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Article

Comparison of the non-invasive monitoring of fresh-cut lettuce condition with imaging reflectance hyperspectrometer and imaging PAM-fluorimeter

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Abstract: We compared two approaches to non-invasive proximal sensing of the early changes in fresh-cut lettuce leaf quality: hyperspectral imaging and imaging PAM-fluorometry of chlorophyll contained in the leaves. The assessments made by the imaging techniques were confronted with the quality assessments made by traditional biochemical assays: relative water content and foliar pigment (chlorophyll and carotenoid) composition. The hyperspectral imaging-based approach provided the highest sensitivity to the decline of fresh-cut lettuce leaf quality taking place within 24 h from cutting. Using of the imaging PAM was complicated by (i) weak correlation of the spatial distribution pattern of the Qy parameter with the actual physiological condition of the plant object and (ii) its high degree of heterogeneity. Accordingly, the imaging PAM-based approach was sensitive only to the manifestations of leaf quality degradation only at advanced stages of the process. Sealing the leaves in the polyethylene bags slowed down the leaf quality degradation at the initial stages (< 3 days) but promoted its rate at more advanced stages, likely due to build-up of ethylene in the bags. An approach was developed to the processing of hyperspectral data for non-invasive monitoring of the lettuce leaves with a potential for implementation in greenhouses and packinghouses.

Keywords: reflectance; dehydration stress; proximal sensing; vegetation indices; pigments.

1. Introduction

Producing high-quality vegetables for the consumer is a priority goal of any grower regardless of the company size and the farm setup. The highest quality of production needs to be assured at all stages of the production chain, from farm to table. This goal calls for the development of affordable and efficient, preferably non-invasive techniques for monitoring the quality of vegetable produce. A wide array of methods has been proposed for this task; currently the most widespread approaches are based on recording of light reflected by or chlorophyll (Chl) fluorescence emitted from leaf and/or fruit [1,2].

Light reflected by plant carries ample information about its biochemical composition, tissue architecture, and physiological condition [3]. Developmental changes in pigment composition as well as those induced by the environmental stresses and attacks of phytopathogens manifest themselves as specific changes in plant reflection properties [4-6] linked with the changes in pigment content and composition. Chlorophylls are central to capture and transformation of light energy in photosynthesis, carotenoids (Car) are important for light harvesting and photoprotection [7]. Attempts to apply optical reflectance

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spectroscopy for assessment of fruit and vegetable quality and their physiological state have been undertaken for decades [2]. Current understanding of plant spectral features stems mostly from the "point-type" measurements with conventional spectroradiometers and spectrophotometers [8]. Recent technical progress paved the way for affordable imaging hyperspectrometers providing spatially resolved spectral information on plants at different scales, from plant organs (leaves and fruits) to individual plants and ecosystems. In the past two decades, the hyperspectral reflectance image (HRI)-based technology has evolved into a powerful noninvasive inspection tool commercially available as instrumentation for packing lines [9,10]. Among the Chl fluorescence-based methods, measurements of the induction of Chl fluorescence and calculation of its derived parameter known as pulse-amplitude modulation (PAM) is the most widespread approach [11,12]. With the advent of imaging spectrometers and PAM fluorometers, a breakthrough was made in this field: devices of these types are capable of capturing spatially resolved information about the object [13].

Nevertheless, the relationship between the changes in the parameters constituting what is perceived as 'quality' of vegetable produce and the spectral reflectance or fluorescent data remains in many cases uncertain. This is exacerbated by a significant heterogeneity of the monitored parameter exhibited by plants even within a single leaf. There were also doubts that imaging hyperspectrometers can be useful for monitoring plant physiological responses [14]. These uncertainties and limitations stem from insufficient understanding of the relationships between biochemistry and morphology of plant tissues and their measured optical properties. Solving these problems is a prerequisite for a confident interpretation of reflectance images, development of computer vision and robotic systems for automated crop harvesting and grading [8].

In view of the challenges outlined above, we have compared two approaches—one based on imaging PAM, and another based on imaging hyperspectral reflectometer—to monitor the early changes in quality of freshly cut lettuce leaves. The first approach is focused on the functional diagnostics of the photosynthetic apparatus and is considered the most sensitive to rapid changes in the state of photosynthetic organs and tissues of plants [12,15]. The second approach is traditional for remote sensing of plants and has significant potential for assessing the productivity of green parts of plants, biomass assessment, chlorophyll content and damage development [1]. We confronted the data acquired using both approaches with the results of traditional biochemical analyses (leaf tissue water content and pigment composition). We have proposed an original approach to process the image data and tested it in the experiment with stored fresh-cut lettuce leaves.

2. Materials and Methods

2.1. Plant material and cultivation

Lettuce (cvr. Revolution) plants were grown from commercially available seeds (Bayer, Nunhems) in two stages. The first stage was carried for 15 d out in a nursery on a turf substrate. The second stage was carried out in 1.2-L vessels in the same substrate for 20 d in vertical greenhouses (Panasonic, Japan). The fresh-cut plants were divided into two equal-sized groups (n = 9): one was stored in conventional air atmosphere; another one was stored in the resealable polyethylene bags. Both sample groups were kept in a climatic camera (Liebherr, Germany) at a constant temperature of 15 °C, relative humidity of 45%, and illuminated by white fluorescent tubes with PAR photon flux density of 50 μmol quanta m^{-2} s^{-1} as measured by LI-850 quantum meter (LiCOR, USA) at the leaf surface level. The plant parameters (see below) were measured at 0, 1, 4, 7, and 11 days of storage non-destructively. At the same time samples (n = 3 per data point) were taken for the destructive relative water content (RWC), total chlorophyll (Chl) and carotenoid (Car) content, and fatty acid assays. The corresponding methods are described below.

