

Effect of Temperature in Color Changes of Green Vegetables

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ABSTRACT

The effect of temperature on colour degradation of green vegetables as well as the chlorophyll content as a factor that contributes to colour variation were studied. The studied vegetables were lettuce (leafy) and broccoli (inflorescence). The results suggested that as the storage temperature increases so the chlorophyll degradation rate increases and so the colour degradation. From the two tested vegetables the lettuce was proved to be more sensitive to chlorophyll and colour degradation.

Keywords: Temperature, chlorophyll, colour, lettuce, broccoli.

INTRODUCTION

Vegetables are an important factor of human nutrition. Following harvesting they undergo a series of physiological changes that affect the quality. These changes can not be stopped but can be controlled within certain limits by using the appropriate post harvest handling. Temperature control is one of the major treatments for increasing the time of their commercial life and maintain their quality. Cantwell *et al* (1998), reports that temperature is the only factor that determines the post-harvest quality of leafy green vegetables. It should be noted that the higher the storage temperature the faster the deterioration and the shorter the duration of the commercial life and this is because temperature affects the metabolism and respiration rate. The low temperature (in tolerance limits) and high relative humidity increase the commercial life of most fresh vegetables (Cantwell and Kasmire, 2002), delaying degradation of chlorophyll (Pogson and Morris,

1997). The degradation of chlorophyll is considered as the first visible symptom of aging, but when it appears in fact aging is already advanced (Ansari and Chen, 2011). Degradation of chlorophyll is a complex multi-pathway as the biosynthesis of which may be summed in two basic group of reactions; the first one produce greenish derivatives while the second one, colourless compounds by an oxidative ring opening (Marquez and Sinnecker, 2007a). The degradation of chlorophyll reduces the intensity of the green color and leads to yellowing. Yellowing of leafy vegetables and broccoli is attributed to the action of the enzymes peroxidase and lipoxygenase (Murcia *et al.*2000). The reduction of the intensity of green vegetables is associated with aging, reduction of the nutritional value and in general their quality (Shewfelt, 2000; Cantwell and Kasmire, 2002).

The initial stage of the degradation of chlorophyll is influenced by external factors such as: the water stress, changes in temperature, light,

ethylene, inadequate lighting or a combination (Heaton and Marangoni, 1996; Nooden, 1988a, 1988b; Okada *et al.* 1992; Taylor and Craig 1971, Thomson, 1988; Agüero *et al.*, 2011).

Consumers choose fruits and vegetables based on appearance (Abbott, 1999). An important parameter of the display is color which is affected by chemical, biochemical, microbiological and physical changes that occur during development, maturation and post harvest handling (Pankaj *et al.*, 2011). Color is used as the maturity or aging criterion and as an indicator of physiological, mechanical or pathological lesions (Kader, 2002).

Green vegetables such as lettuce and broccoli are widely used in the Mediterranean diet. Over the last decade it has been scientifically proved and promoted that these vegetables constitute important functional food components by contributing vitamins, minerals and biologically active compounds which are associated with dietary activities (Kimura & Rodriguez-Amaya, 2003).

Leafy vegetables like lettuce are highly perishable, characterised by a short life-time and high transpiration rates due to high ratio of surface to mass, fact that contributes to accelerating senescence and colour loss. (Marquez & Sinnecker, 2007b).

The parameters for maintaining visual and nutritional quality in broccoli heads after harvest are well understood, with low temperature maintenance being of paramount importance. Recently, much attention has been focussed on the phytochemicals contained within broccoli, glucosinolates in particular, that may help prevent the onset of certain cancers and cardiovascular disease (Jones *et al.* 2006).

The aim of this work is the study of the changes in the color and especially chlorophyll in two green vegetables widely consumed and of high commercial value, lettuce and broccoli, when stored at temperatures of 0°C, 5°C, 10°C and 20°C. According to Watada *et al.* (1996), the recommended storage temperature is 0°C, however vegetables are often stored at 5°C and occasionally at 10°C. The temperature of 20°C is the room temperature.

