


Nondestructive detection of low temperature induced stress on postharvest quality of kápia type sweet pepper

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ABSTRACT

Application of cold storage temperatures below optimum induces a high risk and threat of chilling injury (CI) in the case of sensitive commodities. Sweet pepper belongs to this group of vegetables, so our main objective was to investigate and monitor the effect of non-optimal temperatures (2.5 and 5 °C) induced stress (chilling injury) on kápia type sweet pepper (*Capsicum annuum* L.) during its postharvest storage by nondestructive quality measuring methods. Fresh, semi-matured (reddish-green colored) samples of 'Kapitány F₁' cultivar were stored at 2.5, 5 and 10 °C for 7 d followed by 7 d shelf-life. Nondestructive texture measurements were carried out by a purpose built tabletop acoustic stiffness device. Surface color and chlorophyll content related quality indices were evaluated by a chroma meter, a DA-meter[®] and a

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chlorophyll fluorescence imaging system. High resolution digital pictures were captured and analyzed for possible CI defects by means of surface color values (normalized RGB, hue and saturation). According to our results, the evaluated quality indices (DA-index[®], acoustic stiffness coefficient, surface color parameters; F_0 , F_m , F_v and F_v/F_m chlorophyll fluorescence parameters) clearly represented the temperature dependent quality changes during low temperature storage, subsequently followed by ambient shelf-life. Samples stored under and at 5 °C showed the chilling temperature stressed symptoms of delayed and partly retarded postharvest ripening, even under simulated shelf-life conditions, but without the onset and manifestation of the characteristic visible symptoms of chilling injury. This may raise doubts and suggest possible future research areas regarding the role of non-optimal cold storage temperatures induced stress, the effect of chilling injury contributing factors and consequences.

KEYWORDS

acoustic stiffness, chlorophyll fluorescence, DA-index[®], hue, saturation, color, machine vision system

INTRODUCTION

In order to provide high quality fresh horticultural products, several attempts are made to minimize postharvest quality losses. Even under the most carefully applied operations, different kind of quality losses may occur, especially during postharvest and marketing period. Even the most frequently applied refrigerated storage and/or transport of cold sensitive products such as sweet pepper may cause the low temperature induced stress called chilling injury (abbreviated and later used as 'CI') at below optimal storage temperature (Cen et al., 2016; Patel et al., 2016; Zsom et al., 2018). One of the chilling sensitive vegetables representing high postharvest marketing value is sweet pepper. According to consumers' perception in the European fresh vegetable market, nowadays the kápia type sweet pepper varieties gained a significant market share. Among improper storage temperature conditions under 7–8 °C, chilling injury may occur, especially in the case of unripe and semi-matured sweet peppers (Fallik et al., 1999; Ilić et al., 2012; Lim et al., 2007) representing a higher risk of postharvest quality loss. Due to the complexity of chilling sensitivity and injury phenomena of fruits and vegetables, and the special structure of sweet pepper, the number of possible non-destructive quality determination methods are limited. Quality assessment and control in postharvest technology demand new reliable, objective and quantitative methods (Zsom-Muha and Felföldi, 2007). Nowadays, among the potential quality determination methods mainly optical techniques are applied. According to the already published papers (Baranyai et al., 2020; Cen et al., 2016; Gorbe & Calatayud, 2012; Kaszab et al., 2008; Nguyen et al., 2020; Pinto et al., 2015; Ziosi et al., 2008; Zsom et al., 2016, 2018) the measurement of chlorophyll content related absorbance differences, chlorophyll fluorescence characteristics, machine vision and hyperspectral imaging systems could be used as non-destructive approaches for the evaluation of quality changes of perishable horticultural produces.