2.2. Pigment analysis

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Leaf RWC was assessed gravimetrically as described in [16]. Leaf samples were transferred to a glass-glass homogenizer with a chloroform-methanol (10 mL, 2:1, v/v) mixture and extracted to remove all pigment. The lipid fraction including Chl was separated according to Folch, *et al.* [17] according to the method of Solovchenko, *et al.* [18]. The chloroform phase was used for pigment analysis. Chl *a* and *b* were quantified using absorption coefficients for chloroform [19]. Average values with corresponding standard error values are presented in the figures and tables below. The significance of the average differences was tested using Student's *t*-test in Origin software (OriginLabs, USA).

2.3. Imaging PAM measurements

The spatially-resolved data on Chl fluorescence containing the induction curves of Chl fluorescence and their derived parameters were recorded and analyzed using a FluorCAM 800 imaging Pulse Amplitude Modulated (PAM) fluorometer (Photon Systems Instruments, Drasov, Czech Republic) essentially as described earlier [20]. The maximum potential photochemical quantum yield of photosystem II (Qy) was calculated as Qy = (Fm – Fo)/Fm = Fv/Fm [15]. The images were then masked, and non-fluorescing background was excluded by manual thresholding in the PAM imager software (Figure 1).

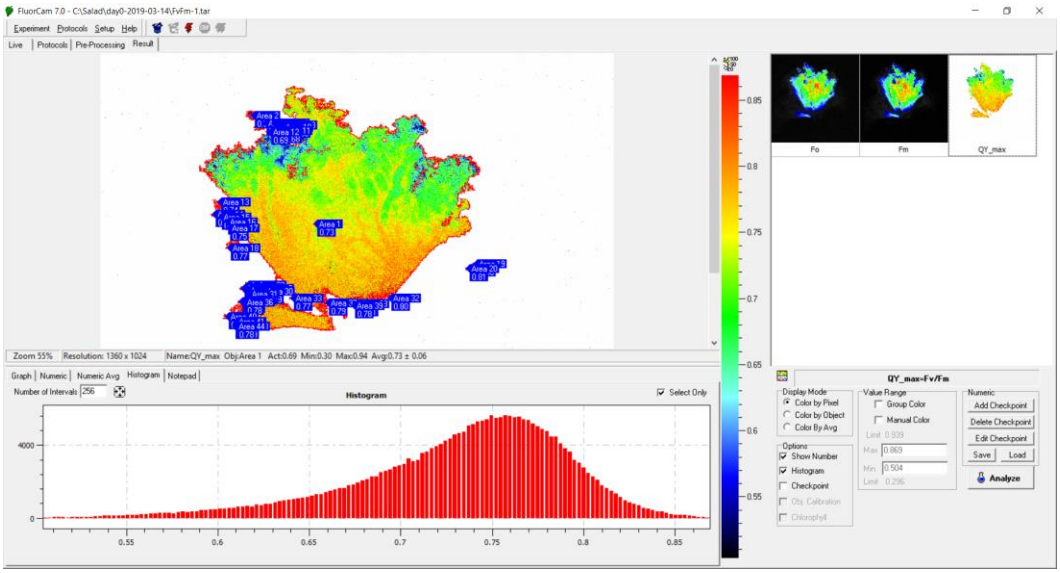

Figure 1. A representative measurement of the spatial distribution of Qy over the lettuce leaf surface with the imaging PAM fluorometer Fluorcam 800. For each image containing the Qy values as pixel values, a histogram was generated (Figure 1) and exported for further analysis in Excel spreadsheet software (Microsoft, USA). The histograms were then re-binned (i.e. integrated within the selected intervals, see text).

2.4. Hyperspectral reflectance image recording and analysis

The hyperspectral reflectance data-containing images of the lettuce plants were captured with a frame-based imaging hyperspectrometer IQ (SPECIM, Finland; Figure 2). For each pixel of the hyperspectral image, a reflectance spectrum (spectral range 400–1000 nm; spectral resolution 1 nm; 500 × 500 pixels/frame) was recorded against a reflectivity standard made of Spectralon (Figure 2) under illumination with two 150 W cold daylight fluorescent lamps.

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Figure 2. Hyperspectral recording of the fresh-cut lettuce leaves/plants with the SPECIM IQ camera (Spectral Images, Finland).

For each pixel of the hyperspectral images, two indices were computed. The first index, CI_{678}, is among the most sensitive spectral indices indicative of Chl content, [Chl], of the sample [21]. It was calculated as follows:

$$\mathrm{CI}_{678} = \frac{R_{800}}{R_{678}}, \quad (1)$$

where R_{800} is the reflectance in a band in the near infra-red (NIR) region unaffected by pigment absorption of light and R_{678} is the reflectance in the band of the red Chl absorption maximum [22].

The second spectral index was PSRI, an index tightly related with [Car]/[Chl] ratio in the samples and, ultimately, indicative of the rate and stage of senescence of plant objects [21,23,24]:

$$\mathrm{PSRI} = \frac{R_{678} - R_{480}}{R_{800}}, \quad (2)$$

where R_{800} is the reflectance in a band in the near infra-red (NIR) region unaffected by pigment absorption of light, R_{480} is the reflectance in a band affected by both [Car] and [Chl], and R_{678} is the reflectance in the band of the red Chl absorption maximum.