MATERIALS AND METHODS

Sample preparation

Lettuce type Romana (*Lactuca sativa*, var. *Paris Island*), were cut at the stage of commercial maturity, (approximately 20 to 24 leaves / head) early in the morning and immediately transferred to postharvest laboratory where sorting was carried out to reduce the natural variability and remove the outer leaves.

Broccoli (*Brassica oleracea*, var. *Marathon*) were harvested when the warheads were fully developed without the florets being deployed. Immediately after cutting which was carried out early morning they were transported to the laboratory where they were screened for size and color.

Both lettuce and broccoli were grown on the farm of TEI Peloponnese in Kalamata. Following sorting they were divided into 4 groups, each of which contained 36 pieces.

The vegetables were stored in the dark to prevent potential interference of photosynthesis on chlorophyll breakdown at four different temperatures: 0, 5, 10 and 20°C and 90% relative humidity. At 0, 5 and 10°C the lettuce storage period was 13 days whereas that of broccoli 23 and at 20 ° C the lettuce storage time was 10 days whereas that of broccoli 12 days.

The parameters studied were: the change in the total chlorophyll content and the change of color. Measurements were made on the 0, 3, 6, 10th, and 13th day in the case of lettuce and 0, 6th, 9th, 12th, 16th, 19th and 23rd in the case of broccoli. Measurements were made at six different samples per temperature for both vegetables.

Total chlorophyll determination

The chlorophyll was extracted with the solvent dimethylsulfoxide (DMSO) in accordance with the method of (Hiscox & Isrelstam, 1979, Barnes *et al.*, 1992). 0.1g of tissue was placed into a test tube containing 10 ml DMSO. The tube loosely capped was placed in a water bath at 60 ° C until complete depigmentation (1h). Cooling was followed at room temperature for 30 min, followed by filtration and

measurement of the absorption at wavelengths 665 nm and 648 nm. Calibration of the instrument (blank) was carried out with DMSO. The concentration of total chlorophyll was expressed in mg / g fresh weight of the sample and was calculated from the formula $Chl\ total = 7,49 A^{665} + 20,34 A^{648}$ (Hiscox & Isrelstam, 1979).

Colour determination

The color changes of lettuces and broccoli were determined by the colorimeter Minolta (Model CR-300, Minolta Co Ltd Osaka). Before each measurement the instrument was calibrated by a white calibration plate ($Y = 92.6$, $x = 0.3135$ and $y = 0,3193$). The color was determined by the parameters L^* , a^* , b^* . L^* parameter gives brightness and changes between 0 (black) to 100 (white). The color parameter a^* gives the green ($-a^*$) or the red ($+a^*$), and the color parameter b^* gives the yellow ($+b^*$) or blue ($-b^*$) (Mc Guire, 1992). From the parameters L^* , a^* and b^* the hue h° [$h^\circ = \tan^{-1}(b/a)$] was calculated (Lancaster *et al*, 1997) and the color difference $\Delta E^* = [(L-L_0)^2 + (a-a_0)^2 + (b-b_0)^2]^{1/2}$. L_0 , a_0 , b_0 are the values at baseline. The ΔE^* is important when the relationship between the visual and the numerical analyses is evaluated while the h° are the qualitative attributes of color (Sant'Anna *et al.*, 2013).

At heads of broccoli color was determined at 5 points (4 in the region and one in the center). In lettuce color was determined at three points of the plate (base, medium, end) in 3 leaves located immediately after the outer sheets. The change of color of both vegetables was attributed by the parameters: L^* , h° and ΔE^* .

Statistical analysis

Experimental data was analyzed with the statistical package Statgraphics Plus (5.1). Comparison of the means was carried out with the Fisher method of least significant differences (LSD), at a confidence level $p = 0.05$.

RESULTS AND DISCUSSION

Changes in chlorophyll

Change in total chlorophyll of lettuce (a) and broccoli (b) is shown in Fig. 1.

Statistical analysis (ANOVA) showed that the factors affecting the degradation of chlorophyll in lettuce and broccoli are: storage temperature, storage life and their interaction ($p = 0.05$).

