLT approach is a useful tool for the rapid estimation of shelf life of fresh-cut produce, as apple (Amodio et al., 2015b), melons (Amodio et al., 2012) even with the use of other empirical models as the Weibull model used for kinetic fitting on fresh-cut melons (Amodio et al., 2013) and fresh rocket leaves (Amodio et al., 2015a).

* Corresponding author.
E-mail address: marialuisa.amodio@unifg.it (M.L. Amodio).

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Degradation process of food products and particularly of fresh-cut produce is a multivariate phenomenon depending from several pre-harvest handling and storage factors, impacting on many quality attributes (Routray and Orsat, 2014; Torres-Contreras et al., 2014; Fernando Reyes et al., 2007). In this regard, chemiometric tools such as principal component analysis (PCA) which operate by data dimensionality reduction, measurement correlation and noise compression (Bro and Smilde, 2014; Brereton, 2009) may be usefully integrated into ASLT to formulate a new procedure named Multivariate Shelf Life Testing (MASLT) aimed to include several quality attributes at the same time (Labuza, 1982; Pedro and Ferreira, 2006).

The first step in MASLT procedure involves the kinetic description of the important degradation reactions based on the PC scores resulting from a PCA model, assuming that degradation reactions are the main sources of variation in the data set, and that PCA will explain the time-related phenomena. Usually, these are calculated using the zero order, first order and second order kinetics (Odriozola-Serrano et al., 2009; Amodio et al., 2015b). Secondly, the temperature dependence of the rate constants is defined using the Arrhenius equation and the third step involves the calculation of shelf life. The MASLT approach has successfully been applied to various food products such as broccoli puree (Kebede et al., 2015), low-fat UHT milk (Richards et al., 2014), sunflower oil (Upadhyay and Mishra, 2015) and tomato paste (Pedro and Ferreira, 2006). This method was applied for the first time on fresh-cut produce by Derossi et al. (2016), who obtained an accurate description of the degradation phenomena occurring during the storage of fresh-cut lettuce at three different temperatures, monitoring several sensorial, physical and chemical changes over time. In the same way, MALST method was applied to estimate the shelf-life of fresh-cut pineapples (Amodio et al., 2016). Shelf-life estimation obtained with MALST method have been proved to be more reliable than ASLT, but generally, the application of these studies by processors is limited by the scarce possibility of carrying out specific quality analysis and collecting data. Therefore, many companies are looking for a possible alternative system for the evaluation of the quality and shelf-life in a faster, simple and eventually non-destructive way. In this regard, hyperspectral imaging is a fast, reliable, objective, economical and non-destructive means of data collection. This technique is a combination or integration of imaging and spectroscopic techniques for the quantitative prediction of physical and chemical characteristics of the food samples as well as their spatial distribution.

Every product has, in fact, a specific spectral signature, which is a function of the structure of the sample, the moisture content, the particle size, the temperature of the sample and most importantly of its chemical composition (Workman and Shenk, 2004). In case of the green leaves, Vis-NIR region retains all the information related to leaf pigments such as chlorophyll, anthocyanin and carotenoid content (Mishra et al., 2017), characterized by a strong absorption by these leafy pigments, particularly chlorophyll which are responsible for photosynthetic activity in plants (Feret et al., 2008). When spectral profiles are collected over time they can be used for the estimation of the quality changes and shelf life of the food products during storage (Gowen et al., 2008; Rajkumar et al., 2012; Løkke et al., 2013). Some applications include monitoring of the ripening of tomatoes (Polder and Heijden, 2010), or banana (Rajkumar et al., 2012), hence providing a promising opportunity for the collection of the information related to the quality of a product in the form of spectral responses as they retain most of the information related to the overall quality.

Standing to these considerations, spectral data and hyperspectral imaging may be usefully integrated into MASLT in shelf-life research studies. Therefore, the objective of the present work was to use the MASLT technique for the estimation of the shelf life of rocket leaves using the spectra as a quality attribute. In addition an alternative method based on the use of partial least squares regression (PLSR) and latent variables (LV) instead of PCA and PC scores was also proposed.

2. Materials and methods

2.1. Experimental design and spectral acquisition

Washed and dried rocket leaves (*Diplotaxis tenuifolia*) were received in the postharvest laboratory of University of Foggia, after being processed in a commercial company. Drying was conducted with a drying tunnel, heating the product at 30 °C for 5 min, and achieving about 95% of added water reduction. Upon arrival the rocket leaves were stored at 5 °C. Representative samples of 100 g were packed in plastic clamshells and stored at three different temperatures (5 °C, 10 °C and 15 °C) in a humidified (99% RH) flow of air. Ten replicates were prepared for each storage temperature. Samples were taken for image acquisition and sensory analysis at 0, 3, 6, 8 and 10 days of storage.

A hyperspectral line scan scanner (Version 1.4, DV srl, Padova, Italy) equipped with a spectrograph, in the visible-near infrared (Vis-NIR) range of 400–1000 nm with a spatial resolution of 1000x2000 pixels and a spectral resolution of 5 nm was used to acquire the images. Twenty leaves were taken for each replicate in a single image and self-developed MATLAB (2012b, version 8.0.0.783) code was used for extracting the mean spectra of these leaves producing one spectrum per replicate. For the extraction of the mean spectrum, the original image was thresholded and the best contrast between the object and the background was found. Image thresholding was performed using the Otsu method, on the image depicting the best contrast between the foreground and background, corresponding to 795 nm for the Vis-NIR and 1495 nm for the NIR. A 2D binary image (mask) was obtained, with 0 value for the background and 1 for the leaves. This mask was imposed to extract the mean spectra of the pixels corresponding to the leaves. A total of 150 spectra were acquired, 50 from each storage temperature

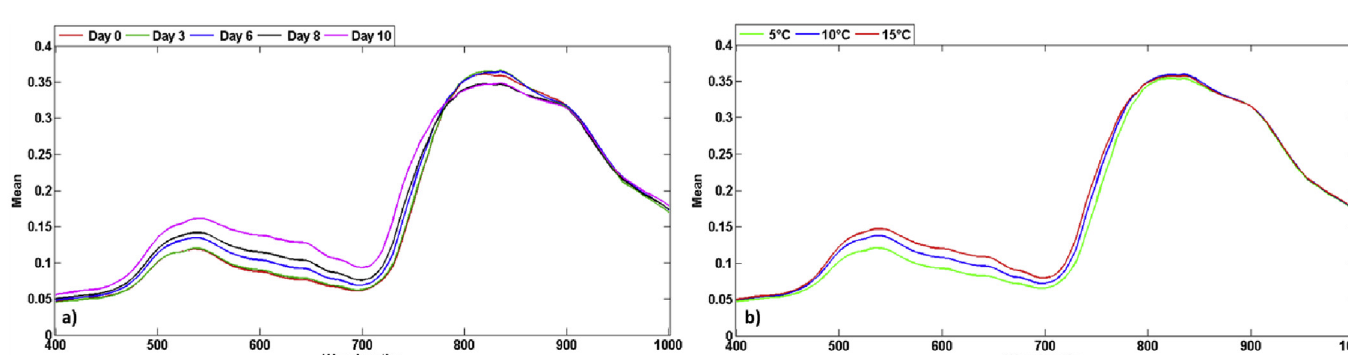

Fig. 1. a) Mean spectra based on days of storage Day0 (red), Day3 (green), Day6 (blue), Day8 (black), Day10 (Violet); b) Mean spectra based on temperatures of storage 5 °C (green), 10 °C (blue), 15 °C (red). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

during 5 different days of acquisition (Fig. 1). Fig. 1a shows the mean spectra based on days of storage and 1b demonstrates the changes in the mean spectra based on the storage temperature plotted in PLS toolbox (version 7.5.2) supported by MATLAB.