For each index and each hyperspectral image, histograms were calculated (Figs. S2 and S4). The histograms were re-binned (integrated) over specific ranges, normalized to the total pixel number in the corresponding image and the time-courses of changes of the corresponding integral values (Figs. A3 and A5) were analyzed. As a result of the analysis, two combined hyperspectral indexes were developed. The first one is the CI_{678}-based index HCI_{678} (hyperspectral chlorophyll index based on 678 nm band):

$$\mathrm{HCI}_{678} = \int_{6}^{10} \mathrm{CI}_{678} \left(\int_{2}^{4} \mathrm{CI}_{678} \right)^{-1} \quad (3)$$

This index is essentially a ratio of the hyperspectral image area corresponding to the leaf surface with a high [Chl] (CI_{678} values above 6) to the area the leaf surface with a low [Chl] (CI_{678} values below 4).

The second one was the PSRI-based index HPSRI (hyperspectral plant senescence reflectance index):

$$\mathrm{HPSRI} = \int_{0.2}^{0.5} \mathrm{PSRI} \left(\int_{0}^{0.1} \mathrm{PSRI} \right)^{-1} \quad (4)$$

This index represents a ratio of the hyperspectral image area corresponding to the leaf surface with a high [Car]/[Chl] ratio (PSRI values above 0.2) to the area the leaf surface

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with a low [Car]/[Chl] content (PSRI values below 0.1). The indices HCI_{678} and HPSRI were used as non-invasively monitored markers of leaf quality to be confronted with the results of traditional biochemical assay and destructive RWC measurements. A more detailed discussion of the indexes and rationale for their construction is presented below.

3. Results

3.1. Changes in water content and pigment composition of the leaves

The non-sealed lettuce leaves lost, under our experimental conditions, approximately a half of their water within one day of storage (Figure 3, closed symbols). Later, the dehydration of the leaves continued but at slower rate. By the end of the experiment the average RWC of the non-sealed leaves was around 0.2 i.e., the leaves lost 80% of their maximum water capacity. The leaves were visually wilted. By contrast, the leaves contained in PE bags retained most of their water content (Figure 3, open symbols) and remained turgescent throughout the observation period.

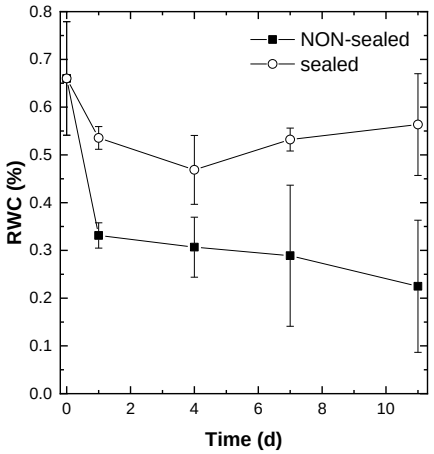

Figure 3. Changes in the relative water content during storage of the non-sealed (closed symbols) and sealed (open symbols) fresh-cut lettuce leaves. Average ± SE values are presented (n = 9).

The storage of the fresh-cut lettuce leaves was accompanied by characteristic changes in their content and composition (Figure 4). The fresh lettuce leaves were characterized by a [Chl] of 1000–1500 μg/g FW and a correspondingly high [Car] (Figure 4c). By contrast, the decline in quality of the stored leaves was accompanied by a decline in [Chl] but the decline in [Car] was slower resulting in the increase of [Car]/[Chl] (Figure 4d). The stored leaves exhibited a decline in their [Chl] regardless of storage conditions; in the non-sealed lettuce leaves Chl degradation occurred at a slightly faster rate (Figure 4a). The changes in [Car] recorded during the experiment followed a more complex pattern (Figure 4b). [Car] increased by 30% for the first 4–7 days of the experiment and underwent a sharp decline during the remaining time of the experiment. Notably, in the PE-sealed leaves the transient increase in [Car] took place later and its magnitude was lower than that observed in the non-sealed leaves. The same patterns of pigment transformation were revealed when the leaf [Car] was plotted vs. [Chl] (Figure 4c).

In frame of a more advanced approach allowing to estimate the rate of plant senescence [23], we also analyzed the trend of [Car]/[Chl] ratio plotted vs. leaf [Chl] (Figure 4D). In this approach, an increase in [Car] over that of [Chl] visually manifesting itself as yellowing of leaves is used as a marker of plant tissue senescence [23,25]. Overall, the studied leaves demonstrated a close rate of pigment transformation typical for senescence,

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but the magnitude of these changes was smaller in the PE-sealed leaves (Figure 4d, open symbols).

