South Western Journal of Horticulture, Biology and Environment
P-Issn: 2067- 9874, E-Issn: 2068-7958

Vol.2 , No.2, 2011
pp.193-212

CHANGES OF ANTIOXIDANT COMPOUNDS OF BROCCOLI (*Brassica oleracea* L.var. *Italica*) DURING STORAGE AT LOW AND HIGH TEMPERATURES

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Abstract. *The influence of storage temperature on the changes of antioxidant compounds of five broccoli cultivar was investigated during two different storage temperatures. Florets were stored three days at 20 °C and 40 days at 0 °C, followed by two additional days at 20 °C. The florets deterioration rate was strongly affected by storage temperature, subsequently, the rapid decrease of, chlorophyll, carotenoid and total flavonoid was observed at 20 °C. The antioxidant protection occurred by total phenol, flavonoid, carotenoid and also peroxidase (POD) enzyme are important for the retention of green colour in broccoli flower buds and the increases in POD were likely related to florets yellowing. The result showed that in both temperatures higher phenolic content, antioxidant capacity and lowest POD activity was associated with maintenance of broccoli quality and chlorophyll and delayed lipid peroxidation. The phenolic content and lowest pod activity in* '*General*' *and* '*Revolution*' *cultivars at 0 °C storage and* '*Liberty*' *and* '*Revolution*' *at 20 °C storage is important for the retention of green colour in broccoli florets.*

Key words: *Broccoli, Antioxidant Compounds, Peroxidase, Senescence, Temperature Storage*

INTRODUCTION

Broccoli (*Brassica oleracea* L. var *italica*) is a floral vegetable with an important nutritional value due to its content of vitamins, antioxidants and anti-carcinogenic compounds (Lemoine et al. 2010, Lemoine et al. 2009). Among these bioactive compounds, phenolics, vitamins and carotenoids deserve special attention (Borowski and Szajdek 2008). Most of the antioxidant potential in plants is due to the redox properties of phenolic compounds which allow them to act as scavenge reactive oxygen spieces (ROS) and the reform their resistance to environmental stresses (Hodges

2003). Flavonoids and their derivatives also are the largest and most important group phenolic compounds in plants (Hounsome et al. 2009) and display free radical scavenging activity and inhibitation of oxidative stress Cell membrane degradation (Koh et al. 2009). Carotenoids which occur in the chloroplast membranes also help prevent oxidative damage from ROS (Hodges 2003). However, limited information is available on the effect of postharvest handling on carotenoids in broccoli (Yuan et al 2010). Thus, Antioxidants is very important to inhibit free radical reactions, and may therefore protect cells against oxidative damage (Kurilich et al. 2002, Soto-Zamora et al. 2005).

Fresh broccoli is highly perishable, with a storage life of 3 to 4 weeks in 0 °C and 95% RH and 2 to 3 days at 20 °C (King and Morris 1994, Jacobsson et al 1994). This is due to broccoli having a relatively high rate of metabolism and consequently a high respiration rate, being extremely sensitive to ethylene (Jacobsson et al 1994). Various studies have demonstrated that shelf life of fruits and vegetables is modulated by antioxidants, suggesting the involvement of ROS in senescence (Hodges 2003). In some previouse study decrease in the antioxidant compounds decreased in fruit and vegetable reported during storage (Yuan et al. 2010; DuPont et al. 2000, Hounsome et al. 2009), however, an increase in antioxidant levels was found during short-term storage of broccoli and tomatoes (Leja et al. 2001, Toor and Savage 2006). The main objective of this study was therefore to investigate the changes of antioxidant compounds during storage during low and high storage temperatures.

MATERIAL AND METHODS

Plant material

Five different broccoli (*Brassica oleracea* var. *Italica*) cultivars, ‘General’, ‘Liberty’, ‘Pilgrim’, ‘Revolution’ and ‘Millady’ were grown at University of Guilan, Rasht, Iran. The inflorescences were harvested in the early morning, when the heads were completely developed and without opened florets (Finger et al 1999). Head separated into florets and stem and disinfected with chlorinate water (150 mL L^{-1} as sodium hypochlorite) for 15 min, followed by repeated washing with distilled water. broccoli florets were dried at room temperature, then five florets of broccoli (include 20 g each florets) were placed in polyethylene bags with overall dimensions 20×20 cm2 and 29.2 pmol/s/m2/Pa oxygen transmission rate film and stored three days at 20 °C and at 0 °C for 40 days, followed by two additional days at 20 °C. The following biochemical characteristics were evaluated at harvest time (day 0), and 40 days and 2 additional days in 0 °C storage, and during three days at 20 °C.