For the reduction of postharvest quality losses and efficient quality determination, postharvest scientific research is continuously searching for possible and the least impact providing methods and storage technology developments. This is also true for possible novel detection and prevention methods concerning postharvest chilling injury analysis (before onset and/or well



before symptom manifestation) in the case of sensitive horticultural produces. For the sake of these postharvest developmental intents, our current research work aimed to investigate and monitor the effect of non-optimal temperatures (2.5 and 5 °C) induced stress (chilling injury) on kápia type sweet pepper (*Capsicum annuum* L.) during its postharvest storage by nondestructive quality measuring methods. Additionally, applicability of the used non-invasive measuring methods was investigated for the assessment of sweet pepper chilling injury related quality changes.

MATERIALS AND METHODS

Kápia type fresh sweet pepper samples (*C. annuum* L. cv. Kapitány F₁) were obtained directly from an orchard (Bugyi, Hungary) in semi-matured ‘turning’ (more green than red) maturity (so called ‘smoky green’ colored stage of ripeness). Samples were transported to laboratory within 6 h after harvest. In this special maturity stage, the dark green ripe pepper samples’ color starts to change partly from homogeneous dark green by hints of greyish-brownish or even reddish-orange color. Harvesting kápia type peppers in this turning or later maturity stage suggests higher possibility and ability to postharvest ripening under appropriate storage conditions. Ninety samples without any surface defects were randomly selected and divided into three groups.

Peppers were stored in temperature-controlled refrigerators at 2.5 ± 0.5 and 5 ± 0.5 °C for 7 d in LDPE bags in order to simulate chilling injury inducing conditions well below optimal cold storage temperature. Average relative humidity was 65 ± 5 and $75 \pm 5\%$ for 2.5 ± 0.5 and 5 ± 0.5 °C, respectively. Control samples were stored for 1 week at 10 ± 0.5 °C (average relative humidity $85 \pm 5\%$). After one week of cold storage, all samples were placed to ambient shelf-life conditions at 20.5 ± 0.5 °C for further one week (average relative humidity $80 \pm 10\%$) in order to stimulate postharvest ripening and simulate retailing conditions. Temperature and relative humidity data were collected by Trotec BL-30 type (Trotec GmbH & Co. KG, Germany) data loggers in every 5 min.

Non-destructive quality measurements were carried out on marked places of the two opposite sides of each pepper using the reddest and the greenest area available on the pepper surface, respectively. These selected points were used for non-destructive surface color, DA-index[®] and chlorophyll fluorescence analysis.

Quality changes caused by cold storage temperatures lower than optimal (2.5 and 5 °C) were intended to be followed and characterized – among others – by the analysis of chlorophyll fluorescence parameters’ change. The change of these indirect internal quality indices represent a close relation to the photosynthetic activity, integrity and efficiency of photosystem II (PSII) in chlorophyll containing plant samples. A FluorCam7 (version 1.2.5.18) controlled Open FluorCam FC 800-O/2020 imaging chlorophyll fluorometer (Photon Systems Instruments, Czech Republic) was used to determine F_0 and F_m (minimum and maximum fluorescence signal, respectively) and F_v (variable fluorescence signal as $F_v = F_m - F_0$) parameters measured at the two opposite sides of each dark adapted pepper. The system provides not just a spot like, but even overall fluorescence data of the selected sample surface(s) (see Fig. 4). The calculated index of F_v/F_m reflects the potential maximum photon yield of photochemistry, i.e. the maximum photochemical efficiency of a photosynthetically active chlorophyll-containing sample. It is a



valuable tool to determine both photosynthetic capacity, stability and to follow e.g. the maturity related chlorophyll degradation or the negative quality effect of external factors such as low storage temperature.

Additionally, DA (or ΔA) index[®] of the pepper samples was acquired by a FRM01-F type Vis/NIR DA-meter[®] (Sintéleia s.r.l., Italy) at the marked points of the two opposite sides of the pepper bodies to characterize the change in surface color related chlorophyll content during storage. This non-destructive maturity index as an index of absorbance difference (I_{AD}) created by Costa and co-workers (Ziosi et al., 2008) is calculated upon the difference in absorbance between the wavelengths of 670 and 720 nm near the chlorophyll-a absorption peak. The value of DA-index[®] varying from 0 to 5 is proportional to the amount of active chlorophyll existing in the sample.

