THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System

Michela Palumbo[1ab], Bernardo Pace[1a], Maria Cefola[a*], Francesco Fabiano Montesano[c], Giancarlo Colelli [b], Giovanni Attolico [d]

[a] Institute of Sciences of Food Production, National Research Council of Italy (CNR), c/o CS-DAT, Via Michele Protano, 71121 Foggia, Italy

[b] Department of Science of Agriculture, Food and Environment, University of Foggia, Via Napoli 25, 71122 Foggia, Italy

[c] Institute of Sciences of Food Production, National Research Council of Italy (CNR), Via Giovanni Amendola 122, 70125 Bari, Italy

[d] Institute on Intelligent Industrial Systems and Technologies for Advanced Manufacturing, CNR-National Research Council of Italy Via G. Amendola, 122/O, 70126 Bari, Italy

[*] Corresponding Author: phone/fax: +39.0881.630201; email address: maria.cefola@ispa.cnr.it

[1] **First Authorship is equally shared**

Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

Abstract

Computer Vision Systems (CVS) offer a non-destructive and contactless tool to assign visual quality level to fruit and vegetables and to estimate some of their internal characteristics. The innovative CVS described in this paper exploits the combination of image processing techniques and machine learning models (Random Forests) to solve these kind of problems on unpackaged and packaged rocket leaves. Its performance did not depend on the cultivation system (traditional soil or soilless). The same CVS was able to build effective models for either the classification problem (visual quality level assignment) and the regression problems (estimation of senescence indicators such as chlorophyll and ammonia contents) just by changing the training data. The experiments showed a negligible performance loss on packaged products (Pearson's linear correlation coefficient of 0.84 for chlorophyll and 0.91 for ammonia) with respect to unpackaged ones (0.86 for chlorophyll and 0.92 for ammonia). The results showed that the CVS (non-destructive and contactless) represents a valid alternative to destructive, expensive and time-consuming analyses in the lab and can be effectively and extensively used along the whole supply chain, even on packaged products that cannot be analyzed using traditional tools.

Keywords: contactless quality level assessment, *Diplotaxis tenuifolia* L., image analysis, packaged vegetables, senescence indicators prediction

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

1. Introduction

Rocket is a green leafy vegetable usually marketed and consumed as fresh-cut salad, alone or mixed to other leafy vegetables. It is well known and appreciated for its pleasant bitter taste and for its high content in bioactive compounds, such as vitamins, minerals and antioxidants. The two species commonly sold on the market are *Eruca sativa* and *Diplotaxis tenuifolia* or wild rocket that is known to have longer shelf-life (Mastrandrea et al. 2016).

The quality loss during the postharvest storage is mainly due to the yellowing of the leaves strictly related to chlorophyll degradation that therefore is the most common index used to evaluate quality and freshness of this product (Limantara et al. 2015; Cavallo et al. 2017). Generally, as reported by Pace et al. (2019), a 30 % loss of total chlorophyll content is considered the shelf-life limit in rocket leaves.

Another important indicator of leaves senescence in fresh-cut rocket is ammonia accumulation in plant tissues, as a consequence of the protein breakdown during early stages of senescence. High concentrations of this component may cause tissue damages with visible senescence effects, that impact on the overall quality evaluation of the product (darkening and browning of detached leaves) (Chibnall, 1939; Mastrandrea et al. 2016; Amodio et al. 2018).

Traditional approaches for chlorophyll and ammonia content measurements in leafy vegetables include destructive methods, based on spectrophotometric assay.

Even if these approaches for a long time have been considered the standard and most used methods for these determinations, they require specific laboratory equipment and destructive sampling and they are expensive and time consuming.

While, for the ammonia analysis, the destructive method is widely applied, for chlorophyll evaluation, modern, handheld and sensor have received a considerable attention in the last decades because of their high accuracy and real time measurement in a non-destructive way directly on field or on minimally processed products.

So, many researchers have developed several types of chlorophyll meters (Novichonok et al. 2016), multispectral and hyperspectral sensors (Chen et al. 2010; Li et al. 2014) to detect the spectral reflectance of leaves to assess the total chlorophyll content. Many of these techniques are costly and complex and require the presence of specialized personnel. Among them, the most developed instruments widely used for chlorophyll content measurement are fluorimeters (Ferrante and Maggiore, 2007) and SPAD meters (Ling et al. 2011; Liu et al. 2012; Yuan et al. 2016).

All these instruments are simpler, faster and cheaper than chemical analysis and other sensor based approaches, but they need to touch the leaf with a probe to carry out a chlorophyll measurement on a limited area of few mm^2 of leaf surface. For these drawbacks, their use is not suitable for application into an industrial line and, additionally, the estimation of chlorophyll content is strictly related to the quality of sampling (Cavallo et al. 2017).

Recently, in order to reduce these disadvantages, image analysis based on common digital camera has been applied as a low cost instrument for the assessment of chlorophyll content on leafy vegetables, becoming a potential approach for smart agriculture (Mohan and Gupta, 2019) and postharvest quality assessment (Pace et al. 2014, 2015; Cavallo et al. 2017).

Machine vision systems have proved to be more robust than area-based instruments as they work at pixel level considering the entire visible surface of the product. Many research works on the use of digital image both during cultivation and postharvest were reported to evaluate total chlorophyll content of leaves of rice (Wang et al. 2013, 2014), soybean (Rigon et al. 2016), corn (Vesali et al. 2017), spinach (Agarwal and Gupta, 2018) and rocket leaves (Cavallo et al. 2017; Pace et al. 2019), also through the use of common smartphone cameras that are often associated to high speed processor

(Mohan and Gupta, 2019). The success of Computer Vision System (CVS) is due to the possibility of establishing relationships between spectral reflectance indices and chlorophyll absorbance and RGB (red, green and blue) components of an image (Santos do Amaral et al. 2019).

Recently, Cavallo et al. (2018) also applied the image analysis technology to packed and unpacked fresh-cut iceberg lettuce to assess the quality levels of the product with similar performances both on packaged and unpackaged samples. The main purpose of this study was to achieve a careful selection of the bag area where the product was visible with a quality suitable for image analysis. All images acquired on packed samples were segmented using a Convolutional Neural Network (CNN), identifying and selecting only pixels belonging to the fresh-cut lettuce without interferences of the affected regions of bag. These Authors recorded a performance loss of only about 3 % due to the presence of packaging (accuracy of 83 % on packed product instead of 86 % on unpacked one), showing the power of image analysis in monitoring the quality level of fresh-cut vegetables. The CNN segmentation method was able to separate the graphical elements and the regions affected by lighting artefacts from the product inside the commercial bag, demonstrating its real applicability on an industrial line, regardless the presence of the packaging. The proposed approach has been the only CVS application in the quality level assessment of vegetables through the packaging and, certainly, further investigations are needed to confirm and implement this emerging technology for a continuous check of the quality of fresh-cut products along the whole supply chain.

Few application regarding the use of CVS for the detection of ammonia content in leafy vegetables, are reported. In Pace et al. (2014), CVS was applied on whole and fresh-cut lettuce to give a non-destructive evaluation of this chemical parameter often used as a senescence indicator in several leafy vegetables (Cefola and Pace, 2015; Tudela et al. 2013). Starting from these considerations, the aims of the research reported in this paper were to verify and assess the capability of the non-destructive and contactless CVS in: i) assessing the visual quality changes during postharvest storage of fresh-

Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

cut rocket leaves; ii) estimating some internal characteristics (chlorophyll and ammonia contents) of fresh-cut rocket leaves; iii) working through the packaging without significant performance loss due to the identification of the regions where the product is properly visible. This region identification, in the experiments described in this paper, has been accomplished using a simplified techniques based on data-driven image processing without the need of training a Convolutional Neural Network (CNN).

2. Materials and Methods

2.1. Plant material and experimental setup

Rocket leaves (*Diplotaxis tenuifolia* L. cv Dallas) were cultivated at the same time in soil or soilless cultivation systems at the CNR-ISPA experimental farm La Noria (CNR-ISPA) located in Mola di Bari (in the South of Italy). Harvests were performed at 55, 70 and 110 days and at 60, 110 and 145 days after sowing for soilless and soil system, respectively.

At each harvest time, fresh-cut rocket leaves, separated per cultivation system were provided to the laboratory for image analysis by CVS and postharvest quality determinations. Thus, fresh-cut leaves were selected to avoid samples with defects and mechanical damages and packed in open PP bags (dimensions 50 x 30 cm, Orved, Musile di Piave (VE), Italy) of about 600 g each one. In detail, 13 bags (replicates) were prepared for samples cultivated on soil system, while 10 bags for rocket leaves cultivated on soilless system. Then, all samples were stored at 10 °C (the storage temperature commonly used in the supply chain) for 16 and 18 days for soilless and soil system, respectively.

2.2. *Sensory visual quality attribution during cold storage of rocket leaves.*

During storage, samples were taken and observed to attribute the visual quality level (QL) according to the scale reported by Palumbo et al. (2021).