In these figure, the characteristic Vis-NIR reflectance spectra of the rocket leaves resulting from the leaf biochemical compounds such as chlorophyll, anthocyanins, carotenoids, water and cellulose in the wavelength range of 400–1000 nm can be observed. In case of green leafy vegetables, the interaction between the plant leaves and electromagnetic radiation yields reflectance spectra in the Vis-NIR region which are mainly representative of the photosynthetic pigments such as chlorophyll and carotenoids (Mohd Asaari et al., 2018). Visually, a green plant spectral curve can be observed in the raw spectra with 550 nm reflectance peak and 680 nm absorbance peak caused by chlorophyll, a major color related pigment (Kong et al., 2016). The sharp rise in the spectra from 700 nm corresponds to the red edge resulting from two special optical properties of plant tissue, the high internal leaf scattering resulting in large NIR reflectance and low red reflectance as a result of chlorophyll absorption (Horler et al., 1983).

2.2. Sensory analysis

Rocket leaves were evaluated by a panel of experts for changes in appearance, including freshness, color and dehydration, during the storage period. Since appearance is the most important quality attribute for the selection of the product at the retail sites for the consumers, the appearance scores were given a scale from 0 to 5 and these sensory evaluations were carried out on each acquisition day. In case of appearance scores, rocket leaves with uniform dark green color with fresh and turgid appearance were given score 5, fresh rocket leaves with a slight loss of turgidity obtained an appearance score of 4, rocket leaves with a significant loss of turgidity and an apparent loss of color (limit of marketability) were set at an appearance score of 3, leaves with significant senescence with the passage of storage time having wrinkled and yellowish blades received an appearance score of 2 and the spoiled rocket leaves with severe wilting, significant yellowing and decay symptoms were given a score value of 1 (Amodio et al., 2015b).

2.3. Multivariate accelerated shelf life testing (MALST) approach

An elaboration of the MASLT algorithm used in this study is presented, and for the sake of convenience, conventional algebraic symbology is followed, where matrices will be represented by boldface upper case letters, vectors with bold face lower case letters, scalar quantities with italic lower case, italic subscripts denote case letters and sequences.

In the first step, a matrix **X (mxn)** where **m** = 150 and **n** = 121, was formulated representing the quality changes in the rocket attributes at three different storage temperatures (5, 10 and 15 °C) with **m** being the number of data points collected during storage on Days 0, 3, 6, 8 and 10 respectively with 10 replicates from each storage temperature and **n** being the vector of variables or spectra in a wavelength range of 400–1000 nm (Fig. 2). Auto-scaling the data is a necessary consideration when various univariate quality attributes with different scales are simultaneously under study (Pedro and Ferreira, 2006; Derossi et al., 2016) and in case where spectral profiles are serving as property attributes, normalization of the data is important for which mean centering was done.

Secondly, PCA was performed on the data matrix **X** after data mean centering (Fig. 3). PCA describes the data by projecting it on a new set of axis in the multivariate space. Principal components are linear combinations of the original variables and each one accounts for the direction of maximum variability in the data (Bro and Smilde, 2014). The loading plots of the time related PCs were observed for the selection of the best variables and the elimination of those who did not contribute to any information in the PCA model. For each storage temperature, the scores matrix (**S**) of every *i*th time related PC were plotted against the storage time to formulate kinetic plots also known as shelf life charts, describing the changes of the PC scores as a function of time. For these time related PCs, reaction order and multivariate kinetic parameters were determined. After the estimation of the multivariate constants, the Arrhenius equation (Labuza, 1982) was used for estimating their temperature dependence for each kinetic model.

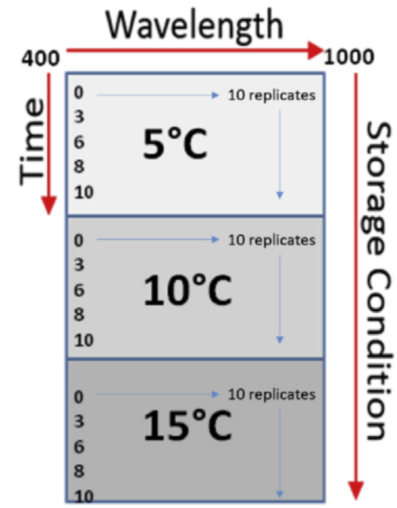

Fig. 2. Data matrix (mxn) with Time = m, Wavelength = n at the 3 storage conditions (5, 10, 15 °C).

Quality degradation kinetics can be represented by equation (1):

$$-\frac{dQ}{dt} = kQ^n \tag{1}$$

Where Q is the measured quality attribute, t represents time, n is the reaction order and k is the reaction or degradation rate.

The reaction rates are significantly temperature dependent, higher the storage temperature the faster the degradation. Therefore, to associate or describe the temperature dependence of the degradation rates, Arrhenius equation is used.

$$\ln(k) = \ln k_{T_{ref}} - \frac{E_a}{RT} \tag{2}$$

Where, Ea is the activation energy, R is the universal gas constant with a constant value of 8.314 J/mol, T_{ref} is the reference temperature usually in shelf life studies is the market shelf temperature of any food product.

The most important and significant aspect of the MASLT technique is the selection of the cut-off criteria for the property under study. In case of study evaluating different quality parameters, individual reference limits are chosen for each quality index, and cut-off criteria (t_c) is calculated as the maximum acceptable value of the vector **t** (Pedro and Ferreira, 2006; Derossi et al., 2016).

$$\mathbf{t} = \mathbf{x_a}^*\mathbf{L} \tag{3}$$

$$t_c = \mathrm{Max}(\mathbf{t}) \tag{4}$$

Where $\mathbf{x_a}$ is the row vector containing the auto-scaled values of the reference limits of each quality attribute that define the threshold of acceptability of the product, while **L** is the loading matrix of the time-related PCs at the market storage conditions.

In this study, the spectra of samples judged unacceptable was used for the calculation of the cut-off value. For visualization of degradation with the passage of storage time for leaves stored at different temperatures, 5 random leaves were collected from the replicates of each acquisition time and were processed in Hypertools (Mobaraki and Amigo, 2018). The images were spatially binned, masked and augmented with each row representing a single acquisition time (Fig. 5).