Sweet pepper surface color and color change, represented as CIELAB color characteristics (L^* , a^* , b^* , C^* and h°), were determined by a portable Minolta Chroma Meter CR-400 (Minolta Europe GmbH, Germany) with \varnothing 8 mm aperture.

Furthermore, non-destructive texture evaluation was carried out by a purpose-built tabletop acoustic texture measuring system (Zsom et al., 2016; Zsom-Muha and Felföldi, 2007). Samples were positioned vertically their tips facing upwards individually on a soft cushioning sample holder. This sample holder contained a microphone located under the sample. The sample characteristic acoustic sound response – simply having been excited by a gentle hit on the peppers' tip – was analyzed by a Custom Fast Fourier Transform software called 'Stiffness'. Acoustic stiffness coefficient (S) was calculated as $S = f^{\text{®}} \cdot m \cdot 10^{-6}$ ($N \text{ mm}^{-1}$), where f is the characteristic resonance frequency in Hz of the excited sample and m is the sample mass in g.

Full high definition (FHD) resolution digital pictures (1920×1080 pixels) were captured by a machine vision system consisting of a Samsung WB350F digital camera mounted on a tripod, homogeneous illumination provided by two studio softboxes and a photo-box. Images were analyzed for surface color change and possible chilling injury defects by means of RGB (Red, Green, Blue) color components, normalized RGB values, HSL (Hue, Saturation, Luminosity) components. Image processing program was developed with GNU Octave (version 4.4.1).

Mass loss (% of initial fresh mass) was calculated based on the measured mass data by a digital laboratory balance of each sample on every measuring day.

Data were converted by means of routines in MS-Excel and were analyzed using the SPSS for Windows (ver. 14) software. Results are presented in figures with mean and bars represent confidence interval (CI) for mean (95% CI). Statistical analysis was performed at 95% significance level ($\alpha = 0.05$). ANOVA tests were carried out to evaluate the effect of factors of storage temperatures and storage time at $P < 0.001$. The ANOVA F value was used to compare effects to the natural variability of collected data.

RESULTS AND DISCUSSION

Considering the calculated mass loss (%) data of the different storage temperature treatments, significant difference was found between samples stored either at 2.5 or at 5 °C and the optimal temperature of 10 °C from 4 d ($F = 6,393$) to 7 d ($F = 8,463$). This clearly proved the positive effect of reduced temperature on mass loss (Fig. 1, left). On the other hand, no significant difference was found between the two, usually chilling injury inducing temperatures of