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

In detail, at each sampling day, an amount of sample was taken from each PP bag and evaluated by a group of 5 researchers using the 5 to 1 rating scale cited above, where 5 = very good (very fresh, no signs of yellowing, bright, dark and uniform green, no defects), 4 = good (fresh, slight signs of yellowing, light green, slight loss of texture), 3 = fair (slight wilting, moderate signs of yellowing, slight discoloration, minor defects, loss of texture), 2 = poor (wilting, evident yellowing, discoloration, severe loss of texture), 1 = very poor (unacceptable quality due to decay, severe wilting and yellowing, complete loss of texture and other evident defects). A score of 3 was considered to be the limit of marketability, while a score of 2 represented the limit of edibility.

Then, images of packaged and unpackaged fresh-cut rocket leaves were acquired by CVS and the quality of the same samples was evaluated through destructive conventional methods as detailed below.

2.3. Colour analysis by colorimeter, total chlorophyll content, ammonia content, and electrolyte leakage

The CIELAB colour parameters (L^*, a^* and b^*) were detected, for each replicate, on 3 random points on the surface of 10 rocket leaves using a colorimeter (CR400, Konica Minolta, Osaka, Japan). The instrument was calibrated with a standard reference having values of L^*, a^* and b^* corresponding to 97.44, 0.10 and 2.04, respectively. Then, the colour parameter of Hue angle ($h°$) was calculated from primary L^*, a^* and b^* readings according the equation below:

$$h° = \tan^{-1}\frac{b*}{a*} \quad (1)$$

The total chlorophyll content was measured according to the spectrophotometric method reported by Cefola and Pace (2015). Five grams of rocket leaves were chopped and extracted in acetone/water

(80:20 v/v) with a homogenizer (T-25 digital ULTRA-TURRAX® - IKA, Staufen, Germany) and then centrifuged at 6440 *x*g for 5 min. To remove all pigments, the extraction was repeated 5 times (10 mL per times) and extracts were combined. The absorbance was read immediately after the extraction procedure on extracts proper diluted using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at three wavelengths, at 663.2 nm, 646.8 nm, and 470 nm. Total chlorophyll content was expressed as mg g^{-1} of fresh weight using the equation reported by Wellburn (1994).

Ammonia content was evaluated according to Fadda et al. (2016). Chopped rocket leaves (5 g) were homogenized for 2 min in 20 mL of distilled water on an ice bath, and then centrifuged for 5 min at 6440 *x*g at 4 °C. Then, the supernatant extract (0.5 mL), was mixed with 5 mL of nitroprusside reagent (phenol and hypochlorite in alkali reaction mixture) and heated at 37 °C for 20 min. The color development after incubation, was determined with the spectrophotometer (reading the absorbance at 635 nm). The content of NH_4^+ was expressed as µg NH_4^+ g^{-1} of fresh weight, using ammonium sulfate as standard (0–10 µg mL^{-1}, $R^2 = 0.99$).

The electrolyte leakage was determined following the method reported by Palumbo et al. (2021). In detail, about 2.5 g of rocket leaves disks obtained using a cork borer (ø 8 mm) were placed in plastic tubes and immersed in 25 mL of distilled water. After 30 min of storage at 10 °C, the conductivity of the solution was measured using a conductivity meter (Cond. 51+ - XS Instruments, Carpi, Italy). Then, the tubes with samples and solution were frozen at – 20 °C and, after 48 h, the conductivity was detected after thawing and considered as total conductivity. Electrolyte leakage was calculated as the percentage ratio of initial over total conductivity.

2.4. *Image analysis by computer vision system*

At each sampling day, a sample of about 60 g of product was taken from each replicate and was placed inside a 20 x 25 cm PP bags (Cartonpack Rutigliano, Italy). Three images of the packaged

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

product were acquired by randomly shuffling the rocket in the bag before each acquisition: this procedure generated three different images from each packaged sample to increase the amount of observed surface and the variability of visual appearance considered by the CVS. Then, the same product was extracted from the bag and three images were acquired by randomly shuffling and stacking the leaves before each acquisition. In this way, for each replicate the CVS acquired 6 images: 3 of the packaged product and 3 of the unpackaged product. Example of the six images acquired from a single sample are shown in Figure 1.

Therefore, 78 images were available at each time for samples coming from soil system (6 images for each of the 13 replicates) and 60 images for samples coming from the soilless system (6 images for each of the 10 replicates).

The final dataset was composed by 429 and 450 image from soil and soilless cultivation respectively after all three harvest date. In Table 1 the number of acquisitions conducted for each harvest time on packaged and unpackaged products are reported. The complete collection was therefore composed by 879 images for each packaged or unpackaged product. We choose to not distinguish images coming from different harvests: therefore, the final image dataset was composed by 207 images for the quality level 5 and by 168 images for each quality level from 4 to 1. These data are reported in the Table 1.

Table 1. Composition of the dataset of images with respect to harvests (H1, H2 and H3), and quality levels (QL).

	Soil							***Soilless***						
	Replicates	**QL**					**Total**	**Replicates**	**QL**					**Total**
		5	**4**	**3**	**2**	**1**			**5**	**4**	**3**	**2**	**1**	
		15	15	15	15	15	**75**		15	15	15	15	15	**75**
H 1	**13**	15	15	15	15	15	**75**	**10**	15	15	15	15	15	**75**
		9	9	9	9	9	**45**		-	-	-	-	-	**-**
H 2	**13**	15	15	15	15	15	**75**	**10**	15	15	15	15	15	**75**

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

		15 9	15 9	15 9	15 9	15 9	**75 45**		15 -	15 -	15 -	15 -	15 -	**75 -**
H 3	**13**	15 15 9	- - -	- - -	- - -	- - -	**15 15 9**	**10**	15 15 -	15 15 -	15 15 -	15 15 -	15 15 -	**75 75 -**
		117	**78**	**78**	**78**	**78**	***429***		**90**	**90**	**90**	**90**	**90**	***450***

2.5. Image processing steps by Computer Vision System

The following paragraphs describe all the processing steps used by the CVS. All the software was developed using Matlab 2019a (Mathworks Inc.). A flowchart of the processing steps (whose sequence is slightly different for packaged and unpackaged products) along with their effects on the images, is shown in Figure 2.

2.5.1. Acquisition of calibrated color images

To acquire calibrated color images, color changes due to environment conditions (lighting, geometry, sensor instability) were evaluated and reduced to the minimum. Images were acquired using the set-up reported in (Pace et al. 2015, 2017; Cavallo et al. 2017, 2018) using a 3CCD (with a dedicated Charged Coupled Device for each color channel) digital camera (JAI CV-M9GE) having a resolution of 1024 x 768 pixels. The imaged area is about 32 x 24 cm. A 3CCD sensor has been used to avoid the artifacts introduced by the demosaicing methods required to record color information using a single CCD. The optical axis of the LinosMeVis 12 mm lens system was perpendicular to the black background. Two DC power suppliers delivered current to eight halogen lamps, placed along two rows at the two sides of the imaged area and oriented at a 45° angle with respect to the optical axis. All the images were saved using the uncompressed TIFF format to avoid the artifacts introduced by compression algorithms.

2.5.2. Color-chart processing and foreground segmentation

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

A small X-Rite color-chart with 24 patches of known colors was placed into the scene to measure color variations due to environmental conditions and sensor characteristics by comparing the expected numerical values released by the manufacturer with the ones acquired by the camera. The color-chart was automatically detected regardless of its position and orientation (Cavallo et al. 2017). All the colors in the color-chart were used to estimate the linear transformation used for color correction.

Image processing worked only on the part of each image belonging to the product at hand (foreground), while the background was discarded. The CVS automatically separated foreground and background without any human intervention: two thresholds were derived from the analysis of the whole image in the HSV color space as described in (Cavallo et al. 2017). This segmentation identified the region belonging to the product as a whole and did not separate its different parts neither discarded any region of leaves. It was designed to be conservative, that is to discard all the background pixels even at the cost of removing some marginal borders of the product. It removed also background area inside the stack of leaves as long as part of leaves too dark (for example for self-shadowing of the product) to provide meaningful color information.

2.5.3. Color correction

Color correction needs to be effective (able to provide consistent color measurements) and efficient (computationally simple enough to be suitable for real applications along the supply chain). The linear correction model proved to be the best trade-off between effectiveness and computational complexity and it was used in the experiments (Palumbo et al. 2021).