From Figure 1 it is shown that at the temperatures of 10 and 20°C a sharp degradation of chlorophyll occurs both in lettuce and broccoli. On the third day of storage at these temperatures (Figure 1a) lettuce has lost approximately 20% of the initial chlorophyll, and at the end of storage (day 13) the percentage of the loss amounted to 46% at 10°C and 64% at 20°C. In the case of broccoli (Fig.1 b) after 6 days of storage at 10 and 20°C a loss of about 44% and 80%, respectively was shown, while at the end of storage (23 days at 10° C and 12-days at 20 ° C) these rates amounted to 93 and 88%, respectively. Our results agree to those reported by (Lemoine *et al* 2007); Page *et al* 2001; Costa *et al.* 2006).

At 5 ° C storage chlorophyll content was constant for the first 3 days in lettuce and 6 days in broccoli. Page *et al* (2001) reported that broccoli presented a significant reduction in chlorophyll after 11 days at 4°C, while Zhuang *et al* (1994) reported that broccoli showed a significant reduction in chlorophyll (~ 40%) on the 6th day at 5°C. At the end of storage lettuce showed a loss of 30% which is consistent with the findings of Singh *et al* (1972), while the broccoli showed a loss of 73%. At temperatures of 5, 10 and 20°C the change of chlorophyll in lettuce and broccoli is practically a linear function of storage time with R^2 ranging between 0.83 and 0.99 in both cases.

At the end of storage at 0°C lettuce (day 13) showed a loss of about 5%, and broccoli a loss of approx. 25%. The temperature of 0°C reduces the respiratory activity, the rate of metabolism and the degradation of chlorophyll (Pogson and Morris, 1997). The degradation of chlorophyll is a symptom of transition of chloroplasts to gerontoplasts, score characteristic of plastids of aging (Sitte *et al.*, 1980). Degradation of chlorophyll brings to the surface colors that preexisted, in this case carotenoids (Gross, 1991).

Colour change

Change in colour is attributed to L^* , h° , ΔE^* parameters, (Table 1)

Statistical analysis (ANOVA) showed that the change of color parameters L^* , h° and ΔE^* in both products is affected significantly ($p = 0.05$) by temperature, storage time and their interaction.

The temperature of 0°C maintained brightness close to the baseline levels at both cases. Therefore, at the end of storage (day 13) lettuce lost 5% of its brightness, whereas the broccoli lost 3% at day 23. The higher the storage temperature the greater the change in brightness. Hence, at the end of storage (13th day) lettuce stored at 5°C showed an increase of about 8%, at 10°C , the increase was

13%, in particular 20°C , the increase recorded was of the order of 36 %.

In broccoli the increase in brightness observed at the end of storage at 5°C was 12%, at 10°C was 19% and at 20°C was 30%. In both cases the change in brightness (L^*) at temperatures of 5, 10 and 20°C was a linear function of storage time with R^2 ranging between 0.71 and 0.98.

The parameter h° (hue) at end of storage at 0°C was maintained at baseline levels in both vegetables. At temperatures of 5, 10 and 20°C in