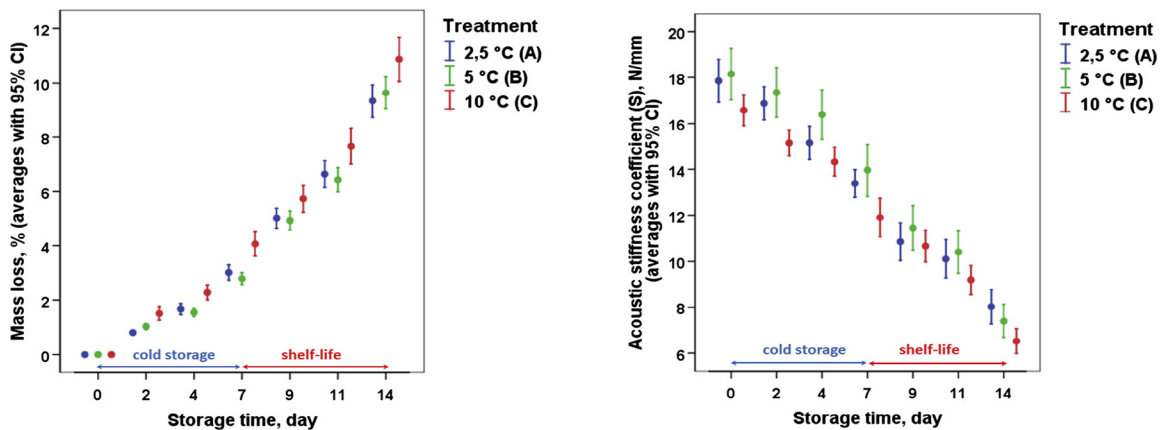


Fig. 1. Average mass loss change (left) and textural change (acoustic stiffness S, right) of sweet pepper samples stored at 2.5, 5 and 10 °C for 7 d, and 7 d subsequent shelf-life



2.5 and 5 °C during the one week cold storage. After removal to ambient conditions, this significant mass loss difference between low (2.5 and 5 °C) and optimal temperature slowly changed from 9 d to only a clear difference between optimal (10 °C) and the lowest storage temperature (2.5 °C) by the end of the storage. Additionally, this is also to be seen in Fig. 1 (right) in the overall texture change of the samples.

In the case of sweet pepper, one of the main quality attributes is the overall texture of the pepper berry. Fig. 1 (right) shows the acoustic stiffness change (S) in storage time revealing the effect of low storage temperature too. Together with the increasing mass loss, the overall texture also changed with a steep decrease resulting in about a 25% loss of initial texture during the 7 d cold storage.

In accordance with mass loss results, similar difference was observed between at non-optimal and optimal temperature stored samples textural change from 9 d until the end of shelf-life storage. Even higher degree of mass and textural loss was prevented by the applied LDPE wrapping providing relatively high humidity around the samples. Shelf-life conditions decreased these differences to a significant difference only between at 2.5 and 10 °C stored samples concerning overall texture.

Fig. 2 clearly represents the close relation between textural changes and mass loss increase during the entire storage period ($R = -0.713$, $P < 0.001$). Relative acoustic stiffness coefficient's change versus mass loss figure (overall texture decrease and softening) reveals a negative exponential relationship, similar to the results shown is Fig. 1.

According to the surface color measurement results (only CIE Lab a^* is shown in Fig. 3), no significant difference was observed among color characteristics of sample groups during the cold storage period, revealing no major effect of optimal and below optimal temperature on surface color.

On the other hand, after removal to shelf-life conditions (at 7 d), significant increase in a^* ($F = 15.106$) and decrease in hue angle (data not shown in figures) values ($F = 10.578$) were observed as normal postharvest ripening in the case of the at 10 °C (control) stored samples compared to the results of CI inducing temperature groups. Additionally, the approximately 3

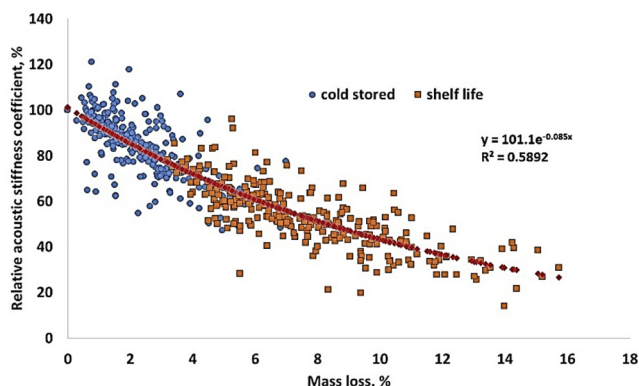


Fig. 2. Relation between the mass loss (%) and calculated relative acoustic stiffness coefficient (%) of sweet pepper samples stored at 2.5, 5 and 10 °C for 7 d, and 7 d subsequent shelf-life