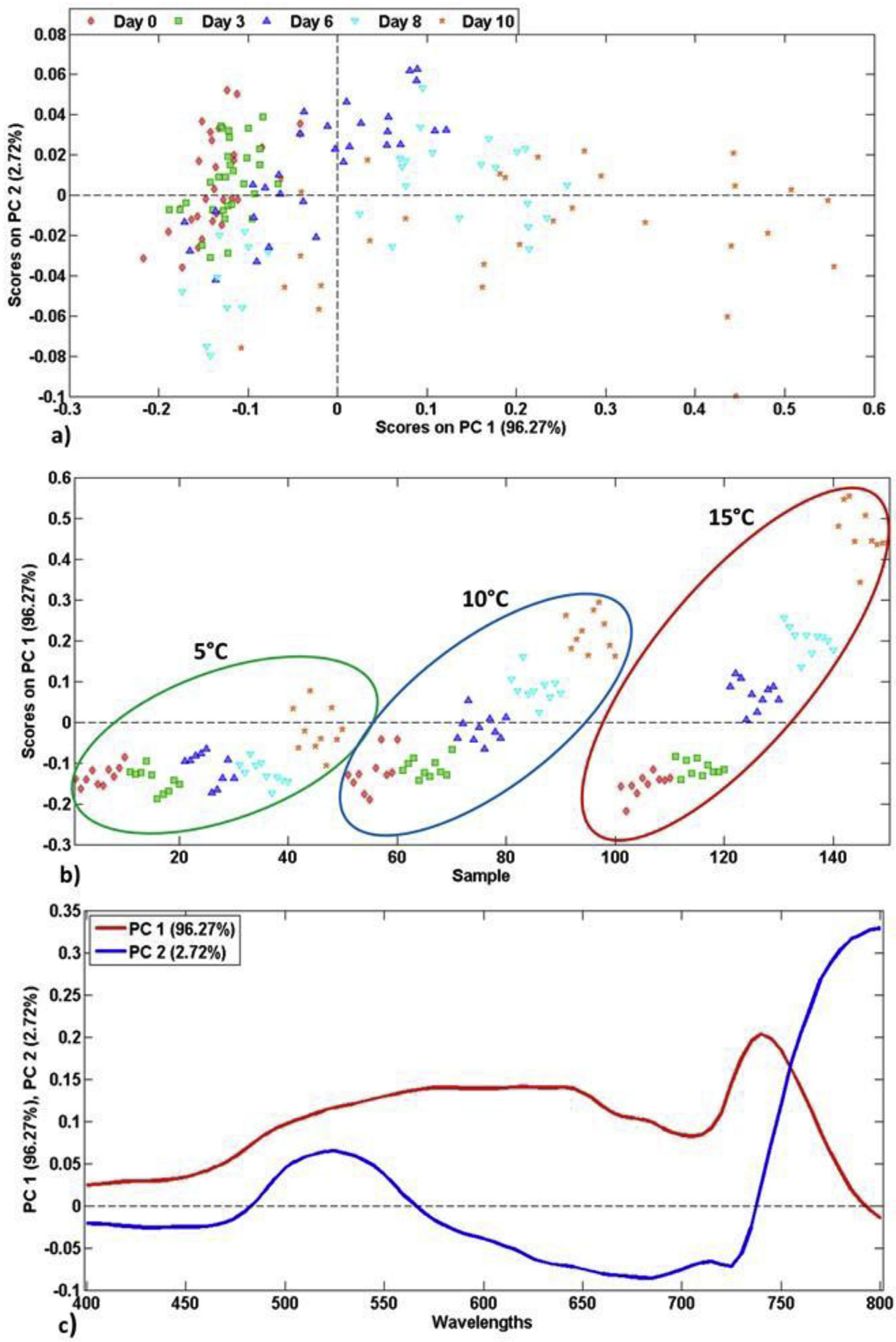

Fig. 3. PC scores plots in the wavelength range of 400–800 nm: a) PC1 vs PC2 for days of storage (0, 3, 6, 8, 10); b) PC1 variation w.r.t time of storage at 5 (green ellipse), 10 (blue ellipse) and 15 °C (red ellipse) respectively; c) Loadings PC1 (red) PC2 (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.4. Partial least squares regression (PLSR)

A slight modification of the MALST algorithm was also proposed in this study by developing PLS models for the spectra against time of storage. PLSR works by maximizing the covariance between the linear functions of the information included in the **X** (**mxn**) matrix and the corresponding vector of storage time in the **m** (ter Braak and de Jong, 1998; Dunn et al., 1989). Random subset cross validation was applied in this case. Firstly, a PLS model to predict days of storage was developed after mean centering the **X** data and auto-scaling **m** using the PLS toolbox. Then the LVs were taken as properties and were plotted against time to formulate shelf life charts. The rest of the procedure remained the same as the conventional MASLT application. In this case, only mean centering was done for data normalization. The model reliability was accessed by the values of R^2 calibration and R^2 cross validation and also by the root mean square errors.

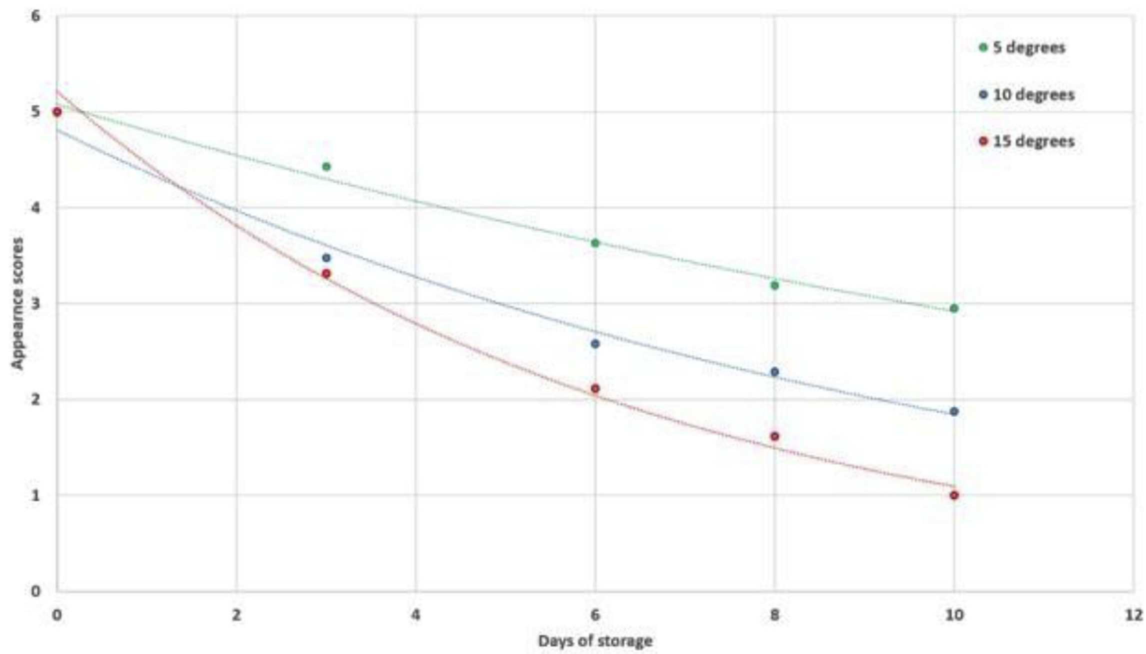

Fig. 4. Changes in the appearance scores of fresh rocket leaves stored at 5 °C (green), 10 °C (blue) and 15 °C (red) over time. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3. Results and discussion

3.1. Principal component analysis and PC relationship with time

PCA of the normalized data in the wavelength range of 400–1000 nm resulted in two PCs covering maximum variance in the data where, PC1 accounted for only 88.24% of the total variability over the entire wavelength range constituting 121 variables. After removing the variables not contributing to the model from the preliminary PCA (MacGregor and Kourti, 1995; Saavedra et al., 2013), a total of 2 PCs accounted for 98.99% of the total variability in the data in the wavelength range of 400–800 nm. In this case, PC1 explained maximum variability of 96.27% followed by the PC2 which accounted for only 2.72% of the total data variability (Fig. 3).