Let it be $[r_e^i\ g_e^i\ b_e^i]^T$ and $[r_m^i\ g_m^i\ b_m^i]^T$ the expected and the measured RGB values respectively for the i-th patch i=1, …, 24. A linear correction (LC) model, a 3 x 3 matrix, was evaluated to reduce the distance between the expected and the measured values on the color chart:

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

$$\begin{bmatrix} r_c \\ g_c \\ b_c \end{bmatrix} = \begin{pmatrix} m_{11} & m_{12} & m_{13} \\ m_{21} & m_{22} & m_{23} \\ m_{31} & m_{32} & m_{33} \end{pmatrix} \begin{bmatrix} r_m \\ g_m \\ b_m \end{bmatrix}$$

where $\begin{bmatrix} r_c \\ g_c \\ b_c \end{bmatrix}$ are the colors corrected using the matrix whose elements were evaluated using a least-square approach and all the patches of the color-chart. The same matrix was therefore used to correct all the foreground pixels of the image.

The linear transformation was different for each image (it was evaluated from the color-chart appearance in each specific image) to adapt to the specific conditions of each acquisition. The linear correction requires 73 ms.

2.5.4. Artefact elimination from packaging

The unpredictable relative orientation of the surface of the bag with respect to light can easily generate artefacts such as reflections. In those regions, the camera cannot measure the true colors of the product at hand: therefore, those pixels are useless to estimate internal components or to assign visual quality. Before feature extraction and classification or regression, those areas must be removed from each image and only the regions where the product is visible with meaningful colors must be maintained. The flowchart in the Figure 2 shows the placement of the artefact elimination step inside the processing chain and its effects on the image. To identify the useless regions, the image converted in the HSV (Hue-Saturation-Value) color space was considered. In this space, it is possible to exploit the characteristics of artefacts that are mainly colorless and much brighter than the product. Two data driven thresholds were automatically extracted on the Hue ($thresh_h$) and on the Value ($thresh_v$) components of the image using the Otsu algorithm: pixels whose hue was greater than $thresh_h$ and whose value was lower than $thresh_v$ were considered as belonging to product and maintained for the

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

following processing. The Figure 2 shows an example of the obtained result. The region left for further processing is significantly reduced: a conservative choice was made also in this case, by preferring to discard more pixels of product instead of leaving saturated pixels in the image. The resulting valid area proved to be large enough to allow the feature extraction and the classification and regression phases.

2.5.5. Features extraction

On the base of previous experiences, the device independent and perceptually uniform CIE *L*a*b** color space was chosen to accomplish color analysis. Because the *L** component is fragile, being too sensible to not uniform illumination levels across the scene, the complete histogram in the *a* b** plane of the foreground pixels was used as feature set for both classification and regression. The color histogram represents the number of occurrences of each color, that is of each (*a**, *b**) pair, in all the foreground pixels: it therefore represents a property of the whole observed product. The continuous (*a**, *b**) plane was discretized using 30 bins for each axis (*a** and *b**): therefore, the complete histogram was a matrix with 900 elements. This representation is more detailed than statistical measures such as mean, median or standard deviation: it describes completely the palette of colors present in the scene and their relative relevance. The hypotheses were that such information was able to represent the appearance of new colors due to senescence, in according with changes in content of internal components such as chlorophyll and ammonia. To avoid any human intervention in the identification of proper color features, the complete matrix containing all the values of the bins of this histogram was reshaped as a vector and passed to the classification phase. The use of a quite large vector with 900 elements was feasible because the ensemble method used for classification and regression can sample for each tree a subset of even a quite large set of features: this approach automatically figures out their best use while keeping reasonable the computational complexity. Even

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

if it is not possible to identify few specific colors suitable to discriminate product quality and internal contents of chlorophyll and ammonia, the ensemble of trees exploits a reasonably large subset of the provided features, that is (a^*, b^*) pairs, to achieve the desired goals.

2.5.6. Image analysis

To assign the visual quality to a sample is an example of a classification problem: the model needs to assign a value out of a finite set of choices (in this case a quality level from 5 to 1 as described above). To estimate the chlorophyll and ammonia contents is instead an example of a regression problem: the model needs to estimate a real number in a continuous interval. In both cases, a supervised learning approach was followed: a model was chosen and its parameters were fixed on the base of the analysis of available samples. Each sample was composed by a set of features (the whole histogram in the a-b plane of the CIE $L^*a^*b^*$ color space as described in the paragraph above) and the expected answers: an integer value (in the set from 1 to 5) defining the visual quality level (used for classification) and two real values for chlorophyll and ammonia respectively (used for regression).
A Random Forest model was used to accomplish both classification and regression (Breiman, 1996, 2001). In case of classification, a model was trained to assign the QL to the product. In case of regression, two different models were trained to estimate the chlorophyll content and the ammonia content of the product respectively. In all the cases, the models shared the same architecture and differ for the free parameters that were fixed for the specific task. The values of the cells of the histogram in the a^* b^* plane (of the CIE $L^*a^*b^*$ color space) of each image provided the vocabulary of features used for training the models. The approach, for training each tree, randomly sampled the available training data (to select the training examples) and randomly selected a set of features (in this case randomly selecting which values of the histogram to use to build the tree at hand). Each tree of the forest was allowed to have a maximum of 10 branches. Due to the limited number of samples, a 10-

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

fold cross validation approach was used: that means to divide the available data into 10 groups (folds), each having approximately the same number of elements. The partitions were made with stratification: therefore, each fold approximated the same distribution of the whole training set. According to the 10-folds validation strategy, at each iteration of the process the training was done 10 times: at each round, a different fold was separated for testing the results while the other nine folds were used for training. The average of the results obtained in the ten rounds estimated the performance of the method at each iteration.

To increase the stability of results, 10 iterations were run for each task (classification for visual quality, regression for chlorophyll estimation, regression for ammonia estimation): the best result of the ten iterations and the average of their results were stored. The best value was very similar to the average, proving that the randomness of the choice of training samples and of features for each tree did not affect significantly the performance of the resulting model.

2.5.6.1 Classification

Accuracy was used as a quick indicator of the performance of classification in the results' section but, to provide a complete description of the obtained results, the confusion matrices were also stored: in fact, they express all the information needed to describe the behavior of the method. The provided confusion matrices and accuracy values represent the average of the values over these 10 different iterations, each corresponding to a new random stratified partition of training data into 10 folds. That increases the significance of the obtained results by making less relevant the effects of chance in sampling training data and features. In spite of the significative number of trees in the resulting forest (200 trees were allowed for each forest) the increase in accuracy provided by their combination did not require high computational costs. The code, written in Matlab without any specific optimization, required about 2 minutes for building the Random Forest model and about 10 milliseconds to apply the model to a new sample and to classify it.

Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

2.5.6.2 Regression

Mean square error (MSE) and Pearson's correlation coefficient between the estimated and the true values measured in the lab were used to quantify the performance of regression. To increase the robustness of performance measures, the 10-fold cross-validation regression was repeated 10 times (iterations). The best values and the average values are provided for both chlorophyll and ammonia estimation. In spite of the significative number of trees in the resulting forest (200 trees were allowed for each forest) the increase in accuracy provided by their combination does not require high computational costs. The code, written in Matlab without any specific optimization, requires about 6 minutes for building the Random Forest model and about 2 milliseconds to apply the model to a new sample and to estimate the desired parameter.

2.6. Statistical analysis

A one-way ANOVA was performed to study the relationship between the most important quality parameters (total chlorophyll content, ammonia content, hue angle and electrolyte leakage) and the QLs during the rocket leaves cold storage (10 °C) with the aim of identifying the physical and chemical parameters able to classify in an objective and consistent way the QLs of rocket leaves.
The mean values were separated using the Student-Newman-Keuls (SNK) test and Statgraphics Centurion (version 18.1.12, Warrenton, Virginia, USA) was used for statistical analyses.
Partial least squares regression (PLSR) was run using the software The Unscrambler X.

3. Results and Discussion

3.1. Changes in quality parameters during storage of fresh-cut rocket leaves

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

During storage the change in the sensory QL from 5 to 1 was mainly due to the yellowness of rocket leaves, as showed in Figure 3.