Evaluation

Weight loss during postharvest storage was determined on the day of harvest and after the different sampling dates and expressed as percentage loss of original weight (Yang et al. 2010). Total chlorophyll and carotenoid content was measured according to Lemoine et al. (2009). Florets were powdered by liquid nitrogen in a refrigerated mill and 0.4 g of the powder obtained was added to 5 mL of acetone/water (80: 20), stirred and then centrifuged at 2800 rcf for 15 min. The supernatant was used to determine the content of chlorophyll and carotenoid according to Lichtenthaler (1987). Results were expressed as mg chlorophyll /g FW and mg carotenoid/100g FW. Total phenolics, total flavonoids and antioxidant capacity was determined by the method of Du et al. (2009). Floret sample (1.25 gr) was homogenised in extracted in 5 mL of ethanol: acetone (7:3, v/v) for 1 h at 37 °C. Therefore, was filtered through whatman paper and rinsed with 1 mL of ethanol: acetone (7:3, v/v) and then stored at 20 °C until used for analysis of the total phenolics, total flavonoids and antioxidant capacity. Total phenols content was determined using the Folin-Ciocalteu method. In a eppendorf tube, 7.9 mL distilled water, 100 µL broccoli extract and 500 µL Folin-Ciocalteu reagent (1:1 with water) were added and mixed. After exactly 1 min, 1500 µL of sodium carbonate (20 g/100 mL) was added, and the mixture was mixed and allowed to stand at room temperature in the dark for 2 h. The absorbance was read at 765 nm by spectrophotometer. Gallic acid was used for calibration curve. Results were expressed as mg GAE/100 g FW.

In order to total flavonoids content 150 µL broccoli extract, 1700 µL 30% ethanol, 150 µL of 0.5 mol/L $NaNO_2$ and 150 µL of 0.3 mol/L $AlCl_3$. $6H_2O$ were added and mixed. After 5 min, 1 mL of 1 mol/L NaOH was added, and the mixture was measured at 506 nm by spectrophotometer. Results were expressed as mg mg/100 g FW.

The antioxidant activity was measured by the scavenging of 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) radicals according to Brand-Williams et al (1995) with minor modifications. Briefly, 800 µL solution of DPPH (6.25×10^{-5} M) in methanol was added to 200 µL of broccoli extracts. In the presence of antioxidant the purple color intensity DPPH solution declined and the change of absorbance is followed spectrophotometer at 517 nm. The content of the tubes were mixed and followed to stand for 30 min and absorbance was measured at 517 nm. The antioxidant activity is expressed in the form of the percentage of free radical scavenging.

Enzyme extract of POD was prepared by first freezing a weighed amount of floret tissue (0.5 g) in liquid nitrogen followed by grinding with 10 mL extraction buffer (50 mM phosphate buffer, pH 7 containing 0.5 mM EDTA and 2% PVPP (w/v). Homogenate was centrifuged for 20 min at 21925 rcf and the supernatant used to determine POD activity. POD activity was assayed by measuring spectrophotometrically the formation of guaiacol in l mL reaction mixture of 450 µL 25 mM guaiacol, 450 µL 225 mM H_2O_2 and 100 µL crude enzymes (In et al 2007). The increase in absorbance was recorded by the addition of H_2O_2 at 470 nm for 2 min (e, 26.6 mM^{-1} cm^{-1}).

Lipid peroxidation (MDA level) was determined by the method of Heath and Packer (1968). Floret sample (0.5 g) was homogenized in 1 mL of 0.1 % trichloracetic acid (TCA). The homogenate was centrifuged at 21925 rcf for 15 min, and then 600 µL of supernatant was added 600 µL ml of 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was heated at 95°C for 30 min and then cooled in an ice bath. After centrifugation at 11200 rcf for 10 min, the absorbance of the supernatant was recorded at 532 nm. The MDA level was calculated according to its extinction coefficient of 155 Mm cm^{-1}.