Plot of PC1 vs PC2 scores clearly indicated as the main source of variance on PC1 was related to time (Fig. 3a). Fig. 3b depicts the change in the PC1 scores for the samples stored at three different temperatures over the storage time. The degradation rate of the rocket leaves stored at 5 °C is much lower than those stored at 10 °C and 15 °C, which as expected, indicates that storage temperature had a significant impact on the shelf life of the rocket leaves. Temperature is, in fact, the most important factor affecting the quality of fresh produce. Low temperature is essential to maintain an optimal product quality because it reduces several physiological activities, such as transpiration, which causes weight loss, and respiration. Senescence can induce chemical and enzymatic changes that may cause tissue softening, pigment loss, ripening and discoloration (Brosnan and Sun, 2001). Moreover, low temperature also reduces the growth rate of spoilage microorganisms on surfaces of vegetable tissues (Rediers et al., 2009; Ukuku and Sapers, 2007). These changes in the PC scores may be directly related to the leaf pigments which are responsible for the color in the rocket leaves since the spectra responses vary with the change in color of the leaves in the Vis-NIR range, as a result of the loss of chlorophyll over the storage period.

As shown in the sample score plot in Fig. 3b, spectral signatures of the leaves at each acquisition time varied with the temperature of storage. From Fig. 3a and b it can also be observed that up to day 3 all the samples at the three different storage temperatures possessed similar scores and therefore similar quality attributes. Regarding appearance, in fact, until day 3 all the leaves possessed a visual score value above 3 and were still marketable, as can be seen in Fig. 4. Starting from day 6 of storage, significant quality differences can be observed in the samples stored at different temperatures.

Therefore, PC scores possess the capability to mark the days of storage during which significant quality changes occur for the samples stored at different storage temperatures. Also in the case of samples stored in modified atmosphere packaging at 5, 10 and 15 °C, differences in appearance score were higher starting from the 6th day of storage (Amodio et al., 2015b), as well as difference in the overall sensorial quality. In the same study, it was found that appearance scores limited the shelf life of the leaves stored at 5 °C, estimated in 7.3 days while an increase in the temperature affected the loss of ascorbic acid more than the appearance and off-odor scores.

Fig. 3c depicts the importance of the variables for the model; all the loadings of the PC1 model above the threshold contributed weight to the model. Since the considered wavelength range is 400–800 nm, this is mainly accounting for the color changes related to the pigments of the rocket leaves such as chlorophyll and carotenoids. The spectral profiles in the NIR region from 800 to 1000 nm are mostly related to the changes in dry matter and textural properties. Moreover, the mid NIR region is usually depicting changes based on the water content as this region is based on the high influence by water (Sánchez et al., 2011).

As for leaf pigments, chlorophyll compounds absorb in the blue and red regions, corresponding to the wavelength peak at 430 and 670 nm for Chlorophyll a, and 460 and 640 nm for chlorophyll b. Carotenoid peak ranges from 470 to 530 nm, whereas anthocyanins has a maximum absorbance at 530 nm. Based on previous research works both the carotenoid and chlorophyll absorbance decreased with the storage time due to the senescence of the leaves (Gitelson and Merzlyak, 1994; Ferrante et al., 2004). Fig. 1a shows lowest reflectance values in the region from 650 to 670 nm for samples at Day0, which means that the leaves possess significant chlorophyll content which with the passage of storage time decreased resulting in higher reflectance (low absorption) values at the end of storage period. This transition from the green to yellow color as a result of chlorophyll breakdown, associated to senescence, results in increased reflectance values.

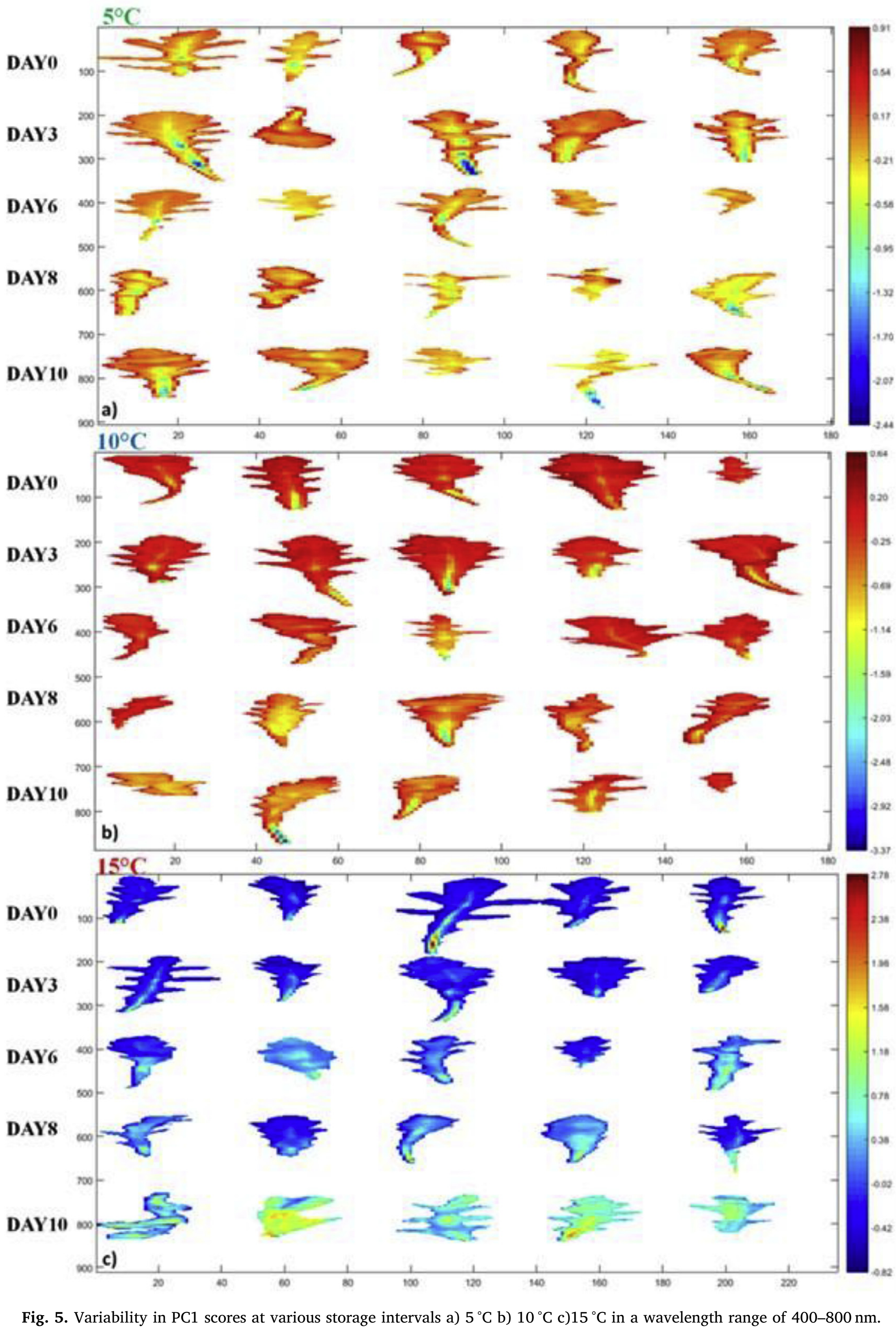

Fig. 5. Variability in PC1 scores at various storage intervals a) 5 °C b) 10 °C c)15 °C in a wavelength range of 400–800 nm.

From Fig. 4 it can be observed that leaves stored at 15 °C showed the highest degradation, followed by samples at 10 °C and 5 °C.