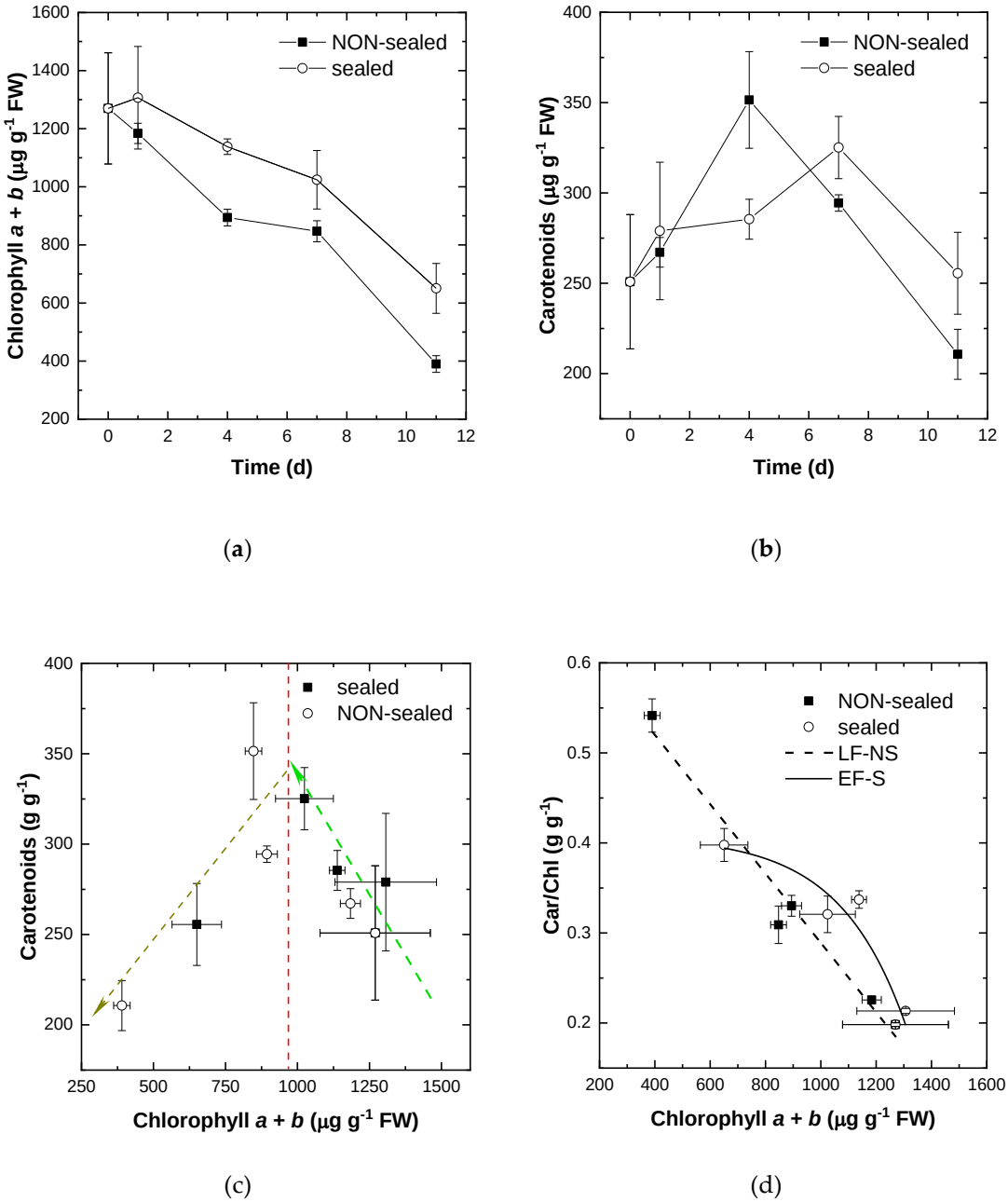

Figure 4. Changes in pigment composition during storage of the non-sealed (closed symbols) and sealed (open symbols) fresh-cut lettuce leaves. (a) Time-course of [Chl]. (b) Time-course of [Car] changes. (c) Relationship between total and [Chl] changes. (d) Changes of [Car]/[Chl] mass ratio plotted vs. changes in [Chl].

3.2. Assessment of leaf condition using imaging PAM fluorometry of Chl

In parallel with the changes in RWC and pigment content, we studied the changes in photosynthetic activity of the leaves as a possible non-invasively measured indicator of the leaf physiological condition and hence its quality [26,27]. Towards this end, we measured the parameters of the induction of Chl fluorescence in and calculated the quantum yield of photosystem II, Q_y for the whole plants using the imaging PAM fluorometer (Figure 5; see also Methods). As a result, the images were obtained whose pixels represented the Q_y values of the leaves at different stages of their storage. The calculated Q_y values

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were divided into three arbitrary chosen ranges according to the level of photosynthetic activity of the leaves [28]: 0.4–0.6 (low-to moderate photosynthetic activity, senescent/damaged leaves), 0.6–0.7 (moderate-to-high photosynthetic activity), and 0.7–0.8 (high photosynthetic activity, intact leaves). Afterwards, the relative are of the leaf image occupied by the pixels in each of the ranges defined above was calculated and plotted as a function of storage time (Figure 5).

Although one can expect an increase of the relative area of leaf surface with declined photosynthetic activity during storage of fresh-cut plants, the analysis of PAM images did not reveal distinct trends of changes in photosynthetic activity of stored leaves (Figure 5). Thus, the PE-sealed leaves demonstrated a considerable increase in the number of pixels corresponding to the expansion of leaf regions with a low photosynthetic activity (Figure 5a, open symbols). Surprisingly, the non-sealed samples showed the opposite trend (Figure 5a, closed symbols). Both experimental sets demonstrated a decline in the leaf area with a high photosynthetic activity; in most cases the trends shown by sealed and non-sealed leaves did not differ significantly (Figure 5b). Unexpectedly, the regions with a high photosynthetic activity remained the same or tended to expand both in the sealed and non-sealed stored leaves throughout the observation period (Figure 5c). Only at advanced stages of leaf dehydration we documented a sizeable decline in Q_y. None of the parameters derived from the PAM images of the stored leaves showed a significant correlation with any of the analytically determined parameters associated with leaf condition and/or perceived quality (not shown).