Statistical analysis

All determination was performed in triplicate. The recorded data were statistically analyzed (ANOVA analysis) using the software of SAS. Sources of variation were five different hybrid broccoli cultivar and two storage conditions. Means were compared with the Tukey's test at $p \leq 0.05$.

RESULTS

Loss weight

The changes in the weight decrease were shown in Table 1 and 2. The rapid decrease of weight was found in broccoli stored at 20° C but low storage temperature delayed weight decrease (Table 1 and 2). The highest weight decrease was found in 'General', 'Pilgrim' and 'Millady' cultivars during storage at 20 °C (Table 1). During storage in low temperature, no significant changes were observed in weight decrease (Table 2). However, weight gradually decreased during storage at 0°C, followed by two additional days at 20 °C in the all broccoli cultivars (Table 2)

Table 1. The weight loss (%) of five broccoli cultivars during three days storage at room temperature.

	General	Liberty	Pilgrim	Revolution	Millady
1 day	1.02 ± 0.18^{efg}	0.86 ± 0.035^{fg}	0.99 ± 0.29^{efg}	$0.7\pm0.0.12^{g}$	0.98 ± 0.05^{fg}
2 day	2.05 ± 0.12^{c}	1.5 ± 0.032^{de}	1.95 ± 0.049^{cd}	1.32 ± 0.042^{ef}	2.18 ± 0.07^{c}
3 day	3.84 ± 0.07^{a}	2.59 ± 0.04^{b}	3.59 ± 0.007^{a}	2.94 ± 0.12^{b}	3.69 ± 0.15^{a}

Means of three replicates followed by the same letters were not statistically significant different (P≤0.05).

Chlorophyll content

Regardless of broccoli cultivars, chlorophyll content of florets declined during storage (Figs 1 and 2). The rapid decrease of chlorophyll was observed only in the case of broccoli heads stored at 20 °C but low storage

temperature delayed yellowing and reduced loss in chlorophyll concentration (Figs 1 and 2). The 'General' cultivar maintained the higher chlorophyll content after 40 days storage at 0°C and two additional days at 20 °C (Fig. 2), but at room temperature (20 °C) storage, chlorophyll content of 'Pilgrim' was higher than two others cultivars (Fig. 1).

Table 2. The weight loss (%) of five broccoli cultivars after 40 days storage at 0°C and two additional days at room temperature.

	General	Liberty	Pilgrim	Revolution	Millady
40 day	1.159±0.26 [a]	1.15±0.14 [a]	1.48±0.09 [a]	0.94±0.24 [a]	1.56±0.15 [a]
40+2 day	1.74±0.087 [a]	2.45±0.04 [a]	2.25±0.08 [a]	1.65±0.94 [a]	2.5±0.04 [a]

Means of three replicates followed by the same letters were not statistically significant different (P≤0.05).

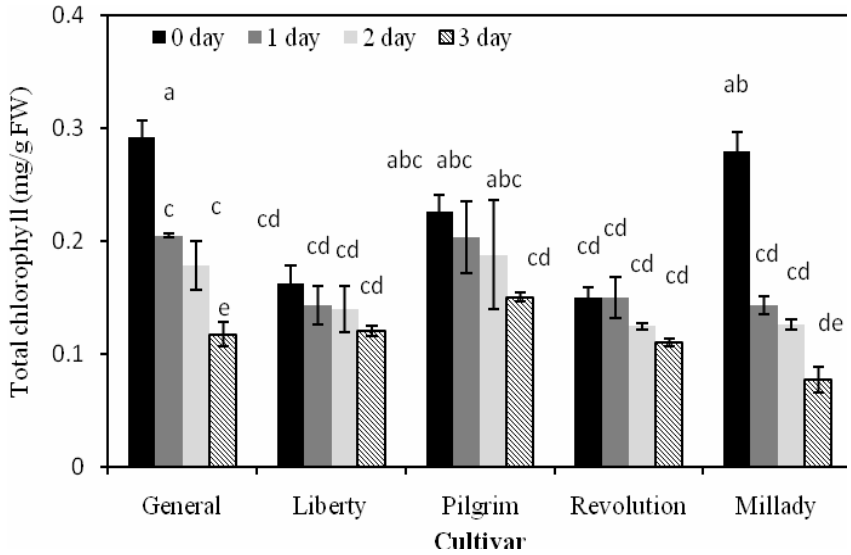

Figure 1. Changes in chlorophyll content of five broccoli cultivars during three days storage at 20°C. Vertical bars represent the average values with ±SE (n = 3).