Fig. 5 represents the variability of the PC Scores in the leaves stored at 5, 10 and 15 °C. As can be seen, the color changes in each row (each acquisition time) at 5 °C are not visually different, but in case of 10 and 15 °C higher changes can be observed. As for 10 °C, starting from the 3rd row corresponding to the 6th day of storage, slight changes in the score colors can be observed. In case of 15 °C, the leaves in row 3 are clearly showing a significant variation of the score values when compared to those at 5 °C and 10 °C. This is totally comparable to the PCA results of the spectra in Fig. 3b where the score sample plot depicts maximum score changes at 15 °C. So Fig. 5 is an image visualization of

Table 1
First order kinetic parameters of PC1 scores as a function of time for fresh rocket leaf samples stored at 5, 10 and 15 °C using equation $A = A_0 * e^{(-kt)}$.

Wavelength Range 400–800 nm							
Temperature		Estimate	Std. error	t-value	p-level	Conf limits	R^2
5 °C	A_0	3.849	0.030	126.570	0.000001	3.753–3.947	0.73
	k_m	0.008	0.004	1.825	0.16	−0.006–0.022	
10 °C	A_0	3.831	0.043	87.299	0.000003	3.692–3.971	0.94
	k_m	0.033	0.006	4.870	0.01	0.011–0.055	
15 °C	A_0	3.776	0.071	52.478	0.00001	3.547–4.005	0.95
	k_m	0.058	0.011	5.276	0.01	0.023–0.094	

Table 2
Estimated parameters of the Arrhenius models describing the temperature dependence of the multivariate degradation rates, k_m, of PC1 score kinetics for fresh rocket leaf samples stored at 0, 10 and 15 °C.

Wavelength range (400–1000 nm)						
PC#	Variance explained (%)	Pretreatment	Storage Temperature (°C)	Multivariate rate constant (k_m)	Acceleration factor (α_m)	Activation Energy (E_a)
1st	88.92	Mean Centering	05	0.0024	…	120.91
			10	0.0085	3.54	
			15	0.0147	6.13	
Wavelength range (400–800 nm)						
PC#	Variance explained (%)	Pretreatment	Storage Temperature (°C)	Multivariate rate constant (k_m)	Acceleration factor (α_m)	Activation Energy (E_a)
1st	96.37	Mean Centering	05	0.0022	…	124.88
			10	0.0082	3.73	
			15	0.0143	6.5	

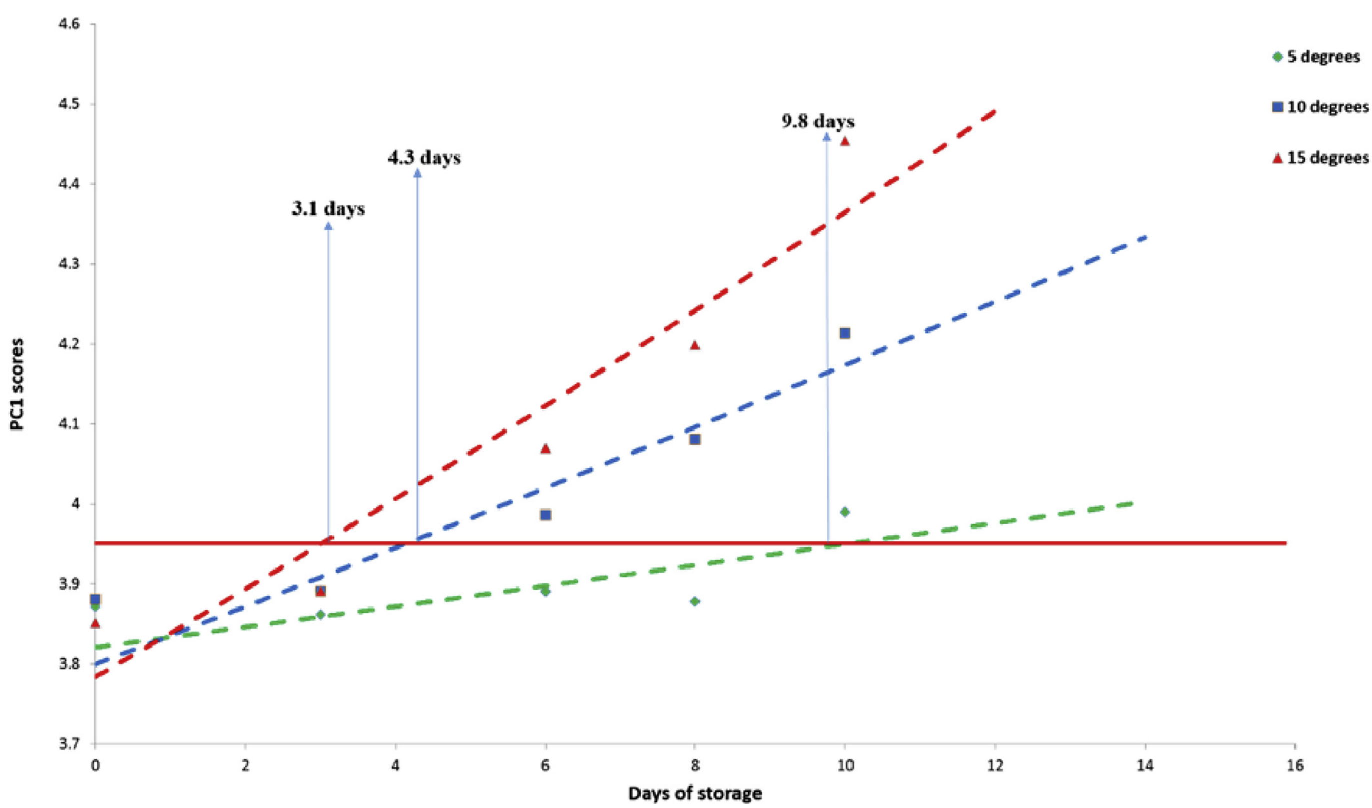

Fig. 6. PC1 scores as a function of time for rocket leaves stored at 5 °C (green), 10 °C (blue) and 15 °C (red). Red full-line represent the shelf-life cut-off value. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the trend of the score changes, obtained using randomly chosen leaves which has been expressed in Fig. 3b for the samples stored at the three temperatures. The differences in Fig. 5a, b and 5c can be directly related to the quality of the leaves. While Fig. 3b shows a plot of the samples against the time related PC1, Fig. 5 shows the relative maps of the leaves stored at all the three different temperatures over a time span of 10 storage days. The PC1 score values correspond to the color changes in the leaves from dark green to yellow over the storage period,