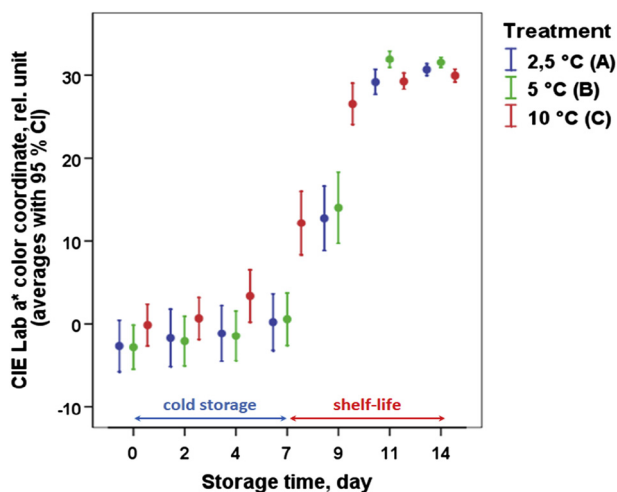


Fig. 3. Average color change represented by CIE Lab a* values of sweet pepper samples stored at 2.5, 5 and 10 °C for 7 d, and 7 d subsequent shelf-life

d long delay in postharvest ripening pattern (sigmoid function) of presumably CI suffered samples (marked with blue and green colors) from 7 d is also noticeable. Furthermore, the difference between the color of cold storage and control group vanished by the end of shelf-life (Fig. 3).

Concerning the normal postharvest ripening process of green to red (e.g. kápia type or bell pepper) or yellow (e.g. banana) surface color change associated horticultural products, the photosynthetically active chlorophyll content's decrease clearly represents the change of maturity. In our case, the pepper surface color change from green to red was confirmed by close correlation between a^* and hue° ($R = -0.966$, $P < 0.001$). This change can be easily and objectively determined by the use of a DA-meter[®], and by a chlorophyll fluorometer. In Fig. 4, characteristic chlorophyll fluorescence images (1360×1024 pixels of resolution) of sweet pepper stored at 2.5 °C for two weeks are shown for a demonstrational purpose. The areas of photosynthetically active chlorophyll containing pepper parts (body – Area no.1; stalk – Area no.2) were separately selected and analyzed for fluorescence parameters (F_0 , F_m , F_v/F_m).

In Figs. 5 and 6 (left), the sensitive chlorophyll fluorescence values change (F_v , F_m and F_v/F_m) are presented. Significant difference concerning the change of F_v and F_m indices was found comparing the behavior of the samples stored at the lowest (2.5 °C) and the two higher cold storage temperatures during the cold storage period. Below optimal temperature induced stress may have affected the integrity and efficacy of PSII's membrane system from as early as 2 d of cold storage for the storage group of 2.5 °C ($F_{Fv} = 12.499$; $F_{Fm} = 10.338$; $F_{Fv/Fm} = 10.414$) represented by the fast and steep decrease either in F_v or F_m values. Furthermore, the change of these chlorophyll fluorescence parameters suggests (and may forecast) and reveals the low temperature stress induced negative quality change as it was earlier published in the case of green banana (Zsom et al., 2018). By the removal to shelf-life conditions, the postharvest maturation process was on the way, but with significantly different intensity (also note Figs. 3 and 6 [right]), especially in the case of at 10 °C (optimal) stored samples (normal ripening).