Although, chlorophyll content of 'General', 'Millady' and 'Pilgrim' was higher at harvest time than two other cultivars, but the decline in total chlorophyll were much greater for these cultivars than 'Liberty'and

'Revolution' at 20 °C storage (Fig. 1). In contrast, 'General' and 'Revolution' retained chlorophyll content over 40 days at 0 °C and two additional days at 20 °C (Fig. 2). Therefore 'General'and 'Revolution' cultivars at 0 °C storage and Liberty' and 'Revolution' at 20 °C retained a stable chlorophyll content during storage.

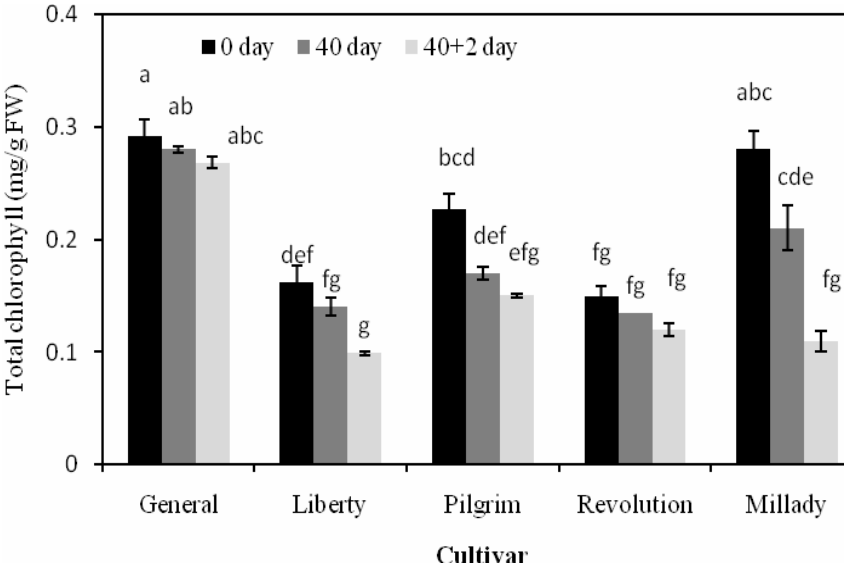

Figure 2. Changes in chlorophyll content of five broccoli cultivars after 40 days storage at 0°C and two additional days at room temperature. Vertical bars represent the average values with ±SE (n = 3).

Carotenoid content

A rapid decline in carotenoid content occurred in florets kept at 20°C, while at low storage temperature delayed and reduced loss in carotenoid level (Figs 3 and 4). The decline in carotenoid were much greater for 'General', 'Pilgrim' and 'Millady' cultivars at 20 °C, while carotenoid content of 'Liberty'and 'Revolution' cultivars was relatively stable over the three days period of the experiment (Fig 3).

In low temperature 'General'and 'Revolution' cultivars retained a stable carotenoid content over 40 days at 0 °C and two additional days at 20 °C but three other cultivar showed a constant decline in carotenoid content (Fig 4)