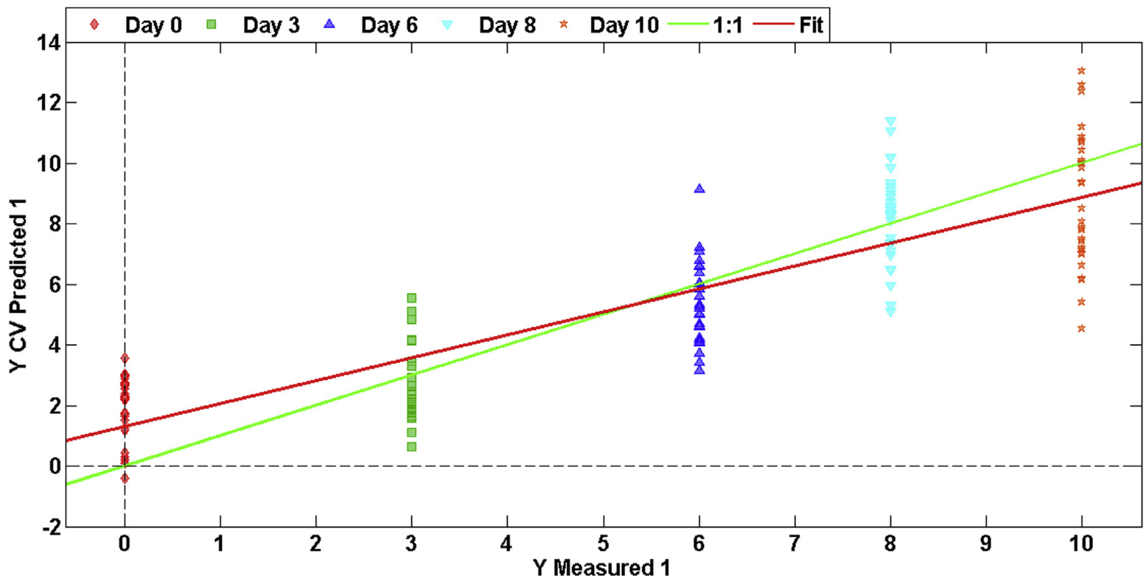

Fig. 7. PLS regression model for prediction of days of storage in the wavelength range of 400–800 nm; Day0 (red), Day3 (green), Day6 (blue), Day8 (light blue), Day 10 (orange). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

which can also be depicted in the PC scores plot along the PC1 axis in Fig. 3a. According to Fig. 3b all the samples at Day1 of storage have the same score values hence they possess the same freshness and green color as can also be observed for the samples of Day1 for all samples in Fig. 5. Since the degradation rate at different temperatures is different, clear quality changes (transition from dark green towards yellowish color) were observed for the samples stored at 10 °C and 15 °C after 6 days of storage, especially for the samples stored at 15 °C.

3.2. Multivariate modeling and shelf life estimation

Table 1 shows the results obtained by applying non-linear fitting to PC1 scores against time; based on R^2 values, results of first order fitting, particularly when a pre exponential factor was also included in the equation, were better than those of the zero order reactions. First order kinetics explained the PC1 score changes with time with an R^2 of 0.73 at 5, 0.94 at 10, and 0.95 at 15 °C for the variables in the range of 400–800 nm. The reason for this low value of R^2 in case of 5 °C can be attributed to the fact that the degradation rate at this temperature was slower as compared to 10 and 15 °C. A_0 refers to the pre exponential factor, and was estimated in similar values among different temperatures, being 3.849, 3.831 and 3.776, respectively at 5, 10 and 15 °C.

Looking also at the confidence values for A_0 values, it can be concluded that all the samples had the same quality attributes upon the beginning of the shelf life estimation and that the variance of overall quality of the fresh samples did not affect the degradation kinetics. On the other hand, a progressive increase in the value of the multivariate rate constants is seen with the increasing temperature which at 5 °C increased with a value of $0.008d^{-1}$, at 10 °C with a value of $0.033d^{-1}$ and at 15 °C it increased with a value of $0.058d^{-1}$. From the confidence intervals, a significant difference can be observed between samples stored at 5 and 15 °C, with an increase of ~7.2 fold in the degradation rate of the appearance scores. Derossi et al., 2016 found higher multivariate rate constants with values increasing with the increasing temperature for fresh-cut lettuce stored in MAP at 0, 5 and 15 °C, but this may be explained with the higher sensitivity of lettuce to browning and quality degradation due to mechanical damages induced with the cutting (Murata et al., 2004) and to the effect of modified atmosphere which at higher temperature may result in improper gas composition and in off-flavor development (Mastrandrea et al., 2017). On the other side, observed values for k_m at 5 and 15 °C were very similar to the value obtained by Amodio et al. (2015b) when fitting color score kinetic, evaluated sensorially, with the same scale system from 5 to 1. These authors reported values of 0.019 and 0.047, at 5 and 15 °C, respectively, fitting the kinetic curve with a Weibull model. Considering that shape factor values were not very different from 1 (1.34), a rough comparison of the constant can be done, and thus it can be hypothesized that observed variations were closely related to the color changes. The decrease in the values of the PC scores with the passage of storage time in the wavelength range of 400–800 nm is indicative of the transition from green color to yellowish as a result of the loss of chlorophyll which at 15 °C was the highest (Fig. 5c) followed by 10 °C (Fig. 5b) and 5°C (Fig. 5a) and color changes can only be observed in the region from 550 to 700 nm.

The multivariate kinetic parameters (k_m, α_m, E_a) calculated using PC scores as properties after various data pretreatments and utilizing two different spectral ranges, are shown in Table 2. These kinetic parameters were obtained after the exponential fitting of the time related PC scores with time of storage using equation $A = A_0{*}e^{(-kt)}$.

From Table 2 it can be seen that the dependence of the rate constant (k_m) on the temperature of storage is clearer in case of mean centered data both for the 121 variables used (400–1000 nm) and 81 variables (400–800 nm) after the spectral cropping. Other data pre-treatments were applied, but a clear difference between the rate constants could not be observed in those cases, since, by extensively preprocessing the data, the baseline effects related to the product degradation cannot be observed (Pedro and Ferreira, 2009), therefore the spectral data were only normalized.

In case of the full wavelength range, the rate constant changed from $0.0024d^{-1}$ to $0.0085d^{-1}$ and $0.0147d^{-1}$, respectively for 5, 10, and 15 °C, depicting the change in the rate constants with respect to temperature. Similarly, after the omission of the undesired variables, the variance explained by PC1 increased but still there was not a significant difference between the rate constant and acceleration factor values from those of the total 121 variables under study. The activation energy calculated for 121 variables was 120.91 kJ/mol whereas for 81 selected variables it was 124.88 kJ/mol. Also in this case the comparison with value found by Derossi et al. (2016) for fresh-cut lettuces, confirmed that degradation reactions in rocket leaves were much slower than for fresh-cut lettuce, requiring much higher E_a.

In Fig. 6, PC1 scores for each temperature were plotted against time in order to estimate the shelf-life. The cut off criterion was calculated

