Quality Maintenance of Broccoli by the use of 1-MCP Treatments

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Abstract: Broccoli's high perishability and its sensitivity to negative quality changes (i.e. mass loss, ethylene induced degreening, abscission of leaves and florets) generates quality problems during postharvest. Freshly harvested samples were stored at 5 °C and 21 °C after separately treated for 24 hours with 625 ppb 1-MCP, 24 hours with 2 ppm ethylene and 1-MCP followed by ethylene. Quality maintenance effectivity of 1-MCP was investigated during cold and room storage by non-destructive optical methods (chlorophyll fluorescence and DA-index®) and by the evaluation of the visual physiological symptoms. The highly positive effects of 1-MCP treatment combined with cold storage were obviously proven on quality maintenance providing better retention of initial quality related to the initial mature green stage as chlorophyll content related DA-index®; F_m, F_v, F_v/F_m and F_m/F_0 chlorophyll fluorescence values. From the practical point of view, the rapid and easy-to-use Sintéleia FRM01-F Vis/NIR DA-meter® could be applied relatively easy for the quality measurement of broccoli. The reproducibility of quality determination could be increased by the enhanced number of measuring points or using computer aided imaging methods (i.e. chlorophyll fluorescence imaging, machine vision system) providing global and more reliable information about quality changes.

Keywords: chlorophyll, degradation, fluorescence, DA-index®, 1-methyl-cyclopropene, ethylene, degreening.

Introduction

Nowadays, postharvest quality issues of fresh horticultural products are continuously under the scope of interest. Among vegetables, broccoli is one of the favorites of the cabbage family. Broccoli is a highly perishable vegetable. According to Cantwell and Suslow (1997), low temperature is extremely important to achieve the adequate shelf-life. Optimal temperature of 0 °C with > 95 % RH is required to optimize broccoli storage life (21-28 days). Heads stored at 5 °C can offer a shorter storage life of maximum 10-14 days; but its storage life at 10 °C is only about 5 days. Ethylene can easily diffuse into and out of plant tissues from endogenous

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(biological) and exogenous (non-biological) sources (Saltveit, 1999) too. Additionally, broccoli is extremely sensitive to exposure to ethylene from natural origin (produced by climacteric fruits) or artificial origin (i.e. from exhaust fumes of internal combustion engines). Due to the ethylene exposure, yellowing (chlorophyll degradation) and abscission of the florets and leaves are the most common postharvest symptoms. Exposure to 2 ppm ethylene at 10 °C reduces the possible shelf-life by 50 % (Cantwell and Suslow, 1997). Additionally, broccoli is also really sensitive to mechanical injury; high mass loss caused softening, mould development and decay among improper transportation and retail conditions. In order to avoid such losses, individual shrink packaging (film wrapping) is the commonly used procedure. Novel postharvest technologies (i.e. the use of ethylene binding packaging materials, or ethylene scavenging agents) could serve effectively for these purposes. 1-MCP (1-methyl-cyclopropene) application as a novel postharvest treatment (Blankenship and Dole, 2003) is proved to be effective against undesired postharvest ripening of several fruits such as apples, bananas, pears, plums, melons (Bagnato et al, 2003; Nguyen et al, 2016; Watkins, 2006), but the possible application for vegetables, especially for broccoli is rather rare lately (Fan and Mattheis, 2000; Fernández-Léon et al., 2013; Sabir, 2012; Toivonen and DeEll, 1998; Yuan et al., 2010). The main effect of 1-MCP is that this highly active and harmless compound is able to fit in and more strongly bind to and block the ethylene receptors than ethylene thus prolonging storage life (especially if used parallel to cold and/or controlled atmosphere storage), preventing ethylene-dependent physiological responses and providing better retail quality. According to Zsom-Muha and Felföldi (2007), the postharvest quality assessment and control is rightly waiting for the new possibilities of reliable, objective and quantitative methods. These novel methods are also welcomed for broccoli quality determination because of its special morphology and structure derived measuring difficulties. So, the now available novel non-destructive optical measuring methods are based on the measurement of chlorophyll content related absorbance differences, chlorophyll fluorescence characteristics, or carried out by machine vision or hyperspectral imaging systems offer the possibility to non-invasively characterize fruits' and vegetables' quality changes (Gorbe and Calatayud, 2012; Ziosi, 2008; Pinto et al., 2015).