Table 2. Hue angle, total chlorophyll content, ammonia content and electrolyte leakage in fresh-cut rocket leaves cultivated on different cultivation systems (soil, soilless or all samples) and stored at 10 °C at each quality level

Parameter	***Quality Level***										***P*-value**
	5 *very good*		**4** *good*		**3*** *fair*		**2** *poor*		**1** *very poor*		
Soil system											
Hue angle (*h*°)	125.6	a	124.7	a	121.3	b	118.1	c	114.4	d	****
Total chlorophyll content (mg g^{-1})	0.9	a	0.7	b	0.6	c	0.5	d	0.4	e	****
Ammonia content (µg NH_4^+ g^{-1})	7.3	c	3.1	c	6.8	c	89.7	b	184.3	a	****
Electrolyte leakage (%)	12.7	e	21.0	d	26.2	c	30.8	b	40.7	a	****
Soilless system											
Hue angle (*h*°)	126.8	a	125.5	b	123	c	119.6	d	116.8	e	****
Total chlorophyll content (mg g^{-1})	0.7	a	0.7	a	0.5	b	0.4	c	0.4	c	****
Ammonia content (µg NH_4^+ g^{-1})	4.3	c	2.9	c	9.9	c	38.5	b	80.1	a	****
Electrolyte leakage (%)	18.9	c	20.3	c	23.5	b	27.2	a	28.0	a	****
All samples (soil and soilless)											
Hue angle (*h*°)	126.1	a	125.2	b	122.2	c	118.8	d	115.6	e	****
Total chlorophyll content (mg g^{-1})	0.8	a	0.7	b	0.6	c	0.5	d	0.4	e	****
Ammonia content (µg NH_4^+ g^{-1})	3.0	c	6.0	c	8.4	c	67.8	b	133.7	a	****
Electrolyte leakage (%)	15.9	e	20.6	d	24.6	c	28.8	b	34.1	a	****

*: limit of marketability.
For each parameter the mean values followed by different letters are significantly different (*P*-value < 0.05) according to Student-Newman-Keuls (SNK) test.
Significance: **** = significant at *P*-value ≤ 0.0001
Quality level: 5=very good; 4= good; 3=fair; 2=poor; 1=very poor

Considering the quality parameters determined during the rocket leaves storage, a significant separation of the visual QL was obtained by colour analysis (Table 2). In detail, for samples cultivated on soil system the *h*° colour parameter separated the leaves very good (QL5) and good (QL4) from

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

fair (QL3), poor (QL2) and very poor (QL1) ones, showing a decrease of about 8.9 % from QL5 to QL1. In soilless cultivation system, all the QLs of rocket leaves were well separated by *h*°. Its value decreased from 126.8 ± 0.9 in QL5 to 116.8 ± 3.2 in waste samples (QL1). The same QL separation was obtained by *h*° value when all samples (soilless and soil) were considered (Table 2). Similar results were reported by Mastrandrea et al. (2016) in which *h*° values decreased from 124 to 115 in rocket leaves stored at 10 °C. Hue angle is considered a qualitative attribute of colour, according to which colours are defined as greenish, reddish, yellowish and so on. Higher *h*° represents a lower yellow trait in the product (Pathare et al. 2013). In the present study, *h*° values decreased during the storage, indicating a gradual yellowing of rocket leaves from QL5 to QL1.

Moreover, a significant relationship was also found between decreasing visual QL and total chlorophyll and ammonia content, which can be considered objective markers of quality loss for rocket leaves (Table 2). In particular, for samples produced on soil system, total chlorophyll content was able to discriminate all the QLs, recording a significant decrease from QL5 (0.9 mg g^{-1}) to QL1 (0.4 mg g^{-1}). The chlorophyll content allowed to have the same QL discrimination when the samples from soil and soilless system were joined (Table 2). For samples cultivated on soilless system, the chlorophyll content at harvest was 24.6 % lower than that measured in samples cultivated on soil system. Many research works proved the influence of pre-harvest factors on the postharvest quality of vegetables and the cultivation system is one of them (Frezza et al. 2010; Elia and Colelli, 2009). In this study, the soilless system produced a lower (-24 %) chlorophyll content at harvest (QL5) with respect to the soil one, reducing the intensity of the green colour of the leaves. On the contrary, at the end of storage (QL1), the samples cultivated on the two different system, showed similar values (about 0.4 mg g^{-1}) with a reduction of about 50 % for the samples from soil system. Additionally, the chlorophyll content in samples grown on soilless system proved to discriminate the very good (QL5) and good (QL4) rocket leaves from fair (QL3), in which a decrease of 16.3 % was recorded;

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

furthermore, marketable samples (QL3) were well discriminated from edible (QL2) and waste (QL1) ones. The chlorophyll degradation during the postharvest storage is strictly related to the senescence of the product. Chlorophyll plays an important role as primary photosynthetic pigment and it is responsible of the specific colour of the plant leaf. It is commonly used as parameter of maturity, quality and freshness of green leafy vegetables because it is involved into leaves senescence process (Limantara et al. 2015; Cavallo et al. 2017). At harvest, rocket leaves are dark or bright green in colour, but during senescence changes into yellow, with a general loss of visual quality. It is well known that one of the factors associated with the loss of freshness and marketability in green leafy vegetables, such as rocket leaves, is an increase of yellowing associated to chlorophyll degradation (Cefola et al. 2010; Watkins, 2006). This process involves many enzymatic reactions in chloroplasts and vacuoles and, particularly, leaves yellowing is strictly related to the activity of chlorophyllase, an enzyme that catalyzes the Type I chlorophyll breakdown in vegetables (Matile et al. 1999; Shi et al. 2016; Li et al. 2017). Many authors suggested that ethylene production in damaged tissues (such as in fresh-cut products) is responsible for the chlorophyll loss because it causes an increase of the chlorophyllase activity (Yamauchi and Watada, 1991). Moreover, some studies about chlorophyll loss in fresh-cut produces reported evidence of the breakdown induced by oxygen radical oxidation of the chlorophyll. Indeed, chlorophyll breakdown take place also when a physiological stress occurs on the tissues, such as the mechanical stress induced by cutting (Toivonen and Brummel, 2008; Torales et al. 2020). This chlorophyll breakdown related to the action of oxidative enzymes is classified as Type II and it can be controlled by antioxidant treatments, as reported by Cefola and Pace (2015) who used oxalic acid treatments to control the chlorophyll oxidation on rocket leaves during storage at 8 °C. In the present study, the chlorophyll decrease, resulting in yellowing of leaves at the end of storage at 10 °C, was probably due to both the types of breakdown (Type I and Type II)

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

above mentioned, in accordance with the evolution of the activity of the enzymes related to the chlorophyll degradation observed by Torales et al. 2020 on fresh-cut rocket leaves during cold stored. The same QL discrimination was observed in the case of ammonia content for both cultivation systems and for all samples, recording a rapid increase from QL3 to QL1 (Table 2). In particular, this parameter well separated the marketable samples (QL5, QL4 and QL3) from the non-marketable ones (QL2 and QL1); moreover, even the waste samples (QL1) were well discriminated from the edible ones (QL2). At QL1 samples cultivated on soil system showed 56.3 % higher ammonia content than that grown on soilless system. Similar results were reported by Pace et al. (2014) who identify in the ammonia content a good classifier for whole and fresh-cut iceberg lettuce, separating the acceptable product (ranged from QL5 to QL3) from the edible (QL2) and waste (QL1) ones. Ammonia accumulation in plant tissues as a consequence of protein catabolism is another aspect associated with the leaf senescence in leafy vegetables. High concentrations of this compound may cause tissue damages with visible senescence effects, that impact on the overall quality evaluation of the product. Chibnall (1939) first reported that ammonia accumulation was the cause of darkening and browning of detached leaves during postharvest, also later demonstrated by Cantwell et al. (2010) on spinach leaves and Mastrandrea et al. (2016) on rocket leaves. Generally, leafy vegetables under stressful condition showed a reduction in the glutamine synthetase activity, an enzyme that leads to the ammonia reintegration during protein synthesis (Chandra et al. 2006). So, large amount of ammonia is accumulated into cells because of its toxicity, but this cause tissue damages (Toivonen, 1997). In addition, ammonia accumulation occurs very often in closed systems, such as a package, where may reach high levels. Indeed, in minimally processed products, deteriorative processes like proteolysis are enhanced by injuries occurred during handling steps, especially in highly active products (such as green leafy vegetables) (Wang et al. 2004; Cefola et al. 2010), and by the activity of phenylalanine ammonia-lyase, that cause lignification of tissues, releasing ammonia (Joy, 1988). Moreover, Yang et al. 1982 demonstrated that also ethylene biosynthesis process from methionine produces little amount of ammonia. Many authors demonstrated that ammonia is a good indicator of rocket senescence as of other leafy vegetables and its accumulation could be the cause of the darkening process linked to leaves senescence (Chibnall,1939). Mastrandrea et al. (2016), and subsequently confirmed by Amodio et al. (2018), reported high correlations ($R^2 > 0.98$) between changes in ammonia content and hue angle variations (that correspond to yellowing) in rocket leaves stored at 10 °C. As for the detection of ammonia content in leafy vegetables by CVS, an application of this non-invasive methodology on whole and fresh-cut lettuce was reported by Pace et al. (2014) to give a non-destructive evaluation of this chemical parameter particularly useful for the objective evaluation of food quality. In particular, the Authors studied the correlation coefficient between the quality levels and the ammonia content of products using the percent brown area of images acquired by CVS to build predictive models.