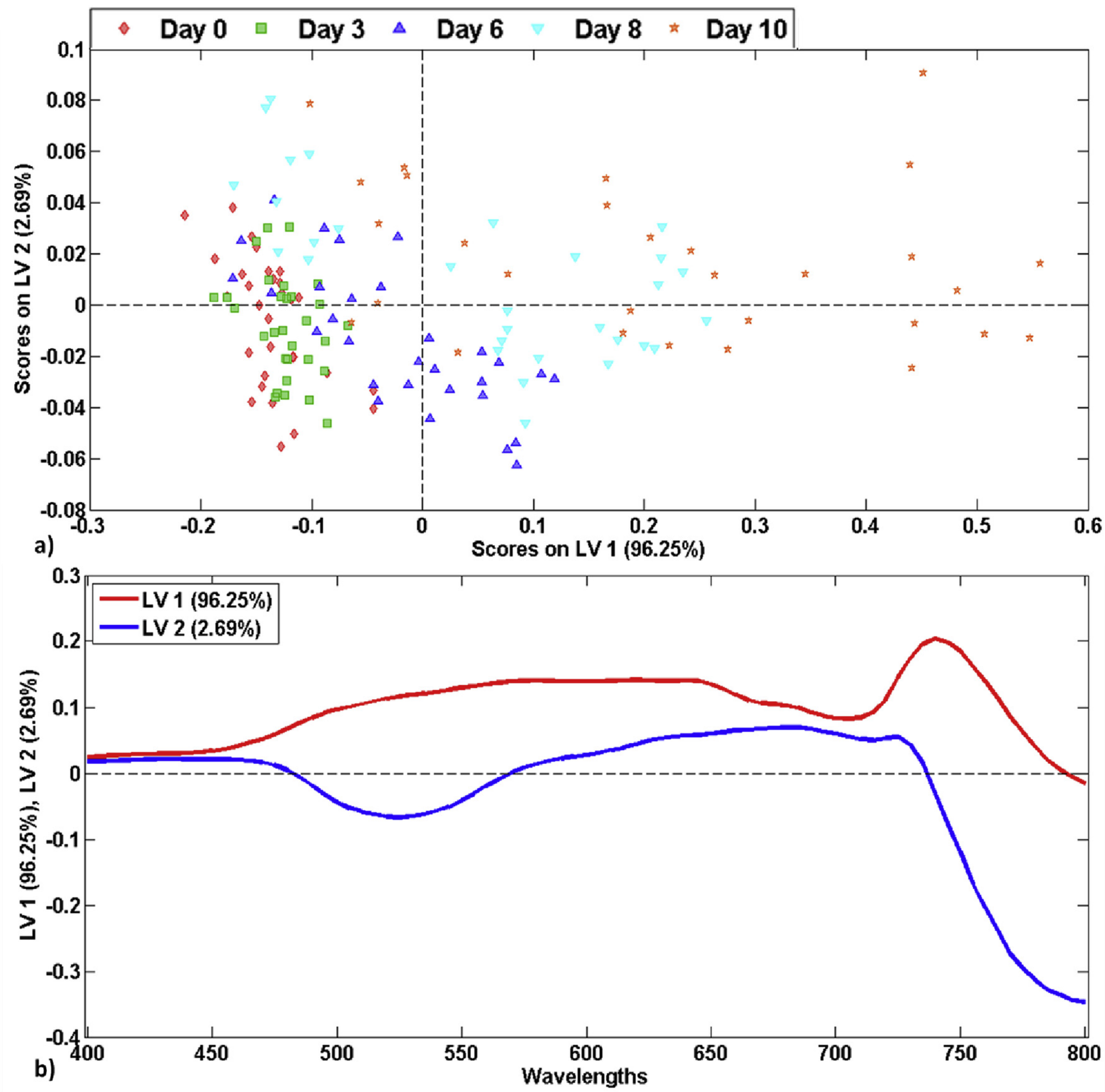

Fig. 8. LV plots in the wavelength range of 400–800 nm; a) LV1 vs LV2 score variability for days of storage (0, 3, 6, 8, 10); b) LV loadings LV1 (red) LV2 (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
First order kinetic parameters of LV1 scores as a function of time for fresh rocket leaf samples stored at 5, 10 and 15 °C using equation A = A0*e(-kt).

Wavelength range (400–800 nm)							
Temperature		Estimate	Std. error	t-value	p-level	Conf. limits	R^2
5	A_0	3.849	0.029	128.814	0.000001	3.754–3.944	0.74
	k_m	0.008	0.004	1.919	0.15	−0.005–0.021	
10	A_0	3.831	0.044	86.470	0.000003	3.689–3.972	0.94
	k_m	0.033	0.006	4.842	0.01	0.011–0.054	
15	A_0	3.775	0.072	51.784	0.00001	3.543–4.007	0.95
	k_m	0.058	0.011	5.218	0.01	0.023–0.095	

based on the number of days needed to reach score 3, corresponding to the marketability limit, applied to the kinetic at 15 °C. This is in line with the MASLT theory of using the maximum acceptable values among shelf-life limit of considered attributes. The spectrum of the unacceptable sample as vector x_a and the loadings of the time related PC were taken and using equation (4) the cutoff criteria was calculated to be 3.955. These appearance score values were in agreement with the studies conducted by Amodio et al. (2015b) and Mastrandrea et al., 2017 in which the rocket leaves reached the limit of marketability (appearance score 3) on the third day of storage when stored at 15 °C.

Estimated values of shelf life were similar to those reported by Amodio et al. (2015b) and were lower when compared to values reported by Koukounaras et al. (2007), but this can be due to the difference in raw material, possibly related with the cultivar, the season, and the number of cutting (Seefeldt et al., 2012; Koukounaras et al., 2007; Martínez-sánchez et al., 2008). Moreover this study was conducted in humidified air flow storage of the leaves and the same leaves were followed over time, being more prone to mechanical damage

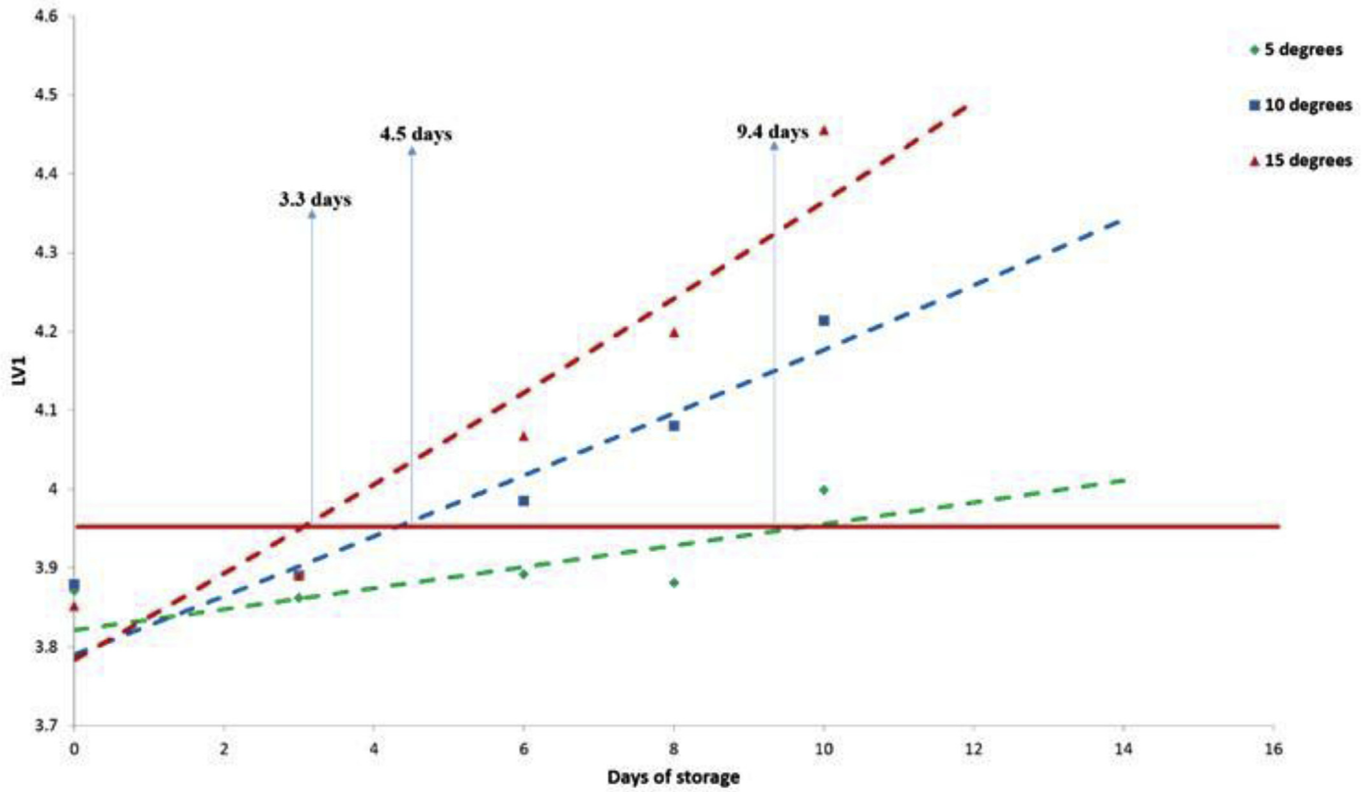

Fig. 9. LV1 scores as a function of time Fig. 6. PC1 scores a for rocket leaves stored at 5 °C (green), 10 °C (blue) and 15 °C (red). Red full-line represent the shelf-life cut-off value. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Estimated parameters of the Arrhenius models describing the temperature dependence of the multivariate degradation rates, k_m, of LV1 score kinetics for fresh rocket leaf samples stored at 0, 10 and 15 °C.