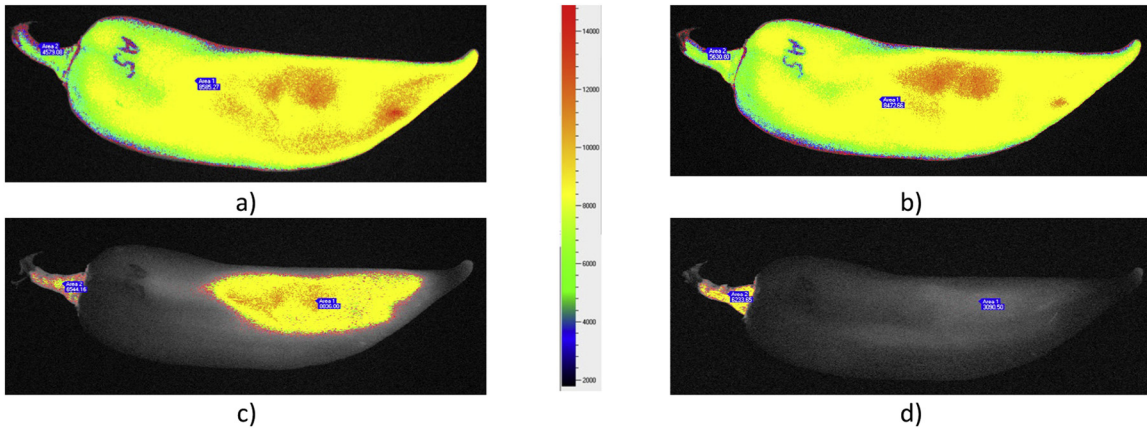


Fig. 4. Chlorophyll fluorescence images (F_m , relative unit) of sweet pepper sample stored at 2.5 °C for 7 d, and 7 d subsequent shelf-life: a) day 0, b) day 7, c) day 11, d) day 14. In these false colored images, the redder is a pixel, the greener is the pepper, so the higher is the photosynthetically active chlorophyll content related fluorescence light emission sensibly captured by the measuring device



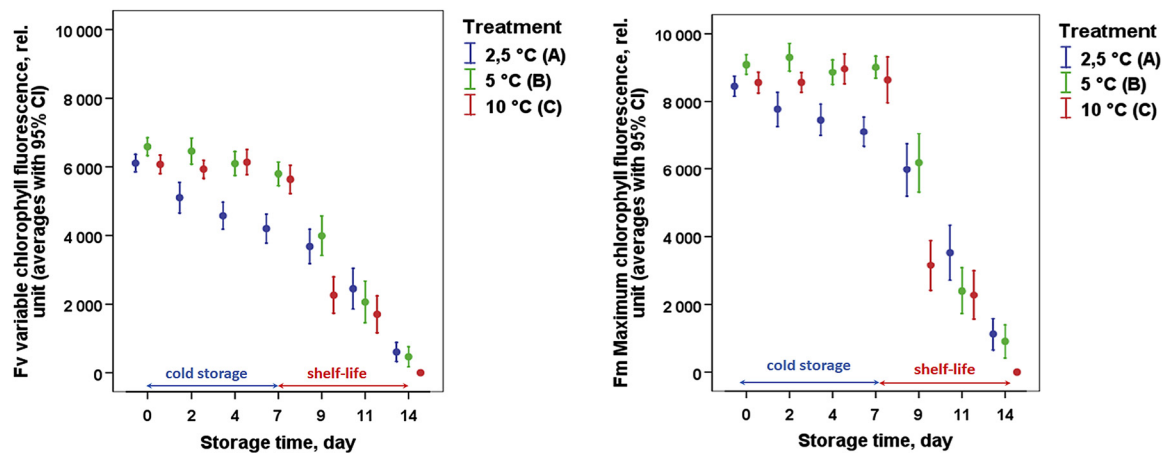


Fig. 5. Average chlorophyll fluorescence change represented by F_v (left) values and F_m (right) of sweet pepper samples stored at 2.5, 5 and 10 °C for 7 d, and 7 d subsequent shelf-life