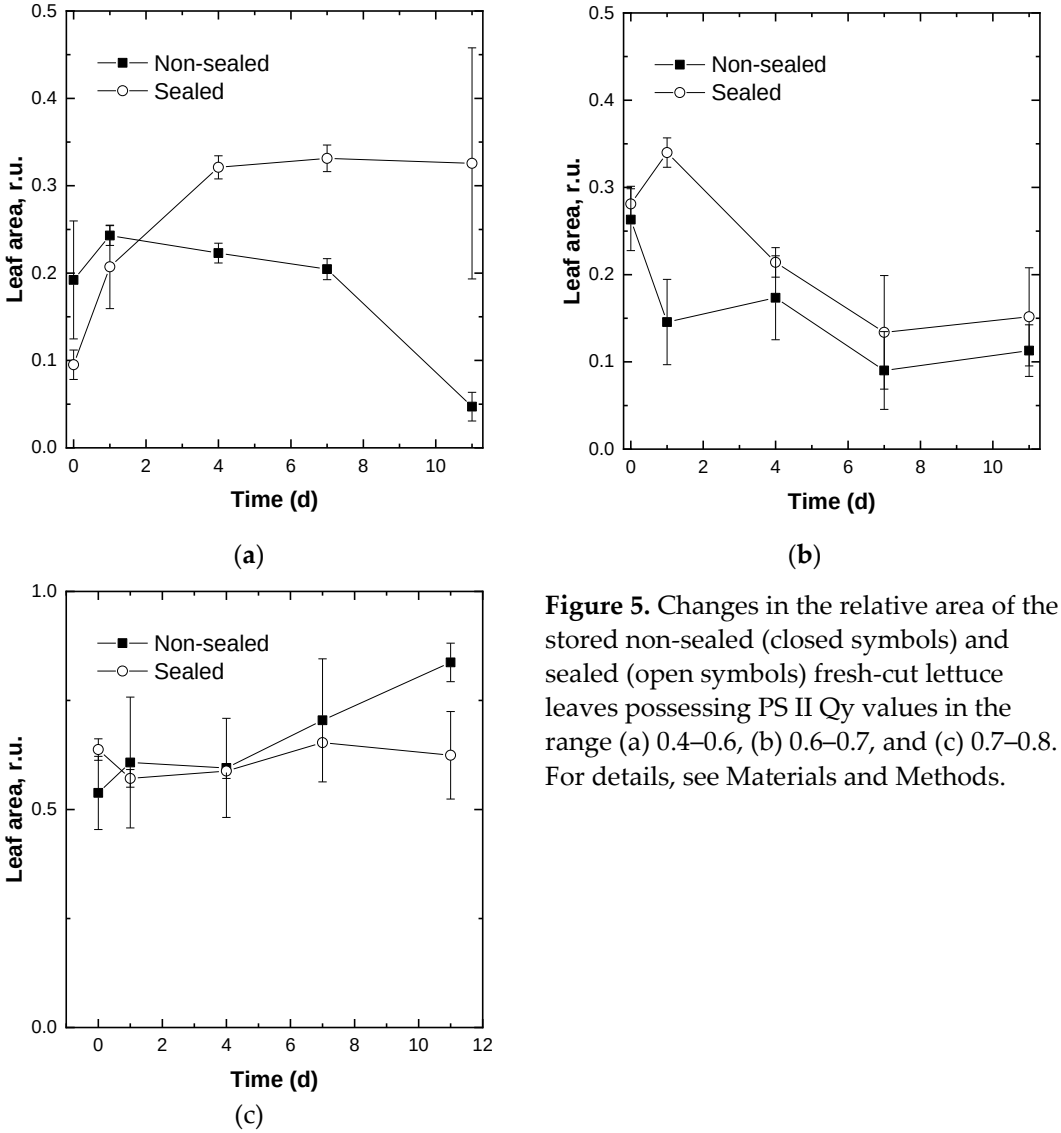

Figure 5. Changes in the relative area of the stored non-sealed (closed symbols) and sealed (open symbols) fresh-cut lettuce leaves possessing PS II Qy values in the range (a) 0.4–0.6, (b) 0.6–0.7, and (c) 0.7–0.8. For details, see Materials and Methods.

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Assessment of leaf condition using hyperspectral reflectance imaging

As an alternative approach to non-invasive monitoring of the stored leafy vegetables, the hyperspectral reflectance images (HRI) of the plants have been recorded. To find the approach to extract quantitative information about the leaf condition from the HRI, the distribution of CI_{678} index indicative of [Chl] [4,21] for each pixel corresponding to leaf surface were analyzed in different leaf samples as a function of time (Figures. 6 and S2). Overall, the HRI of the stored leaves exhibited a decline in the number of pixels corresponding to the leaf regions with a high [Chl] along with an increase in the number of pixels corresponding to the leaf regions with a lower [Chl]. This process manifested itself by the shift of the maximum of the distribution of the CI_{678} values to the left (Figure 6).