Wavelength range (400–800 nm)						
LV#	Co-variance explained (%)	Pretreatment	Storage Temperature (°C)	Multivariate rate constant (k_m)	Acceleration factor (α_m)	Activation Energy (E_a)
1st	96.25	Mean Centering	05	0.0023	…	121.95
			10	0.0082	3.57	
			15	0.0143	6.22	

during image acquisitions.

Generally shelf-life of wild rocket leaves stored at 0 °C was extended to about 3 days if compared to storage at 4 °C and 6 days compared to storage at 7 °C (Hall et al., 2013). Amodio et al. (2015b) reported value of 7.3, 5.8 and 3.7 days for samples stored at 0, 5 and 15 °C, respectively, when appearance score was used as marketability limit. In the same study, the shelf life was of 12.6, 10.4 and 3.1 when calculated on texture score, and even higher if calculated on the ascorbic acid losses. The authors, showed, in fact that appearance score was the limiting factor for shelf life at 0 and 5 °C, but that at 15 °C, ascorbic acid content, followed by the texture, were more critically contributing to the marketability, because the degradation rates of these attributes increased with temperature more than the appearance and off-odor. These findings confirm the needing of a multivariate approach for shelf-life estimation. When MALST method was applied on fresh-cut lettuce, it was found that the degradation of samples stored at 15 °C was mainly attributed to the off-odor rather than to the appearance score and color score, which, on the other hand, were the most determinant for samples stored at 0 and 5 °C, respectively (Derossi et al., 2016).

3.3. PLSR for the estimation of days of storage and shelf life

This method is a slight modification of the MASLT approach. In this case instead of doing a PCA, a PLS model was developed for the prediction of days of storage from the spectra acquired during the storage period at all three storage temperatures. The spectra were mean centered and PLS regression model for days of storage in the full wavelength range resulted in an R^2_{cal} of 0.86 and R^2_{cv} of 0.83 with the RMSC of 1.32 and RMSECV of 1.48. The first latent variable (LV) accounted for 88.82% of the covariance and the second LV described 8.31% of the covariance in the data. Variables having minimal or negligible weight in the PLS model as shown in the loading plots were removed, resulting also in this case, in the elimination of the variables from 800 to 1000 nm. PLS regression model for prediction of days of storage was developed again with 81 variables from 400 to 800 nm after data normalization (Fig. 7).

LV1 explained 96.25% of the covariance, whereas LV2 only accounted for 2.69% of the covariance in the data. The plot of LV1 versus LV2 shows the variability of the LV scores along the LV1 axis (Fig. 8a). The cause of this variability can be found in the differences of the Y values due to the different storage temperatures. Fig. 8b depicts the loadings of LV1 and LV2.

Compared to the PCA in Fig. 3a a similar dependence of LV values with time of storage can be observed; the changes in the spectral properties are evident in this case as well. For this reason, this can also be considered as a new MASLT approach and its results can be compared to the conventional MASLT based on PCA. As expected, LV1 was found to be time related and the loadings of LV1 and PC1 possessed negligible difference. Both the LV1 and PC1 loading profiles hold similarity with the mean centered spectral profiles, hence explaining maximum covariance and variance, respectively.

If compared to Table 1, the results obtained by applying non-linear fitting to the LV1 against time using the first order kinetics are not significantly different as the PC scores approach in the conventional MASLT studies (see Table 3). LV1 score changes with time resulted in an R^2 of 0.74 at 5 °C, slightly higher than the R^2 obtained in the case of PC scores at the same storage temperature, 0.94 at 10 °C and 0.95 at 15 °C, similar to those of the PC approach. The A_0 values for all three temperatures is almost similar in this case and negligibly different from those of the PC scores for each storage temperature. Moreover, the multivariate rate constant values are exactly the same in both cases, with the increasing temperature which at 5 °C was $0.008d^{-1}$, at 10 °C was $0.033d^{-1}$ and at 15 °C was $0.058d^{-1}$. If compared to the A_0 values in Table 1 it can be seen that the results of the PLS regression coupled with the MASLT analysis are the same as the conventional MASLT approach. Also in this case the negligible differences in the A_0 values signify that the leaves in the start of the analysis had the same quality attributes which deteriorated with the passage of time at different rates stored at different temperatures.

The kinetic charts were developed for the LV scores at each storage temperature against the days of storage (Fig. 9).

If compared to Fig. 6, in which the PC1 scores were plotted as a function of time, it can be observed that the results are not very different. Equation (4) was used for the calculation of cutoff criteria using the unacceptable spectrum and the loadings matrix of the time related LVs, and this cutoff criteria possessed negligible difference from that of the PC scores approach. The estimate of shelf life in this case for samples stored at all three temperatures had minute differences but was slightly better if compared to the PCA, since values of 3.3, 4.5 and 9.4 days were closer to the experimental values at 5, 10 and 15 °C, obtained with sensory evaluation.

Comparing Figs. 6 and 9, as well as Table 4 and Table 2, it is clear that LV can be used as an alternative approach for the shelf life estimation in the MASLT method since the differences in the values of the rate constants, of the activation energy, as well as of shelf-life estimation are not different. The advantage of using this approach instead of using a PCA is that it would be much more flexible for further validation experiments. In fact, while PCA distribution of variables may be more sensitive to other sources of variation, a PLS prediction model based on days of storage will be more robust since PLS takes into consideration the covariance between the spectral profiles and the predictor values. Hence, the co-variability of the spectra with respect to days of storage are more accurately represented by PLS. This will facilitate the comparison of data obtained with new samples, just by plotting them versus the regression line plot, without the need of rerunning a PCA every time, and also the adding of new calibration data into the model.

4. Conclusions

For the first time a multivariate approach using the spectral fingerprints for the estimation of the shelf life of fresh cut rockets was used. The changes in the spectra with the passage of storage time for the samples stored at three different temperatures served as the property under study. Comparing the MASLT approach with the conventional ASLT methods the use of PCA yielded valuable information regarding the variables contributing towards the weight in the model and accounting for the quality losses of the product. It was highlighted that the wavelength range of 550–700 nm held great significance while estimating shelf life based on appearance scores. The conventional MASLT approach using the PC scores was also compared with a new method using PLS and LV for the development of the kinetic or shelf life charts. Comparing both the approaches it was concluded that no significant difference exist between the results yielded by both the techniques. On the other side, the PLS model can be more robust as compared to a PCA model with the allowance of new samples to be added in the calibration and can serve as a tool for better validation. MASLT approach with PLS, if implemented ocan enable the processors to better estimate the shelf life of their products and access the market with better product quality by improving the logistics.

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