The aim of our research was to evaluate and prove the possible positive effects of 1-MCP treatment on keeping quality of fresh broccoli at cold and simulated shelf-life storage temperatures by non-destructive measuring methods. Additionally, we were also keen to investigate the applicability of possible non-invasive measuring methods for the measurement of broccoli quality changes.

Materials and Methods

Mature green and fresh broccoli samples (*Brassica oleraceae cv. botrytis var. italica*) were obtained from an experienced broccoli grower in uniform maturity within 12 hours after harvest. According to uniformity, 30 broccolis were selected and randomly divided into four groups according to further treatments. Broccolis were stored in temperature controlled refrigerators at 5 ± 0.5 °C for 9 days. In case of ambient temperature of 21 ± 0.5 °C, samples were stored only for only 5 days due to the fast quality degradation. Broccoli samples were wrapped in commercially available LDPE bags. 15 equally distributed individual measuring points were selected on the head of the broccoli samples. These 15 points were selected for chlorophyll fluorescence analysis and DA-index® evaluation.

In three different series, the following treatments were carried out. Four-four pieces of fresh broccolis per storage temperatures were treated for 24 hours with 1-MCP (625 ppb gaseous 1-methyl-cyclopropene concentration, released from a tableted form in distilled water according to the SmartFresh℠ system application requirements), 2 ppm of ethylene and the consecutive combination of the earlier two (24hs of 1-MCP followed by 24hs of ethylene) in an air-tight plastic cabinet equipped with a small electric fan for well air distribution inside, respectively.

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

Broccoli overall quality, maturity and color related photosynthetically active chlorophyll content changes - referring to the photosynthetic activity, integrity and efficiency of photosystem II (PSII) - were characterized by the measurement of chlorophyll fluorescence parameters. F_0 (dark fluorescence signal), F_m (maximum dark fluorescence signal) and F_v (variable fluorescence $F_v = F_m - F_0$) parameters at the above mentioned points of each broccoli were measured by a PAM WinControl-3 controlled MONI-PAM multi-channel chlorophyll fluorometer (Heinz Walz GmbH, Germany). The calculated index of F_v/F_m reflects the potential maximum photon yield of photochemistry, i.e. the maximum photochemical efficiency. It is a valuable tool to determine both photosynthetic capacity, stability and follow the maturity related chlorophyll degradation. Additionally, the ratio, F_m/F_0 was also calculated.

In order to characterize the change in surface color or tissue related chlorophyll content, the DA (or ΔA) index® was measured by a FRM01-F type Vis/NIR DA-meter® (Sintéleia s.r.l., Italy) on every 22 measurement points of the broccoli samples. This non-destructive maturity index (index of absorbance difference, I_{AD}) created by G. 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 it is proportional to the amount of chlorophyll present in the fruit, and varies from 0 to 5.

Overall visual quality information about the effects of the treatments, digital images were taken by an Olympus SP-350 digital camera (3264*2448 pixels of resolution, Olympus Corporation, Japan) mounted on a vertical stand in a photo-box.

Data were converted by means of routines in MS-Excel and were analyzed using the SPSS for Windows ver. 14. Statistical analysis was performed at 95 % significance level (in figures marked with 95 % CI).