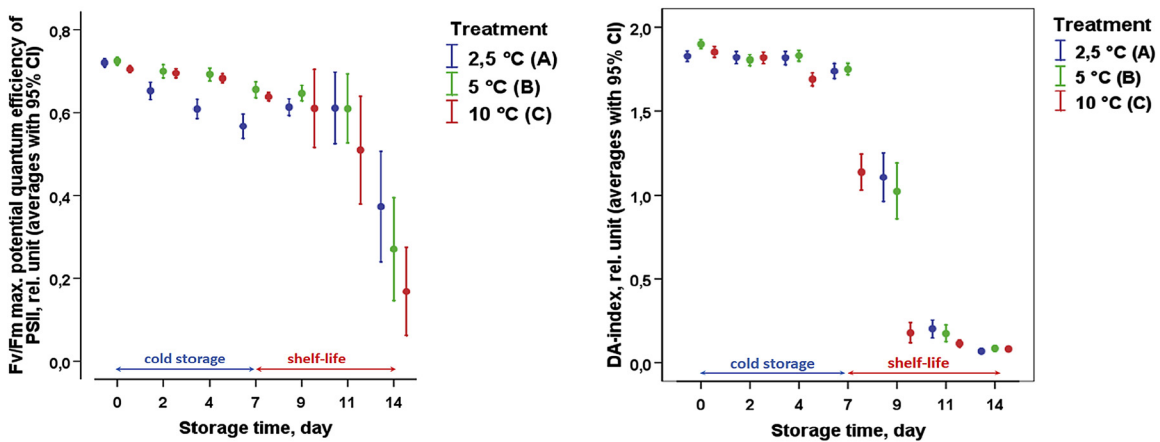


Fig. 6. Average photosynthetically active chlorophyll content related changes represented by F_v/F_m (left) values and DA-index[®] (right) values of sweet pepper samples stored at 2.5, 5 and 10 °C for 7 d, and 7 d subsequent shelf-life



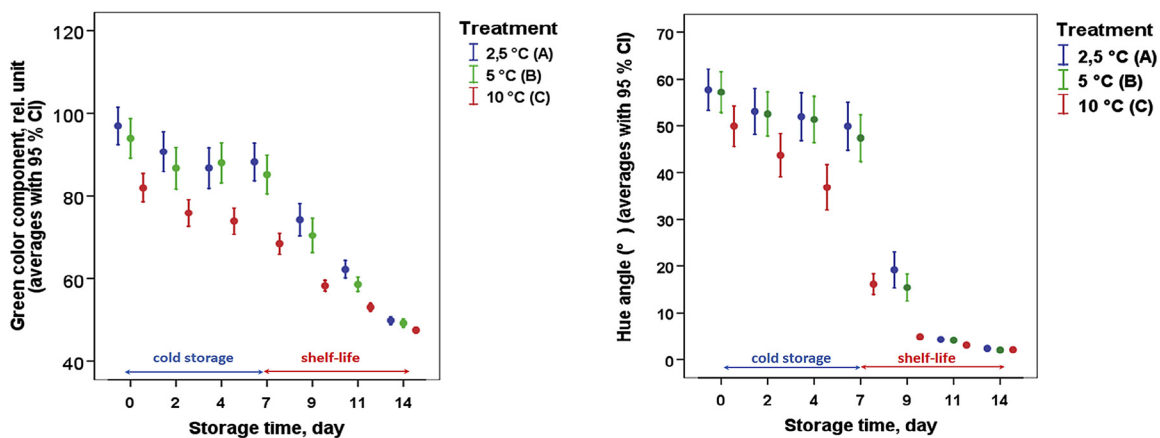


Fig. 7. Digital image processing results of sweet pepper samples stored at 2.5, 5 and 10 °C for 7 d, and 7 d subsequent shelf-life



Remarkably, on the other hand, no direct and visible symptoms of the presumed chilling injury (e.g. surface pitting, sunken areas) were detected in the case of this type and maturity stage of sweet pepper contrary to the green banana chilling injury results (Zsom et al., 2018). Concerning these green banana results, the chlorophyll fluorescence parameters' steep and rapid change – so to say – clearly referred to chilling injury well before the visible symptoms emerged. This suggestion was also confirmed by the results of digital image analysis. Chlorophyll fluorescence parameters correlated with DA-value[®] (F_m , $R = 0.840$, $P < 0.001$; F_v , $R = 0.814$), mass loss (F_v , $R = -0.835$ and F_m , $R = -0.830$, $P < 0.001$) and hue angle ($R = 0.818$, $P < 0.001$).