The electrolyte leakage was closely related to the quality and shelf life of fresh-cut produce and it is a physical parameter commonly used to measure the intensity of oxidative damages to cell membranes due to reactive oxygen species development in fresh-cut tissues (Kou et al. 2014). So, higher values in electrolytic leakage indicate higher physiological stress of leaves tissues. In this research, electrolyte leakage significantly increased in rocket leaves obtained by soil system (from 12.7 ± 4.2 % in QL5 to 40.7 ± 7.6 % in QL1), well discriminating all the five QLs (Table 2). The same QL separation was observed considering all samples, with a significant increase of electrolyte leakage from the QL5 (15.9 ± 4.9 %) to QL1 (34.1 ± 9.1 %). On the contrary, this parameter in rocket leaves cultivated on soilless system proved to significantly discriminate very good (QL5) and good (QL4) from fair (QL3), recording an increase of 24.3 %. Moreover, in this cultivation system, the QL3 samples were well separated from edible (QL2) and waste ones (QL1). Similar results were reported by Palumbo et al. (2021) on rocket leaves cultivated under soilless cultivation system and stored at 10 °C, in which this parameter was able to discriminate the marketable samples (QL5 and QL4) from the fair (QL3) and the waste (QL2 and QL1) ones. In the present research work, the percentage increase of electrolyte leakage along the different QLs in rocket leaves grown on soil system was higher (220.5 %) than that detected on samples cultivated under soilless system (48.1 %), pointing out that the last cultivation system was probably more efficient in terms of reduction of induced oxidative stresses on cell membranes (Bonasia et al. 2017).

3.2. Non-destructive quality evaluation of packed and unpacked rocket leaves by CVS

Computer Vision System was applied to estimate the visual QL of packaged fresh-cut rocket and to predict the total chlorophyll and the ammonia content in packaged and unpackaged rocket leaves. Regarding the first task, table 3, 4 and 5 show the results obtained considering three different cases: i) to separate non-marketable product (QL 1 and 2) from marketable product (QL from 3 to 5); ii) to increase the resolution to separate edible but not marketable product (QL 2) from waste (QL 1); iii) to further increase the resolution to separate also the limit of marketable product (QL 3).
In all these case, the CVS was able to operate through the packaging with negligible loss of accuracy. Moreover, the increase in class separation (from Table 3 to Table 5) showed a reduction in accuracy on soilless samples (and therefore when all the samples were considered). Instead, the accuracy remained at high level for samples coming from the soil system.

Table 3. Confusion matrix obtained separating 2 class (QL 1-2, QL 3-4-5).

		Unpacked			***Packed***		
	QL	**QL**		*Accuracy* (r)	**QL**		*Accuracy* (r)
		1-2	**3-4-5**		**1-2**	**3-4-5**	
All samples	**1-2**	273	63	0.899	281	55	0.907
	3-4-5	26	517		27	516	

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

Soil	**1-2**	152	4	0.981	141	15	0.947
	3-4-5	3	270		7	266	
Soilless	**1-2**	147	33	0.893	146	34	0.891
	3-4-5	15	255		15	255	

Table 4. Confusion matrix obtained separating 3 class (QL 1, QL2, QL 3-4-5).

	QL	***Unpacked***				***Packed***			
		QL			*Accuracy* (r)	**QL**			*Accuracy* (r)
		1	**2**	**3-4-5**		**1**	**2**	**3-4-5**	
All samples	**1**	116	34	18	0.831	122	37	9	0.826
	2	22	86	60		28	79	61	
	3-4-5	1	13	528		0	18	525	
Soil	**1**	72	6	0	0.954	69	9	0	0.905
	2	6	68	4		9	53	16	
	3-4-5	0	4	269		0	6	267	
Soilless	**1**	74	13	3	0.829	69	18	3	0.826
	2	14	40	36		10	43	37	
	3-4-5	0	11	259		0	10	260	

Table 5. Confusion matrix obtained separating 4 class (QL 1, QL2, QL 3, QL 4-5).

QL	***Unpacked***	***Packed***
	QL	**QL**

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

		1	2	3	4-5	*Accuracy* (r)	1	2	3	4-5	*Accuracy* (r)
All samples	**1**	119	42	6	2	0.738	121	42	3	1	0.724
	2	24	99	16	29		28	101	11	27	
	3	1	23	72	72		0	29	57	81	
	4-5	0	1	14	360		0	7	12	356	
Soil	**1**	71	7	0	0	0.909	68	10	0	0	0.86
	2	6	68	4	0		10	59	7	2	
	3	0	4	62	12		0	9	55	14	
	4-5	0	0	6	189		0	1	7	188	
Soilless	**1**	74	15	1	0	0.703	70	20	0	0	0.673
	2	14	51	15	10		12	52	8	18	
	3	0	19	22	49		1	16	15	58	
	4-5	0	1	9	170		0	6	8	166	

The results obtained by the CVS in estimating the chlorophyll and ammonia contents of all the samples and of items cultivated on soil or soilless are reported in Table 6. For each case, the Pearson's correlation coefficient and MSE are shown. For each value, the best result and the average of results obtained over 10 repetitions are reported. The tables compare the results obtained on unpackaged product with the ones obtained on packaged product. Figure 4 plots the values estimated by the CVS against the values measured in the lab for ammonia on unpackaged (A) and packaged (B) products and chlorophyll content on unpackaged (C) and packaged (D) samples. In figure 4 all the samples are drawn, regardless their cultivation system. The negligible differences between the best values and the average ones show that the randomness used in the construction of the model does not affect significantly the performance of the method. The small differences between the values related to unpackaged and packaged products confirm that the method is able to operate also through the bag.

Table 6. Mean Squared Error (MSE) and Pearson's correlation coefficient (r) measured to predict by CVS total chlorophyll and ammonia content of unpackaged and packaged fresh-cut rocket.

Samples		***Total Chlorophyll***				***Ammonia Content***			
		Unpackaged		Packaged		Unpackaged		Packaged	
		Best	Average	Best	Average	Best	Average	Best	Average
Soil and Soilless	MSE	77.62	78.65	90.23	91.41	575.1	588.7	643.0	656.3
	r	0.87	0.87	0.84	0.84	0.92	0.92	0.91	0.91
Soil	MSE	69.55	70.45	72.20	74.05	746.9	782.1	872.5	908.0
	r	0.90	0.90	0.90	0.89	0.94	0.94	0.93	0.92
Soilless	MSE	66.52	68.42	83.81	84.68	284.3	289.0	314.6	326.0
	r	0.83	0.83	0.78	0.78	0.87	0.86	0.85	0.84

3.3. Estimation of visual quality level of packed fresh-cut rocket using as predictors total chlorophyll and ammonia measured by conventional methods or by CVS.

Three PLS models were built to predict the visual QL using as predictors the total chlorophyll and the ammonia contents obtained by destructive methods (Model I), CVS trough packaging (Model II) and CVS without packaging (Model III). The points in the graphics, for each model, represent the average of the data for each QL for Soil and Soilless systems.

The Figure 5 shows the biplot and the comparison between predicted and reference values for calibration (blu) and validation (red) step for three model: Model I is related to data measured in the laboratory, Model II uses data estimated by the CVS on packaged products, Model III uses data estimated by the CVS on unpackaged products. In the graphics, the two factors are a linear combination of both the predictors (chlorophyll and ammonia) even if Factor 1 and Factor 2 weight more the ammonia and the total chlorophyll contents respectively. The predictors estimated non-destructively and contactless by the CVS (Model II and Model III) provide better performances in predicting the visual QL than the ones measured by the destructive analysis in the laboratory, in both calibration and validation. This is probably due to the wider area of product observed by the CVS,

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

larger than the amount of product used by the destructive methods. It is also evident that the difference between Model II (related to the CVS applied to packaged product) and Model III (related to the CVS applied to unpackaged product) is negligible. These results further confirm the ability of the CVS to evaluate the product also through the bag, even working only on the regions of the image that provide meaningful colour information about the product's surface.

4. Conclusion

The research described in this paper aims to develop and validate a CVS that can assess the quality changes of fresh-cut rocket leaves and estimate their total chlorophyll and ammonia contents during postharvest storage, in a non-destructive and contactless way. All the quality parameters determined by destructive conventional methods during the fresh-cut rocket leaves storage were significant to separate the visual QLs and, among them, total chlorophyll and ammonia contents were very useful marker parameters in the assessment of all the considered QLs.

The CVS was able to operate even through the packaging material, enabling these controls along the whole supply chain.

The proposed CVS is based on the use of calibrated colour images and on the proper combination of image processing techniques and machine learning models. It was able to solve the classification problem (assigning the visual quality level) and the regression problems (estimating the chlorophyll and ammonia contents) using the same supervised learning methodology (Random Forest) applied to proper training data. The results proved that the system can be a valid alternative to conventional destructive measures, offering the advantage of being non-destructive, contactless, fast and cheap. Moreover, experiments showed that the loss in performance due to the observation of product through the packaging is negligible.

The PLS models built to predict the visual QL using as predictors total chlorophyll and ammonia content further confirmed the ability of the CVS to operate also through the packaging. The predictors estimated non-destructively and contactless by the CVS (Model II and Model III) outperformed the ones measured by destructive analysis in the laboratory in predicting the visual QL in both calibration and validation.

Therefore, the developed CVS represents a cheap, fast, non-destructive and contactless tool to monitor packaged and unpackaged products along the whole supply chain.