To further analyze the nature of the changes and diversity of the pigment content recorded for the entire plant (leaf) surface, the observed range of the CI_{678} variation (0–10 encompassing > 99% of all values of the index) was divided into five equal intervals. Then the changes of the number of HRI pixels in these intervals were analyzed (Figure S3). The upper limit of the underlying index CI_{678} was set to 10 (see the integral in the numerator of the Eq. 3) since higher values of the index would not give an adequate assessment of the [Chl] due to saturation of the R_{678} vs. [Chl] relationships and non-linearity resulting from it [22]. The lower limit of the CI_{678} index was set to 2 (see the integral in the numerator of the Eq. 3) since the index values of 2 and below are typical for damaged and/or senescent plant tissues or tissues inherently low on [Chl] [23,29]. Accordingly, the most conspicuous changes were comprised by a decline of the number of pixels with CI_{678} values in the range 6–10 taking place along with an increase of the number of pixels with CI_{678} in the range 2–4. These changes were ascribed to degradation of Chl manifesting itself as expansion of the leaf area with a low [Chl] at the expense of the leaf area featuring a high content of the pigment. As a result, the index for monitoring of changes in [Chl] via reflectance data incorporated in HRI was suggested in the form of the HCI_{678} index taking into account the changes in the relative leaf areas with a higher and lower [Chl]. Indeed, the HCI_{678} index demonstrated the kinetics close to that of analytically measured leaf [Chl] (Figure 7a). Moreover, this index was closely related to actual leaf [Chl] in a wide range of its variation regardless of the storage mode (sealed vs. non-sealed) as shown in Figure 7b.

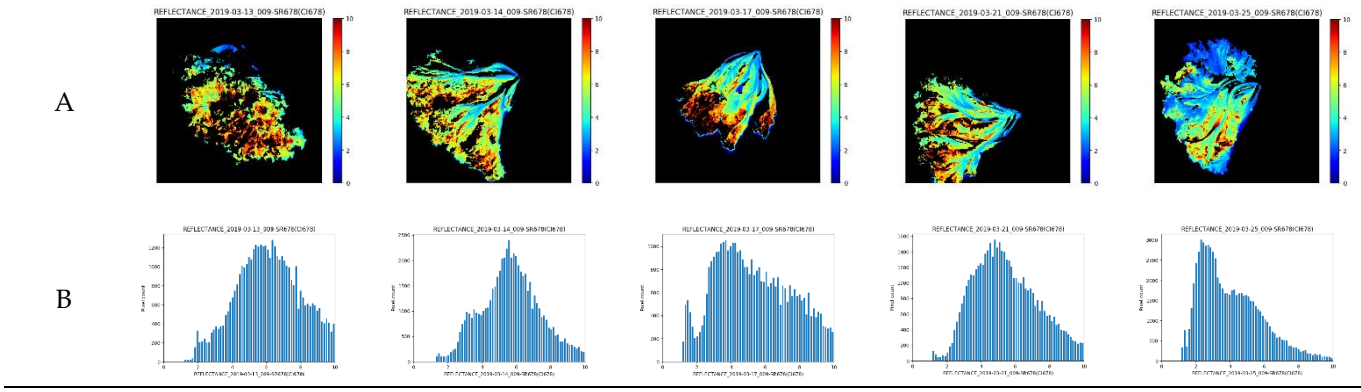

Figure 6. Representative changes in (a) leaf area exhibiting different values of CI_{678} index (color-coded) indicative of Chl content and (b) the corresponding distribution of the pixel values of the color-coded images in the stored non-sealed fresh-cut lettuce leaves. The complete dataset presented in Figure S2 of the Appendix. For details, see Materials and Methods.

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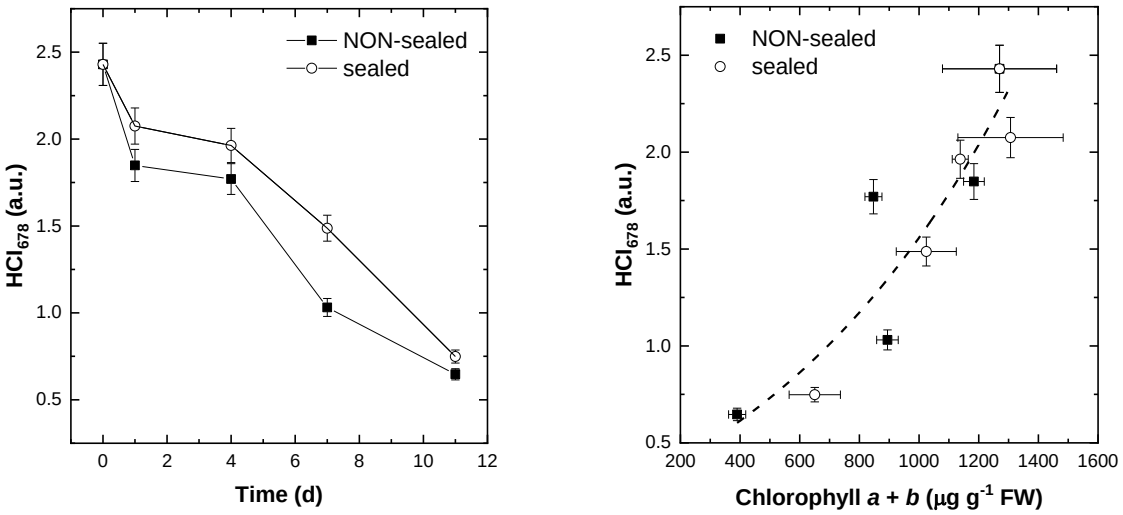

(**a**) (**b**)

Figure 7. Changes in the average values of the index HCI678 during (a) storage of and (b) decline of Chl (indicative of leaf bleaching) in the stored non-sealed (closed symbols) and sealed (open symbols) fresh-cut lettuce leaves. See also Figs. S2 and S3.