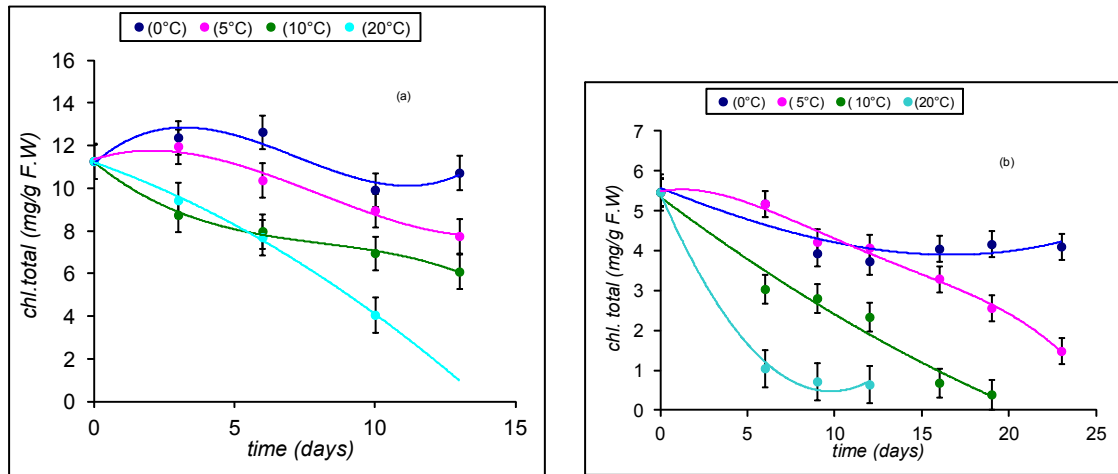


Fig. 1: Change in total chlorophyll of lettuce (α) and broccoli (β) at 4 different temperatures (N=6) (I= LSD)

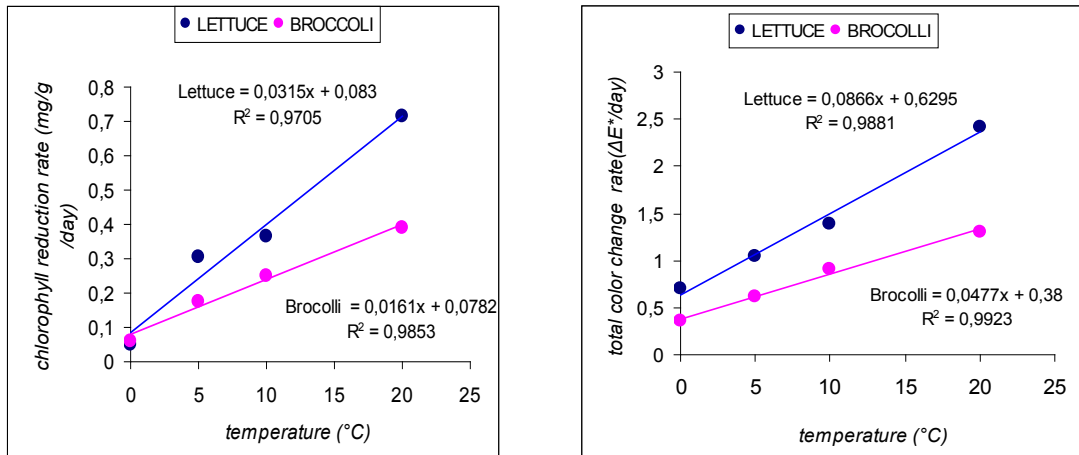


Fig. 2: Change in the rate of chlorophyll reduction and rate of change in total colour for lettuce and broccoli

both vegetables a reduction was observed which was stronger as the storage temperature was higher. Thus at temperatures 5, 10 and 20°C the reduction of the parameter values h° for lettuce was 5, 11 and 10% respectively, while for broccoli was 13, 16 and 28%.

An increase in the total color change ΔE^* was observed from the first day of the storage. At 0°C the variation was maintained at low levels while at higher temperatures the variation was more pronounced.

At a temperature of 0° C a slight decrease in brightness (L^*) was observed along with maintenance of the hue values (h°) close to baseline levels for both vegetables. The slight decrease in L^* values indicates that the color became darker during storage, while the maintenance the h° values shows evidence that there was no reduction in the intensity of green color. Our results agree with those of Chandra *et al* (2008) in lettuce and Gnanasekharan *et al* (1992) in broccoli. At higher storage temperatures an increase in the L^* values was observed along with a reduction in the h° values. The increase in L^* values is a sign of reduction

of the intensity of green color due to appearance of yellow pigments, while low values of h° is an indication of degreening associated with aging. These results agree with those of Chandra *et al* (2008), Suthumchai *et al.* (2007) in lettuce and Lemoine *et al* (2008) in broccoli.

Broccoli stored at 20°C showed values of h° less than 90° due to the color parameter a^* taking positive values. This result agrees with Gnanasekharan *et al* (1992).

It should be noted that at day 3 in lettuce and day 6 in broccoli samples stored at 10 and 20°C there was shown a significant reduction in chlorophyll, whereas the change in h° was not analogous, indicating that the degradation of chlorophyll starts before the color change is perceived. Measurement of chlorophyll is a more sensitive parameter for studying the aging of plant tissues. This result agrees with those of Costa *et al* (2006) and Finger *et al* (1999) in broccoli.

