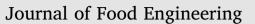
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Hyperspectral imaging and multivariate accelerated shelf life testing (MASLT) approach for determining shelf life of rocket leaves



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

ARTICLEINFO	A B S T R A C T
Keywords: PCA PLS Cutoff Kinetic Appearance	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 offodors 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).

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

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^{*} Corresponding author.

Degradation process of food products and particularly of fresh-cut produce is a multivariate phenomenon depending from several preharvest 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 timerelated 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 selfdeveloped 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

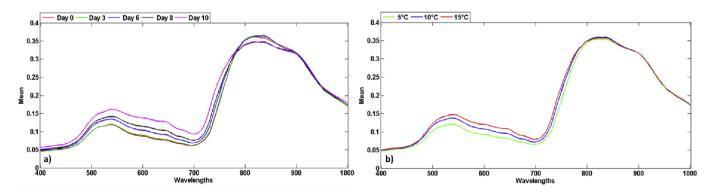


Fig. 1. a) Mean spectra based on days of storage Day0 (red), Day3 (green), Day6 (blue), Day8 (black), Day10 (Violet); b) Mean spectra based on temperatures of storage 5 °C (green), 10 °C (blue), 15 °C (red). (For interpretation of the references to color in this figure legend, the reader is referred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

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