Acknowledgments

The authors thank Michele Attolico of STIIMA-CNR and Arturo Argentieri of ISASI-CNR for the technical support to the configuration of the experimental set-up, Massimo Franchi and Mariella Quarto of CNR-ISPA for the technical and administrative support, respectively.

This research was funded by the project Prin 2017 “SUS&LOW-Sustaining low-impact practices in horticulture through non-destructive approach to provide more information on fresh produce history and quality” (grant number: 201785Z5H9) from the Italian Ministry of Education University.

References

Agarwal, A., Gupta, S.D., 2018. Assessment of spinach seedling health status and chlorophyll content by multivariate data analysis and multiple linear regression of leaf image features. Comput. Electron. Agric. 152, 281-289. https://doi.org/10.1016/j.compag.2018.06.048

Amodio, M.L., Colelli, G., Cantwell, M.I., 2018. Ammonia accumulation in plant tissues: a potentially useful indicator of postharvest physiological stress. Acta Hortic. 1194, 1511–1518. doi:10.17660/actahortic.2018.1194.211

Bonasia, A., Lazzizera, C., Elia, A., Conversa, G., 2017. Nutritional, biophysical and physiological characteristics of wild rocket genotypes as affected by soilless cultivation system, salinity level of nutrient solution and growing period. Front. Plant Sci. 8, 300. https://doi.org/10.3389/fpls.2017.00300

Breiman, L., 1996. Bagging predictors. Mach. Learn. 24, 123–140

Breiman, L., 2001. Random forests. Mach. Learn. 45, 5–32

Cantwell, M., Hong, G., Nie, X., 2009. Using tissue ammonia and fermentative volatile concentrations as indicators of beneficial and stressful modified atmospheres for leafy and floral vegetables, in: X International Controlled and Modified Atmosphere Research Conference. 876 (pp. 165-172). 10.17660/ActaHortic.2010.876.20

Cavallo, D.P., Cefola, M., Pace, B., Logrieco, A.F., Attolico, G., 2018. Non-destructive automatic quality evaluation of fresh-cut iceberg lettuce through packaging material. J. Food Eng. 223, 46-52. https://doi.org/10.1016/j.jfoodeng.2017.11.042

Cavallo, D.P., Cefola, M., Pace, B., Logrieco, A.F., Attolico, G., 2017. Contactless and non-destructive chlorophyll content prediction by random forest regression: A case study on fresh-cut rocket leaves. Comput. Electron. Agric. 140, 303-310. https://doi.org/10.1016/j.compag.2017.06.012

Cefola, M., Pace, B., 2015. Application of oxalic acid to preserve the overall quality of rocket and baby spinach leaves during storage. J. Food Process. 39(6), 2523-2532. doi:10.1111/jfpp.12502

Cefola, M., Amodio, M.L., Rinaldi, R., Vanadia, S., Colelli, G., 2010. Exposure to 1-methylcyclopropene (1-MCP) delays the effects of ethylene on fresh-cut broccoli raab (Brassica rapa L.). Postharvest Biol. Technol. 58(1), 29-35. https://doi.org/10.1016/j.postharvbio.2010.05.001

Chandra, D., Matsui, T., Suzuki, H., Kosugi, Y., 2006. Postharvest changes in some physiological traits and activities of ammonia-assimilating enzymes in lettuce during storage. Asian J. Plant Sci. 5 (2), 378–384. https://doi.org/10.3923/ajps.2006.378.384.

Chen, P., Haboudane, D., Tremblay, N., Wang, J., Vigneault, P., Li, B., 2010. New spectral indicator assessing the efficiency of crop nitrogen treatment in corn and wheat. Remote Sens. Environ. 114(9), 1987-1997. https://doi.org/10.1016/j.rse.2010.04.006

Chibnall, A.C., 1939. The role of proteins in the respiration of detached leaves, in: Chibnall, A.C. (Ed), Protein Metabolism in the Plant. New Haven, CT, USA: Yale University Press, pp. 211–243.

Colelli, G., Elia, A., 2009. I prodotti ortofrutticoli di IV gamma: aspetti fisiologici e tecnologici. Italus Hortus. 16(1), 55-78.

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

Fadda, A., Pace, B., Angioni, A., Barberis, A., Cefola, M., 2016. Suitability for ready-to-eat processing and preservation of six green and red baby leaves cultivars and evaluation of their antioxidant value during storage and after the expiration date. J. Food Process. 40(3), 550-558. https://doi.org/10.1111/jfpp.12634

Ferrante, A., Maggiore, T., 2007. Chlorophyll *a* fluorescence measurements to evaluate storage time and temperature of Valeriana leafy vegetables. Postharvest Biol. Technol. 45(1), 73-80. https://doi.org/10.1016/j.postharvbio.2007.02.003

Frezza, D., Logegaray, V.R., León, A.P., Harris, M., Chiesa, A., 2009. Rocket (*Eruca sativa* Mill.) quality affected by preharvest and postharvest factors, in: Southeast Asia Symposium on Quality and Safety of Fresh and Fresh-Cut Produce. 875 (pp. 357-364). 10.17660/ActaHortic.2010.875.45

Joy, K.W., 1988. Ammonia, glutamine, and asparagine: a carbon–nitrogen interface. Can. J. Bot. 66(10), 2103-2109. https://doi.org/10.1139/b88-288

Kou, L., Yang, T., Luo, Y., Liu, X., Huang, L., Codling, E., 2014. Pre-harvest calcium application increases biomass and delays senescence of broccoli microgreens. Postharvest Biol. Technol. 87, 70-78. https://doi.org/10.1016/j.postharvbio.2013.08.004

Li, D., Li, L., Ge, Z., Limwachiranon, J., Ban, Z., Yang, D., Luo, Z., 2017. Effects of hydrogen sulfide on yellowing and energy metabolism in broccoli. Postharvest Biol. Technol. 129, 136-142. https://doi.org/10.1016/j.postharvbio.2017.03.017

Li, F., Mistele, B., Hu, Y., Chen, X., Schmidhalter, U., 2014. Reflectance estimation of canopy nitrogen content in winter wheat using optimised hyperspectral spectral indices and partial least squares regression. Eur. J. Agron. 52, 198-209. https://doi.org/10.1016/j.eja.2013.09.006

Limantara, L., Dettling, M., Indrawati, R., Brotosudarmo, T.H.P., 2015. Analysis on the chlorophyll content of commercial green leafy vegetables. Procedia Chem. 14, 225-231. https://doi.org/10.1016/j.proche.2015.03.032

Ling, Q., Huang, W., Jarvis, P., 2011. Use of a SPAD-502 meter to measure leaf chlorophyll concentration in Arabidopsis thaliana. Photosyn. Res. 107(2), 209-214. https://doi.org/10.1007/s11120-010-9606-0

Liu, Z.A., Yang, J.P., Yang, Z.C., 2012. Using a chlorophyll meter to estimate tea leaf chlorophyll and nitrogen contents. J. Soil Sci. Plant Nutr. 12(2), 339-348. http://dx.doi.org/10.4067/S0718-95162012000200013

Mastrandrea, L., Amodio, M.L., Cantwell, M.I., 2016. Modeling ammonia accumulation and color changes of arugula (*Diplotaxis tenuifolia*) leaves in relation to temperature, storage time and cultivar. Acta Hortic. 1141, 275-282. DOI: 10.17660/ActaHortic.2016.1141.34

Matile, P., Hörtensteiner, S., Thomas, H., 1999. Chlorophyll degradation. Annu. Rev. Plant Biol., 50(1), 67-95. https://doi.org/10.1146/annurev.arplant.50.1.67

Mohan, P.J., Gupta, S.D., 2019. Intelligent image analysis for retrieval of leaf chlorophyll content of rice from digital images of smartphone under natural light. Photosynthetica. 57(2), 388-398. DOI: 10.32615/ps.2019.046

Novichonok, E.V., Novichonok, A.O., Kurbatova, J.A., Markovskaya, E.F., 2016. Use of the atLEAF+ chlorophyll meter for a nondestructive estimate of chlorophyll content. Photosynthetica. 54(1), 130-137. DOI: 10.1007/s11099-015-0172-8

Pace, B., Cavallo, D.P., Cefola, M., Burbaci, S., Attolico, G., 2019. Colour analysis to predict the total chlorophyll content of rocket leaves, in: VI International Symposium on Applications of Modelling as an Innovative Technology in the Horticultural Supply Chain Model-IT 1311 (pp. 107-112). 10.17660/ActaHortic.2021.1311.14

Pace, B., Cavallo, D.P., Cefola, M., Attolico, G., 2017. Automatic identification of relevant colors in non-destructive quality evaluation of fresh salad vegetables. Int. J. of Food Processing Technol. 4(1), 1-5. DOI: http://dx.doi.org/10.15379/2408-9826.2017.04.01.01