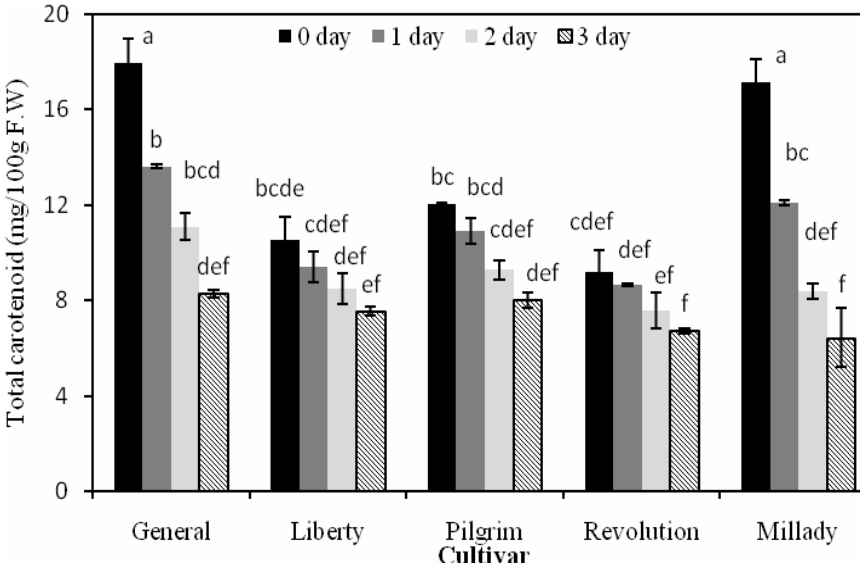

Figure 3. Changes in carotenoid content of five broccoli cultivars during three days storage at 20°C. Vertical bars represent the average values with ±SE (n = 3).

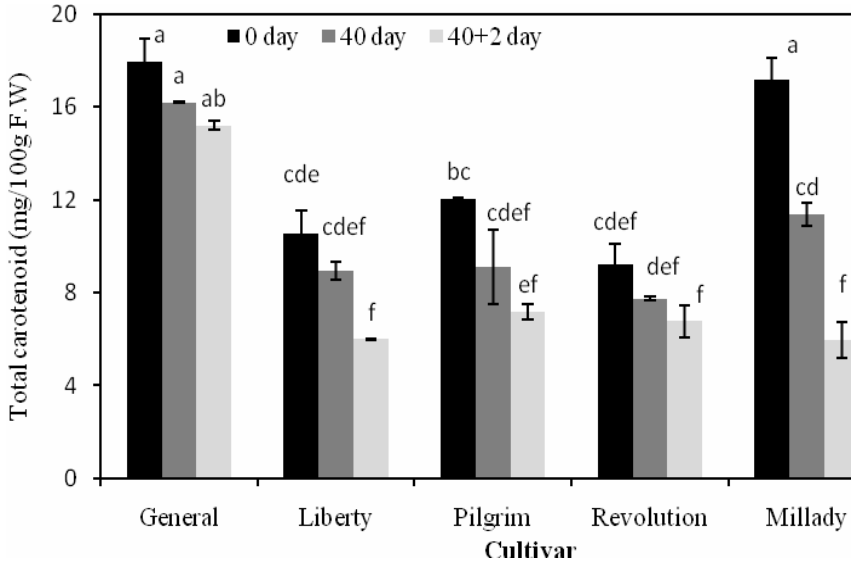

Figure 4. Changes in carotenoid content of five broccoli cultivars after 40 days storage at 0°C and two additional days at room temperature. Vertical bars represent the average values with ±SE (n = 3).

Total phenol

The changes of total phenol content in broccoli florets during three days storage at 20 °C and during 40 days storage at 0°C, followed by two additional days at 20 °C are found at Figs 5 and 6. Storage of the most broccoli cultivars at room temperature (20 °C) caused the significant increase of total phenol content (Fig 5). Total phenol content in 'General' cultivar increased 1 day after florets broccoli were harvested, but decreased towards the end of storage (Fig 5). Also, Total phenol content in 'Millady' cultivar decreased during of storage (Fig 5). The highest total phenol was found in the 'Liberty' and 'Revolution' cultivars in the end storage (Fig 5). Total phenol content increased in 'General' and' Revolution' cultivars by the 40 days, and thereafter, decreased after transfer to room temperature (Fig 6). In 'Millady' cultivar with over time total phenol content was decreased, while in 'Liberty' and 'Pilgrim' cultivars was not significant (Fig 6).

Total flavonoid

The total flavonoid level decreased during storage at both of room temperature and low temperature storage followed by two additional days at room temperature in florets broccoli cultivars (Figs 7 and 8). The highest total flavonoid level was found in 'Liberty' and 'Revolution'cultivars in the end storage at room temperature (Fig 7). In contrast, General' and 'Revolution' cultivars was observed highest flavonoid total content at 0 °C (Fig 8)

Antioxidant capacity

The changes of antioxidant capacity in broccoli florets during two different storage conditions were summarized at Figs 9 and 10 .The results have been showed that storage of broccoli florets at room temperature (20 °C) caused the significant increase of antioxidant capacity in 'Revolution' cultivar (Fig 9). In 'Millady' cultivar decreased while,' General', 'Liberty' and 'Pilgrim' cultivars no significant change during storage (Fig 9). The highest antioxidant capacity was found in the 'Liberty' and 'Revolution' cultivars in the end storage (Fig 9). In low temperature storage the antioxidant capacity in 'General' and 'Revolution' cultivars with over time was increased but in 'Liberty' and 'Millady' cultivars decreased (Fig 10). In 'Pilgrim' cultivar increased after 40 days but decline after transferring to room temperature (Fig 10).