According to the DA-meter[®] results shown in Fig. 6 (right), the same characteristic chlorophyll content and storage temperature related changes were detected concerning the DA-index[®] values as it was shown already in the case of surface color measurements (Fig. 3). DA-index[®] obtained the highest correlation with a^* ($R = -0.867$, $P < 0.001$). No significant relation between DA-index[®] and the below optimal storage temperature stress was detected. The higher temperature of shelf-life storage induced the characteristic postharvest ripening, but the ripening process at below optimal temperature was found to be retarded and delayed by approximately 3 d (note also Figs. 3 and 5), represented by a reduced intensity color change as a possible negative effect of chilling injury.

Analysis of digital color images have shown similar tendencies (Fig. 7) to previously mentioned results. The green color intensity decreased and the group of 10 °C separated from others during the first 7 d of cold storage ($F = 7.935$). The same separation was observed for luminosity and hue angle values as well. All color parameters obtained close mean values for storage groups by the end of shelf-life.

Red intensity, saturation (data not shown) and hue angle were in close agreement with DA-value[®] (see Fig. 6) according to significant correlation of -0.855 , -0.841 and 0.809 , respectively. These confirmed the expected color change from initial smoky green to chocolate brown and final red surface color, especially during simulated shelf-life conditions.

CONCLUSIONS

In the case of postharvest low storage temperature, chilling injury represents a possible postharvest risk of undesired quality loss for sensitive products, such as sweet pepper. Sensitivity and intensity of chilling injury depend on many internal and external factors, and not all the sensitive species respond the same way to this stress. The effects of chilling injury on postharvest quality, induced by below optimal cold storage temperatures of 2.5 and 5 °C, on kápia type sweet pepper samples' postharvest quality were investigated mainly by non-destructive optical methods during one week cold storage followed by one week shelf-life. The evaluated quality indices objectively represented the temperature dependent quality changes at low (2.5 and 5 °C) and around optimal (10 °C) temperature storage of 7 d and during subsequent shelf-life of one week.

Samples stored below and at 5 °C showed the chilling temperature stressed symptoms of delayed and retarded postharvest ripening, even under shelf-life conditions. Remarkably, the characteristic surface pitting – as a general visual symptom of chilling injury of unripe or semi-ripe pepper samples – was not detected either during 7 d cold storage at 2.5 and 5 °C or during subsequent shelf-life. This may raise doubts whether this special behavior was due to



acclimatization to the temperature stress caused by the applied non-optimal cold storage temperatures, or it may suggest a less chilling injury sensitive behavior of this cultivar especially in these maturity stages, or the onset of the commonly known chilling injury symptoms would have needed longer exposure time at the applied non-optimal cold storage temperatures.

On the other hand, in the case of chlorophyll fluorescence parameters (e.g. F_v , F_m and F_v/F_m), the steep and fast decrease from 2 d of cold storage at 2.5 °C may indicate the possible chilling injury onset. Results may also suggest the onset of chilling injury in unripe or turning stage of ripeness of kápia type sweet pepper well before the visible signs appear at all. Additionally, results confirm the possibility of objective and early detection of below optimal storage temperature induced chilling injury by chlorophyll fluorescence imaging devices. The changes in overall texture, mass loss, photosynthetically active chlorophyll content related parameters (represented well by F_0 , F_m , F_v , F_v/F_m and DA-index[®]) were found to characterize reliably the effect of temperature treatments. Furthermore, in order to analyze more precisely and possibly detect the onset of chilling injury phenomena prior to symptom manifestation, the applied chlorophyll fluorescence analysis method may serve as a suitable method for further investigations related to low storage temperature stress.

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