Pace, B., Cavallo, D.P., Cefola, M., Colella, R., Attolico, G., 2015. Adaptive self-configuring computer vision system for quality evaluation of fresh-cut radicchio. Innov. Food Sci. Emerg. Technol. 32, 200-207. https://doi.org/10.1016/j.ifset.2015.10.001

Pace, B., Cefola, M., Da Pelo, P., Renna, F., Attolico, G., 2014. Non-destructive evaluation of quality and ammonia content in whole and fresh-cut lettuce by computer vision system. Food Res. Int. 64, 647-655. https://doi.org/10.1016/j.foodres.2014.07.037

Palumbo, M., Pace, B., Cefola, M., Montesano, F.F., Serio, F., Colelli, G., Attolico, G., 2021. Self-configuring CVS to discriminate rocket leaves according to cultivation practices and to correctly attribute visual quality level. Agronomy, 11(7), 1353. https://doi.org/10.3390/agronomy11071353

Pathare, P.B., Opara, U.L., Al-Said, F.A.J., 2013. Colour measurement and analysis in fresh and processed foods: a review. Food Bioproc Tech. 6(1), 36-60. https://doi.org/10.1007/s11947-012-0867-9

Rigon, J.P.G., Capuani, S., Fernandes, D.M., Guimarães, T.M., 2016. A novel method for the estimation of soybean chlorophyll content using a smartphone and image analysis. Photosynthetica. 54(4), 559-566. https://doi.org/10.1007/s11099-016-0214-x

Santos do Amaral, E., Silva, D.V., Dos Anjos, L., Schilling, A.C., Dalmolin, Â.C., Mielke, M.S., 2019. Relationships between reflectance and absorbance chlorophyll indices with RGB (Red, Green, Blue) image components in seedlings of tropical tree species at nursery stage. New For. 50(3), 377-388. https://doi.org/10.1007/s11056-018-9662-4

Shi, J., Gao, L., Zuo, J., Wang, Q., Wang, Q., Fan, L., 2016. Exogenous sodium nitroprusside treatment of broccoli florets extends shelf life, enhances antioxidant enzyme activity, and inhibits chlorophyll-degradation. Postharvest Biol. Technol. 116, 98-104. https://doi.org/10.1016/j.postharvbio.2016.01.007

Toivonen, P.M., Brummell, D.A., 2008. Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. Postharvest Biol. Technol. 48(1), 1-14. https://doi.org/10.1016/j.postharvbio.2007.09.004

Torales, A.C., Gutiérrez, D.R., Rodríguez, S.D.C., 2020. Influence of passive and active modified atmosphere packaging on yellowing and chlorophyll degrading enzymes activity in fresh-cut rocket leaves. Food Packag. 26, 100569. https://doi.org/10.1016/j.fpsl.2020.100569

Tudela, J.A., Marín, A., Garrido, Y., Cantwell, M., Medina-Martínez, M.S., Gil, M.I., 2013. Off-odour development in modified atmosphere packaged baby spinach is an unresolved problem. Postharvest Biol. Technol. 75, 75-85. https://doi.org/10.1016/j.postharvbio.2012.08.006

Vesali, F., Omid, M., Mobli, H., Kaleita, A., 2017. Feasibility of using smart phones to estimate chlorophyll content in corn plants. Photosynthetica. 55(4), 603-610. DOI: 10.1007/s11099-016-0677-9

Wang, Y., Wang, D., Shi, P., Omasa, K., 2014. Estimating rice chlorophyll content and leaf nitrogen concentration with a digital still color camera under natural light. Plant methods. 10(1), 1-11. https://doi.org/10.1186/1746-4811-10-36

Wang, Y., Wang, D., Zhang, G., Wang, J., 2013. Estimating nitrogen status of rice using the image segmentation of GR thresholding method. Field Crops Res. 149, 33-39. https://doi.org/10.1016/j.fcr.2013.04.007

Wang, Y.T., Yang, C.Y., Chen, Y.T., Lin, Y., Shaw, J.F., 2004. Characterization of senescence-associated proteases in postharvest broccoli florets. Plant Physiol. Biochem. 42(7-8), 663-670. https://doi.org/10.1016/j.plaphy.2004.06.003

Watkins, C.B., 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnol. Adv. 24(4), 389-409. https://doi.org/10.1016/j.biotechadv.2006.01.005

Wellburn, A.R., 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144(3), 307-313. https://doi.org/10.1016/S0176-1617(11)81192-2

Yamauchi, N., Watada, A.E., 1991. Regulated chlorophyll degradation in spinach leaves during storage. J. Am. Soc. Hortic. Sci. 116(1), 58-62. https://doi.org/10.21273/JASHS.116.1.58

Yang, S.F., Adams, D.O., Lizada, C., Yu, Y., Bradford, K.J., Cameron, A.C., Hoffman, N.E., 1982. Mechanism and regulation of ethylene biosynthesis, in: Plant growth substances. Springer, Berlin, Heidelberg (pp. 219-229). https://doi.org/10.1007/978-3-642-67720-5_21

Yuan, Z., Ata-Ul-Karim, S.T., Cao, Q., Lu, Z., Cao, W., Zhu, Y., Liu, X., 2016. Indicators for diagnosing nitrogen status of rice based on chlorophyll meter readings. Field Crops Res. 185, 12-20. https://doi.org/10.1016/j.fcr.2015.10.003

Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

Figure captions

Figure 1. Example of the set of 6 images acquired for each sample. Three images were acquired with the product inside a bag, shuffling the product between acquisitions. No care was given to the position of the product in the bag nor to the position of the bag in the scene and to the highlights created by the illumination on the surface of the bag. Then, the bag was open and three images of the same product were acquired. The leaves were randomly shuffled and stacked before each acquisition. In all the images a color-chart was placed in the scene to enable the color correction step needed to increase the consistency of color evaluation.

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

Figure 2. The flow chart of the processing done on the images. It is possible to appreciate the effects of each step on the image. The flowchart on the left is related to images of unpackaged products. The flowchart on the right describes the processing of images of packaged products. The difference is only in the artefact elimination step applied to packaged products: it is required to select the pixels where meaningful colors can be measured by the camera. Data extracted from the patches of the color chart was used to evaluate the parameters of the linear color correction model used. The final result is a histogram of colors in the a-b plane of the CIE $L^*a^*b^*$ color space: the complete histogram is used as feature vocabulary by the Random Forest classification and regression models used to assign the visual quality to samples and to estimate their chlorophyll and ammonia contents.

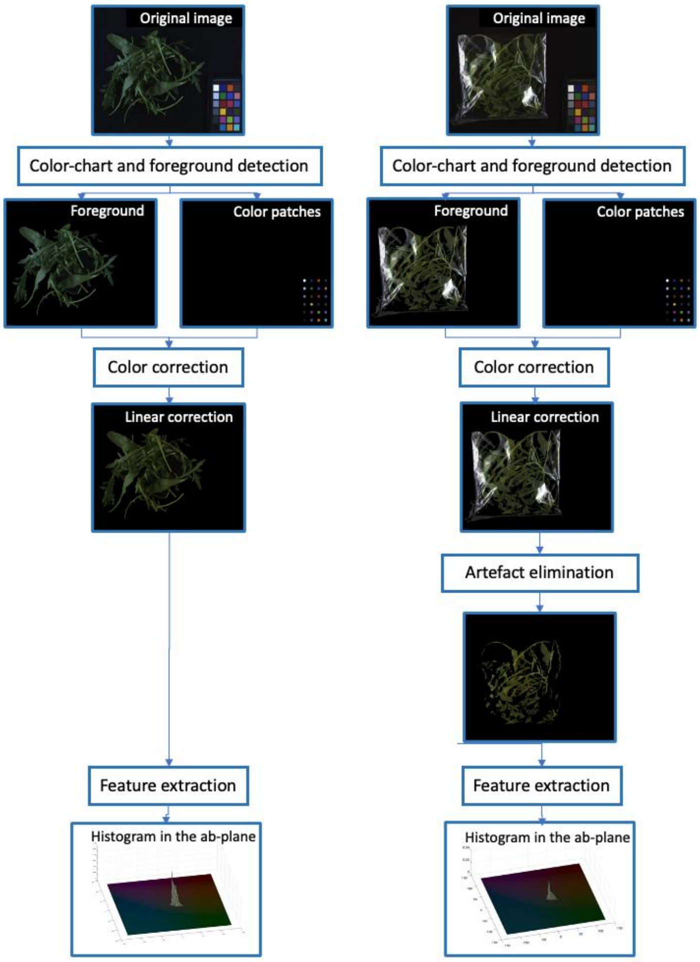

Figure 3. Changes in the sensory quality level (QL) of fresh-cut rocket leaves during the storage at 10 °C according to the 5 to 1 rating scale reported by Palumbo et al. (2021). In detail, QL5=very good; QL4= good; QL3=fair; QL2=poor; QL1=very poor.