Results and Discussion

Based on mass loss data of the treatments, significant difference was found between the cold (5 °C) and ambient temperature (21 °C) stored samples from day 4, clearly proving the positive effect of reduced temperature on mass loss and quality retention (*Fig. 1*). No significant difference can be observed at 5 °C between the 1-MCP-treated and the 1-MCP + C_2H_4-treated samples throughout the whole storage period. This obviously revealed the extremely positive effect of 1-MCP due to its much higher affinity to fit in and bind to ethylene receptors than ethylene, providing less possibility to ethylene to cause leaf abscission related mass loss and quality degradation. As awaited, the highest mass loss was observed in case of ethylene treated and control samples stored at 21 °C due to the additive negative effect of higher temperature and ethylene caused excessive floret and leaf abscission (especially from the 4th day). The lowest mass loss was observed in case of 1-MCP-treated samples together with the best preservation of initial mature green color. Remarkably, control samples may have been slightly exposed to ethylene due to the impossibility of full hermetically separate samples from ethylene. From day 5, in case of 1-MCP-treated samples stored at 21 °C, a slight yellowish-brownish discoloration of the heads could be observed, but with less intensity of floret abscission. Contrary to this, ethylene treated samples were almost unable to measure due to the massive floret abscission and beginning mould development resulting in bad overall quality. All the above and later mentioned changes are clearly supported by the digital pictures taken (*Fig. 2).*

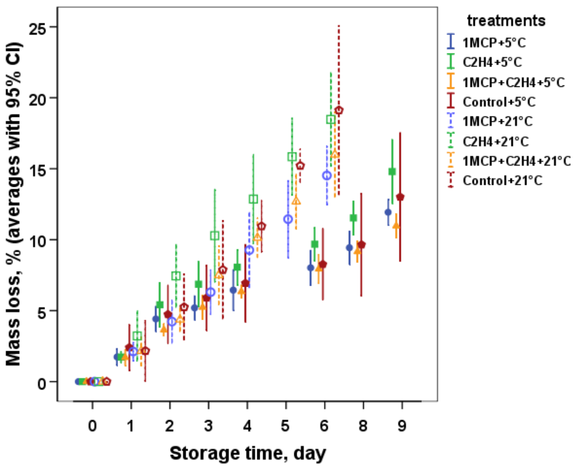

Fig. 1. Change of average mass loss of the broccoli samples at 5°C cold and at 21 °C storage.

Fig. 2. Overall broccoli quality changes represented by the digital pictures taken during storage

Fresh broccoli's original mature green color is one of its main quality features. According to our results gained by chlorophyll fluorescence and DA-index® measurements, the photosynthetically active chlorophyll content related parameters showed also clearly the positive effect of cold temperature (5 °C) combined with 1-MCP treatments (*Fig. 3-4*). Having compared the two temperature treatments, in case of minimal (F_0) fluorescence (data not shown) clear and significant difference was found between the 1-MCP-treated sample and the only ethylene treated samples and untreated control samples. In case of 21 °C stored samples the positive effect of 1-MCP is kept the samples until the 3rd day, but it was followed by a rapid decrease. In case of control and ethylene treated samples, this steep and rapid decrease occurred right after the 1st day as negative quality change and rapid degreening of the florets.

The same characteristic change was observed in case of maximal (F_m), variable (F_v) fluorescence values (data not shown), and additionally in case of F_v/F_m and F_m/F_0 values (*Fig. 3-4*). Additionally to the results of F_0, these later parameters show more clearly the significant and positive effect of low temperature and 1-MCP-treatment on photosynthetically active chlorophyll related green color retention (*Fig. 3-4*). It is also to be seen that the rapid degreening related decrease of control and ethylene treated samples was associated with the fluorescence parameters' steep decrease from the 2nd and 4th day in case of 21 °C and 5 °C, respectively (*Fig. 3-4*). These changes are also to be followed in the digital pictures taken (see *Fig. 2*).

The 1-MCP-treated samples stored at 5 °C retained higher photosynthetic activity during the entire storage period. The average initial F_v/F_m fluorescence values (> 0.7) represented the mature green and highly photosynthetically active chlorophyll containing intact broccoli tissue

(*Fig. 3*). The less significant change of F_0 revealed that this parameter was found to be a less sensible parameter than the F_m, F_v, F_v/F_m or F_m/F_0 (see *Fig. 3-4*).