A similar approach was taken to test the applicability of PSRI, a reflectance index previously developed for the assessment of plant development and senescence [23,30], to gauge the pigment transformation taking place during storage of the cut lettuce leaves. As in the case of development of an index suitable for monitoring of [Chl] via HRI, the analysis of the HRI pixel distribution according to the value of their corresponding PSRI values was carried out (Figures S4 and S5). After that, sub-ranges with the largest amplitude of PSRI index variation in the stored leaves were suggested (Figure S5). The upper limit of the underlying index PSRI was set to 0.5 (see the integral in the numerator of the Eq. 4) since higher values of the index would belong to deeply senescent and/or damaged leaf areas non-relevant for the monitoring of fresh-cut leaf vegetables quality. The lower limit of the CI_{678} index was set to 0.1 (see the integral in the numerator of the Eq. 3) since the index values below 0.1 are typical for green tissues with a high [Chl] and do not allow for discerning the regions with a high [Chl]. As a result, the HPSRI index suitable for monitoring of pigment transformation in storing leaves via HRI data was suggested (see Methods).

Further analysis revealed that the kinetic of HPSRI changes was similar to that of [Car]/[Chl] ratio (Figs. 4d and 8a). Expectedly, HPSRI was found to be closely related with analytically determined [Car]/[Chl] ratio in a broad range of its variation although the relationship HPSRI vs. [Car]/[Chl] tended to saturate at high [Car]/[Chl] values (Figure 8b). Nevertheless, HPSRI was capable of revealing even a slight difference in the rate of the pigment transformation in the differently stored leaves (cf. open and closed symbols in Figure 8).

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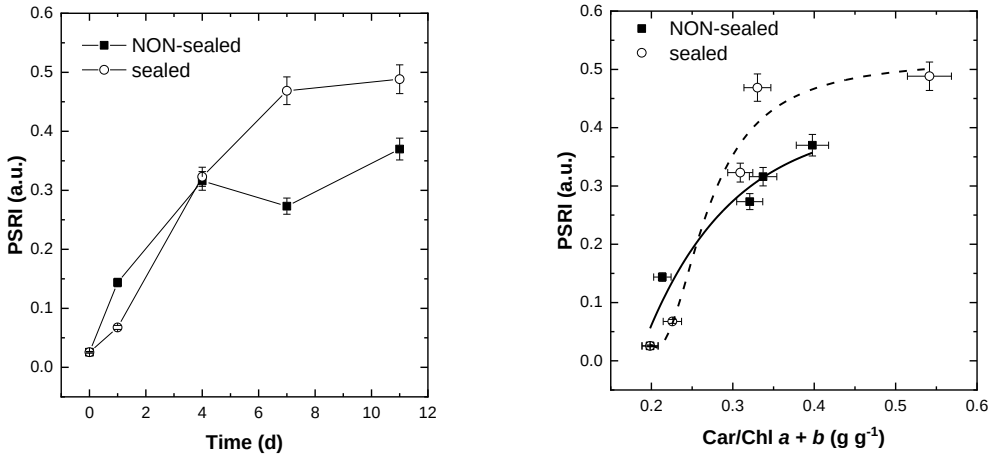

(**a**) (**b**)

Figure 8. Changes in the average values of the indicative of leaf tissue senescence index PSRI during (a) storage of and (b) decline of [Chl] (indicative of leaf discoloration) in the stored non-sealed (closed symbols) and sealed (open symbols) fresh-cut lettuce leaves. See also Figs. S4 and S5.

4. Discussion

In this study we attempted to comparatively assess the two techniques based on Chl fluorescence PAM or optical reflectance imaging as non-invasive gauges for physiological condition and pigment composition of plants using fresh-cut lettuce leaves as a model. A prerequisite for achieving this goal is finding suitable "benchmark" parameters connected with the condition of plant objects and their perceived quality. Such parameters are normally assessed analytically (destructively) by "wet" analytical methods yielding objective information about the plant objects and the degree of their degradation. These parameters comprise the "ground truth" essential for calibration and/or interpretation with confidence of any non-invasively measured parameter yielding the same information about plant object.

We tested as such relative water content (RWC) and pigment (Chl and Car) content of the leaves. Although RWC is an easily measured, straightforward parameter, its usage for monitoring of lettuce leaf condition under our experimental conditions was complicated by its essentially non-linear behavior. Thus, the major changes in RWC occurred very rapidly within the first 24 h of the experiment; afterwards RWC changed quite slowly (Figure 3). At the same time, visual inspection of the leaves did not reveal a pronounced dehydration. In our experiment, it became readily observable only at advanced stages of leaf storage without packaging (after the 7th day). On the contrary, in the PE-sealed leaves the symptoms of water deficiency were not pronounced until the end of storage period.

By contrast, pigment composition exhibited directional changes gradually taking place throughout the observation period (see e.g. Figure 4a). Thus, [Chl] content was suggested as a suitable internal marker of physiological condition and developmental changes of plant objects including leaves and fruit taking into account possible irregularities of environmental conditions [25,31]. The ratio of [Car] and [Chl] is a more specific marker of stress and senescence in plants reflecting (i) retention of Car pigments and (ii) accelerated degradation of [Chl] under adverse conditions [32].