THIS IS THE SUBMITTED VERSION.
Published version: https://doi.org/10.1016/j.postharvbio.2022.111910
Palumbo, M., Pace, B., Cefola, M., Montesano, F. F., Colelli, G., & Attolico, G. (2022). Non-destructive and contactless estimation of chlorophyll and ammonia contents in packaged fresh-cut rocket leaves by a Computer Vision System. Postharvest Biology and Technology, 189, 111910.

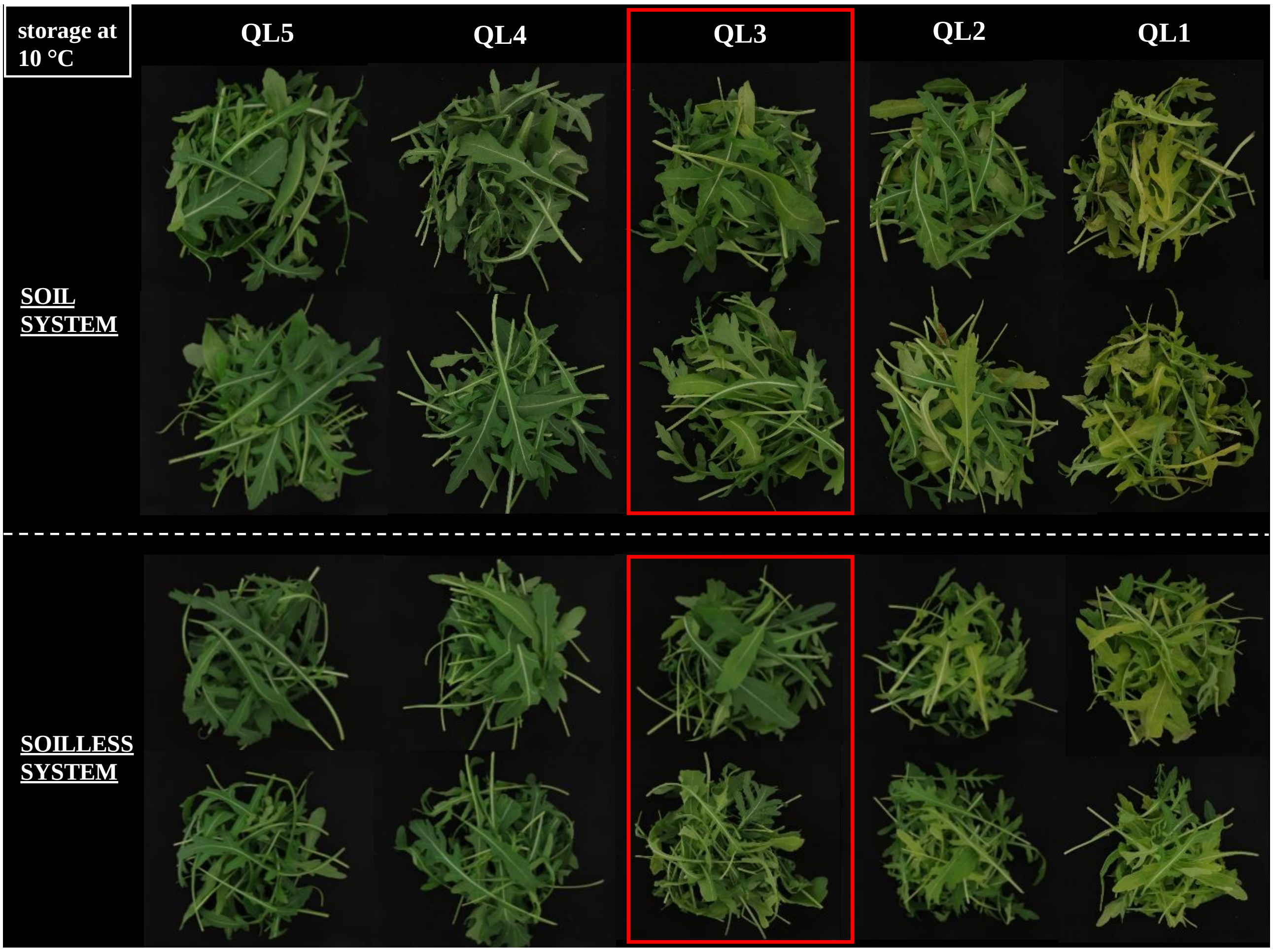

Figure 4. Values estimated by the CVS (abscissa) vs. values measured in the laboratory (ordinate) for ammonia content on unpackaged (A) and packaged (B) rocket leaves and for total chlorophyll content on unpackaged (C) and packaged (D) samples.

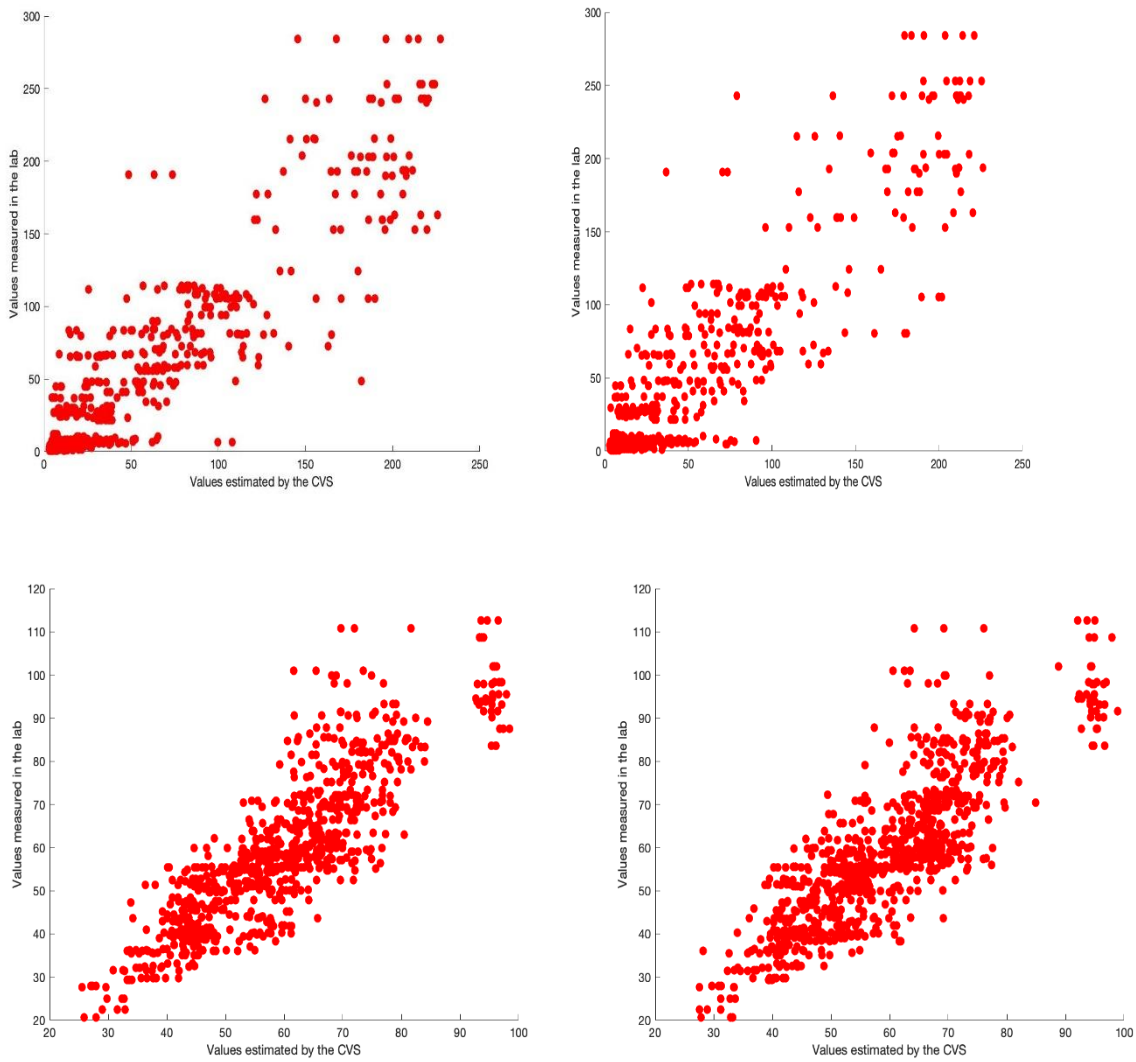

Figure 5. Models obtained by partial least squares regression (PLSR) to predict the sensory QL of fresh-cut rocket leaves through total chlorophyll and ammonia contents. For each model, the biplot and the comparison between predicted and reference for calibration (blue) and validation (red) step are reported. In the first line, the chlorophyll and ammonia data come from the laboratory (Model I),

in the middle one, the same data are estimated by the CVS on packaged product (Model II). In the last line, the data are estimated by the CVS on unpackaged product (Model III). The graphics show the validity of chlorophyll and ammonia contents estimated by the CVS. Moreover, the observation through the packaging does not decrease significantly the effectiveness of the method.