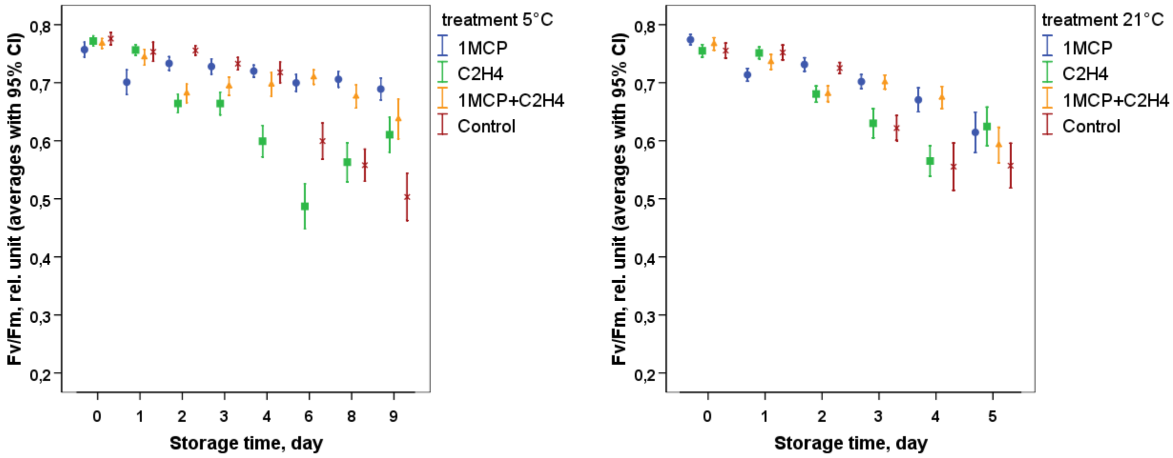

Fig. 3. The F_v/F_m chlorophyll fluorescence change of broccoli samples stored at 5 °C (left) and at room temperature of 21 °C (right).

The data revealed that they characterize more reliably and sensibly the ethylene induced degreening related chlorophyll fluorescence changes and the 1-MCP provided green color (quality) preservation/retention, especially during cold storage at 5 °C.

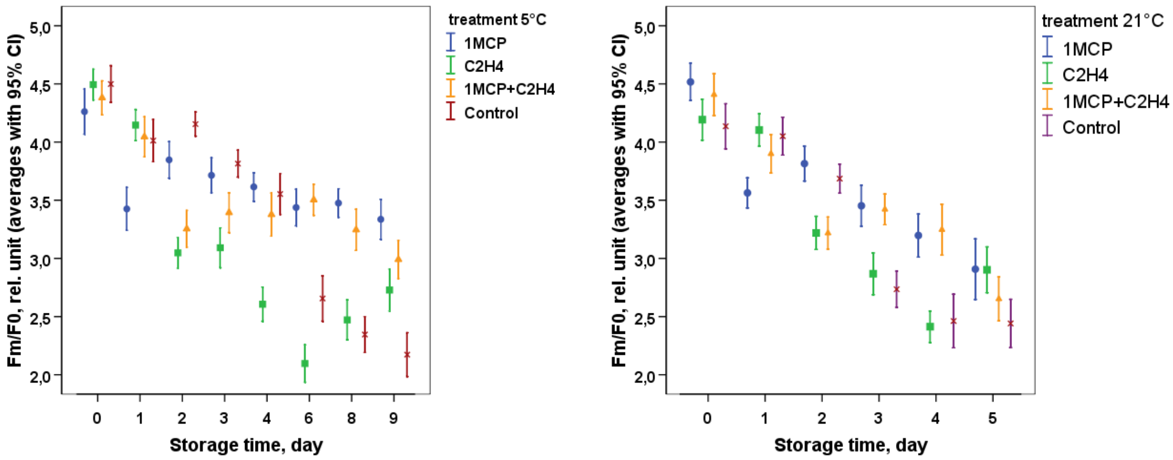

Fig. 4. The F_m/F_0 ratio's change of broccoli samples stored at 5 °C (left) and at 21 °C (right).