For the purpose of this study, we also considered photosynthetic activity (specifically, potential maximal photochemical quantum yield of photosystem II, Q_y) as a potential marker of the physiological condition of the studied plant objects [12,28]. This parameter reflects the proportion of the absorbed light energy that can be potentially channeled into photochemical reactions of light reactions of photosynthesis. In our pilot assessments, the lettuce leaves exhibited a broad range of Q_y variation (0.2–0.75) in response to

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dehydration. However, monitoring of Q_y-based images of the lettuce leaves under our experimental conditions demonstrated a large degree of heterogeneity of the Chl fluorescence signal and hence Q_y over the leaf surface (see e.g., Figure 5). Overall, the analysis of PAM Q_y images recorded in the present study did not reveal a sensible pattern relating spatially resolved Q_y with changes of the leaf pigment content measured during storage. It can be explained, on one hand, by loose relationship of the variable Chl fluorescence parameters with biochemically assayed content of the pigment. On the other hand, plants often demonstrate a high physiological plasticity under limited water availability allowing them to maintain functionality of their photosynthetic apparatus [26]. Therefore, one can observe a high efficiency of photochemical reaction and hence a sizeable Q_y until advanced stages of drought stress, as was the case in the present study: on an average, more than a half of leaf surface exhibited a high Qy values up to the advanced stages of the experiment (Figure 5c) regardless of a significant loss of water. In addition to that, even the intact lettuce leaves featured a high degree of spatial heterogeneity of Q_y (see e.g. Figure 1) which increased during storage of fresh-cut plants. Likely, the changes in Q_y spatial distribution, at least during the initial period of observations, were "masked" by this high heterogeneity. This suggestion is supported by the fact that the differences in Q_y between the non-sealed and the PE-sealed leaves were mostly statistically insignificant (Figures. 5b, c).

In contrast to Chl fluorescence-based parameters, the optical reflectance-based indexes related with [Chl] and [Car] as well to their ratio (Eqs. 1–4; Figure 7) conveyed more consistent information about the condition of the studied lettuce leaves (Figures 8, 9). It might be due to (i) induction of gradual degradation of Chl and (ii) coordinated degradation or retention of Car pigments [23,29,31]. These processes are well-known manifestations of other biochemical and physiological changes accompanying senescence and storage of detached fruits and leaves [31]. Interestingly, [Car] demonstrated a bi-phasic pattern of changes in our experiments (Figure 4c), although [Chl] declined stepwise. Since Car serve for the protection of plants against oxidative stress, one may speculate that a slower degradation of Car reflects acclimation of the leaf photosynthetic apparatus to the stress caused by cutting the plants. At more advanced stages of storage, a synchronous degradation of Car and Chl was observed typical for the senescence of certain plant species [23,31].

To gain a deeper insight into the patterns of pigment transformation in the stored leaves and to estimate the applicability of the indexes developed in this work to extract the relevant information from the HRI, we used the approach previously developed by our group for monitoring of senescence of leaves and ripening of apple fruits. This approach is based on [Car]/[Chl] ratio as an internally normalized robust senescence marker scarcely influenced by environmental factors *per se* [25]. As a result, we were able to monitor non-invasively the characteristic changes of pigment transformation accompanying the loss of perceived quality in stored leafy vegetables regardless of leaf water content. Moreover, this approach was sufficiently sensitive to pinpoint the differences in timing of storage-associated changes in PE-sealed vs. non-sealed leaves (Figure 8). These differences can be tentatively associated with the buildup of ethylene, the senescence hormone, inside the PE packaging. Collectively, our results also support the applicability of the spectral indexes CI and PSRI indicative of leaf development and senescence [23] initially suggested for "point-based" measurements with conventional spectroradiometers for the processing and subsequent interpretation of the hyperspectral data obtained in our experiments.

5. Conclusions

We compared the two approaches to monitoring the condition of plants, using leafy vegetables as a model, via spatially resolved techniques based on imaging PAM and hyperspectral reflectance. Using of the imaging PAM was complicated by (i) weak correlation of the spatial distribution pattern of the Qy parameter with the actual physiological

condition of the plant object and (ii) its high degree of heterogeneity. On the contrary, the indexes HCI_{678} and HPSRI based on the earlier developed "point-based" indexes CI_{678} and PSRI, were able to extract consistent information about the changes in the condition of and pigment transformation in fresh-cut lettuce plants. In view of the obtained findings, hyperspectral reflectance imaging can be suggested for monitoring of plant objects such as fresh-cut vegetable produce in greenhouses and packinghouses. More research is needed to find an approach to harness imaging PAM for the same purpose.

In particular, the indices HCI_{678} and HPSRI were found to be suitable for the non-invasive proximal sensing of the fresh-cut lettuce leaf quality. Further studies are needed to optimize the spectral bands used for these indices.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Changes in (A) [Chl] and (B) [Car]/[Chl] ratios during storage of the non-sealed (closed symbols) and sealed (open symbols) fresh-cut lettuce leaves., Figure S2: Changes in leaf area exhibiting different values of CI678 index (the color-coded images) indicative of [Chl] and (histograms immediately below the corresponding images) the corresponding distribution of the pixel values of the color-coded images in the stored non-sealed fresh-cut lettuce leave. For details, see Materials and Methods, Figure S3: Time-course of changes in the relative area of (top graph) non-sealed and (bottom graph) exhibiting the values of the CI678 index in the ranges indicated in the graph annotation, Figure S4: Changes in leaf area exhibiting different values of PSRI index (the color-coded images) indicative of [Chl] and (histograms immediately below the corresponding images) the corresponding distribution of the pixel values of the color-coded images in the stored non-sealed fresh-cut lettuce leave. For details, see Materials and Methods, Figure S5: Time-course of changes in the relative area of (top graph) non-sealed and (bottom graph) exhibiting the values of the PSRI index in the ranges indicated in the graph annotation.

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