Comparison between lettuce and broccoli

In order to compare the degradation of chlorophyll in a leafy vegetable such as lettuce

Table 1: Change in color parameters L^* , h° , ΔE^* of lettuce and broccoli stored at 0, 5, 10 and 20°C

| Days | L^* | | | | h° | | | | ΔE^* | | | |
|----------|-------|-------|--------|-------|-----------|--------|--------|--------|--------------|-------|-------|-------|
| | 0°C | 5°C | 10°C | 20°C | 0°C | 5°C | 10°C | 20°C | 0°C | 5°C | 10°C | 20°C |
| Lettuce | | | | | | | | | | | | |
| 0 | 42.0 | 42.0 | 42.0 | 42.0 | 121.4 | 121.4 | 121.4 | 121.4 | 0 | 0 | 0 | 0 |
| 3 | 39.7a | 42.7b | 44.4bc | 45.5c | 120.7a | 120.9a | 120.8a | 117.9a | 5.0a | 4.8a | 4.3a | 5.6a |
| 6 | 42.7a | 43.4a | 43.4a | 56.8b | 122.2a | 120.9a | 119.7a | 114.6b | 6.2a | 6.3a | 8.6b | 9.6c |
| 10 | 37.8a | 42.5b | 50.8c | 57.0d | 120.4a | 118.9a | 115.8b | 109.6c | 6.3a | 7.2a | 9.0b | 16.8c |
| 13 | 39.8a | 45.4b | 47.4b | | 120.0a | 114.7b | 107.8c | | 6.3a | 9.5b | 11.7c | |
| Broccoli | | | | | | | | | | | | |
| 0 | 40.0 | 40 | 40 | 40 | 117.7 | 117.7 | 117.7 | 117.7 | 0 | 0 | 0 | 0 |
| 6 | 39.3a | 38.4a | 42.5b | 45.3c | 118.1a | 118.0a | 114.8a | 87.8b | 5.1a | 4.7a | 6.5b | 12.4c |
| 9 | 40.0a | 40.1a | 45.1b | 47.7c | 119.1a | 116.7a | 107.7b | 86.1c | 6.1a | 6.3a | 10.3b | 12.4c |
| 12 | 39.0a | 40a | 48.3b | 52c | 117.3a | 116a | 100.4b | 83.9c | 5.0a | 6.1a | 15.0b | 16.2b |
| 16 | 40.2a | 43.6b | 48.9c | | 117.6a | 111.5b | 96.5c | | 6.4a | 10.3b | 15.7c | |
| 19 | 37.8a | 44.6b | 47.9c | | 118.5a | 105.3b | 99.2c | | 6.4a | 12.3b | 16.6c | |
| 23 | 38.9a | 44.9b | | | 118.8a | 102.3b | | | 6.0a | 14.1b | | |

Means, lettuce= 6 samples × 3leaves × 3 measurements/leaf=54

Means, broccoli= 6 samples × 5 measurements / sample = 30

Values within small letters show statistically significant changes from other values within the same row.

and one inflorescence like broccoli the average daily rate of chlorophyll was calculated along with the corresponding rate of total color change with temperature.

From Figure 2 it is evident that the lettuce shows a greater rate of chlorophyll reduction and change in total color. It should be noted that the color change patterns vary depending on the type of plant organ probably because of differences in the surface with the object interaction of incident light (Gnanasekharan *et al* 1992).

CONCLUSIONS

From the study results show that:

- In terms of colour criterion the lettuce can

be stored in marketable condition at 0 ° C for 13 days, at 5 ° C for 10 days, at 10 ° C for 6 days and at 20 ° C for 3 days, a storage which agrees with literature data (Suthumchai *et al.* (2007), Chandra *et al* (2008).

- Broccoli if not stored at low temperature presents aging symptoms and degradation of chlorophyll. Therefore, at 0°C it can be stored at marketable condition for 23 days, at 5 ° C for 12 days, at 10 ° C for 6 days and at 20 ° C for 2 days, which is consistent with the observations of Cantwell and Kasmire (2002) and Finger *et al* (1999).

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