The DA-index® values of the treatments also showed clearly (notice the different horizontal scaling of *Fig. 5*) the earlier mentioned significant difference between the two temperature treatments and the 1-MCP or ethylene treatments, respectively. This temperature dependent, rapid and steep decrease in DA- index® values of at 21 °C stored samples clearly started from day 2 (*Fig. 5, right*) due to the negative effect of higher temperature and ethylene, compared to the less moderate change observed at 5 °C (*Fig. 5. left*) started later only about the 4th -5th day provided by the 1-MCP.

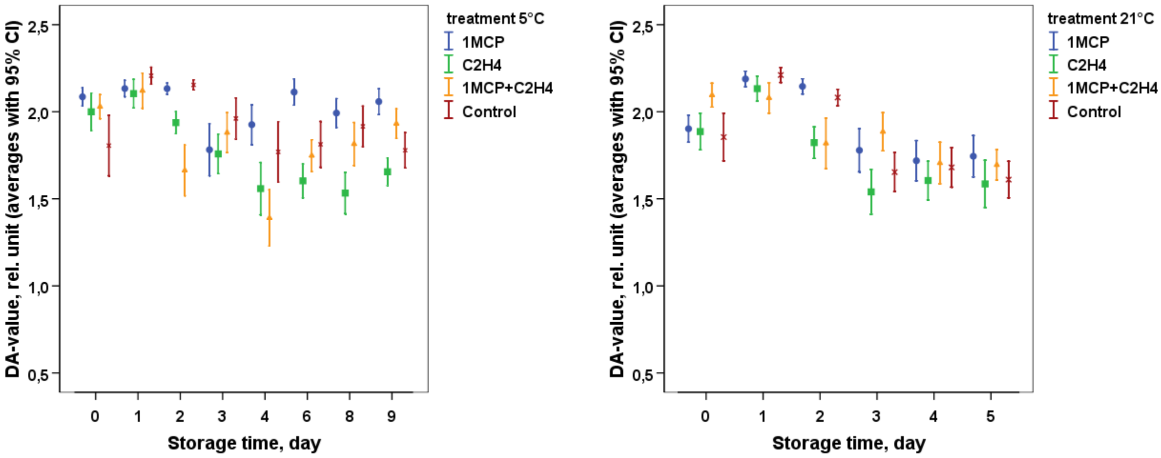

Fig. 5. The Change of broccoli's DA index® during storage at 5 °C (left) and at 21 °C (right).

Conclusions

The effects of 1-MCP, ethylene and 1-MCP followed by ethylene treatments on fresh, mature green broccoli samples' quality were investigated by non-destructive optical methods during the 5 and 9 days long storage at 5°C and 21°C, respectively. The temperature and treatment dependent changes in overall and keeping quality, mass loss, photosynthetically active chlorophyll content related characteristics (represented well by F_m, F_v, F_v/F_m, F_m/F_0 and DA-index®) were found to characterize reliably the highly positive effect of 1-methyl-cyclopropene (1-MCP) treatment on fresh mature green broccoli applied within 12 hours after harvest. Having looked at the results of our experiment, it is clearly to be seen that the quality preserving combined effect of 1-MCP and cold storage together was proven to prevent or at least minimalize the highly deteriorative effect of gaseous ethylene (increased mass loss, chlorophyll breakdown caused yellowing, leaf and florets abscission, etc.). Even the 24 hours long 2 ppm ethylene treatment was not able to eliminate the effect of 1-MCP treatment (24 hours, 625 ppb) due to the complete and effective blockage of ethylene receptors during the 9 days long cold storage at 5°C. Our results coincidence with the results of Fan and Mattheis (2000), Fernández-León et al. (2013), Sabir (2012) and Yuan et al. (2010) suggesting the application of combined treatments with 1-MCP and low temperature cold storage for quality maintenance of fresh broccoli. Concerning reproducibility of the applied measurements for broccoli quality determination, the increased number of measuring points or the use of computer aided imaging methods (i.e. chlorophyll fluorescence imaging, machine vision system) providing global and more reliable information related to quality changes could serve more reliable data.

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