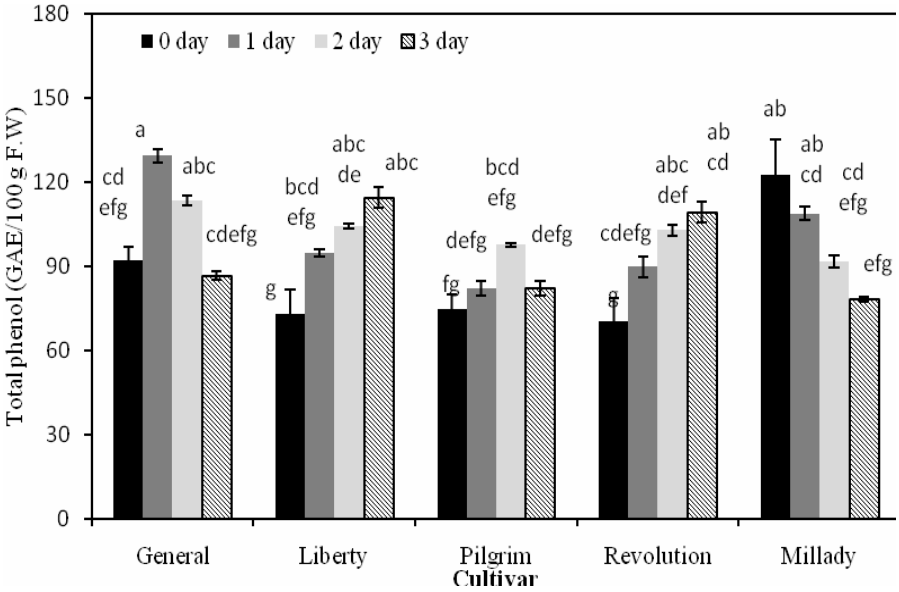

Figure 5. Changes in total phenol content of five broccoli cultivars during three days storage at 20°C. Vertical bars represent the average values with ±SE (n = 3).

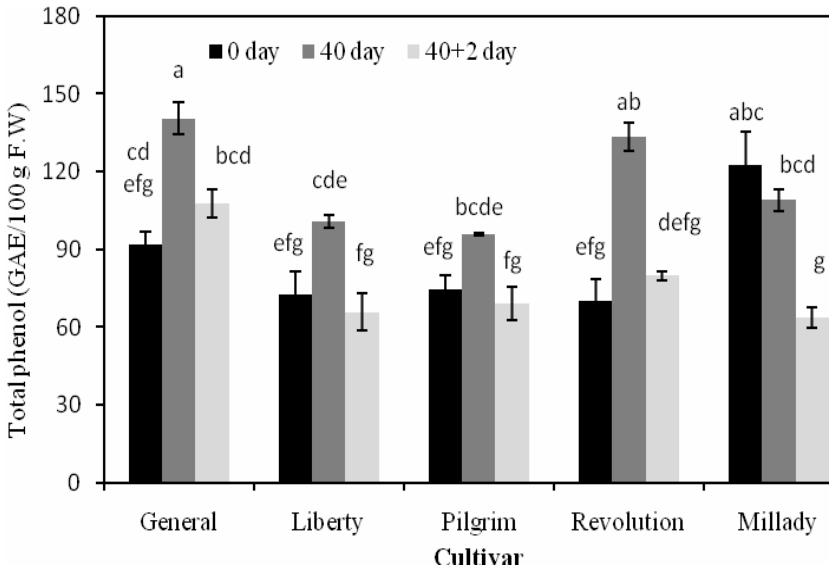

Figure 6. Changes in total phenol content of five broccoli cultivars after 40 days storage at 0°C and two additional days at room temperature. Vertical bars represent the average values with ±SE (n = 3).

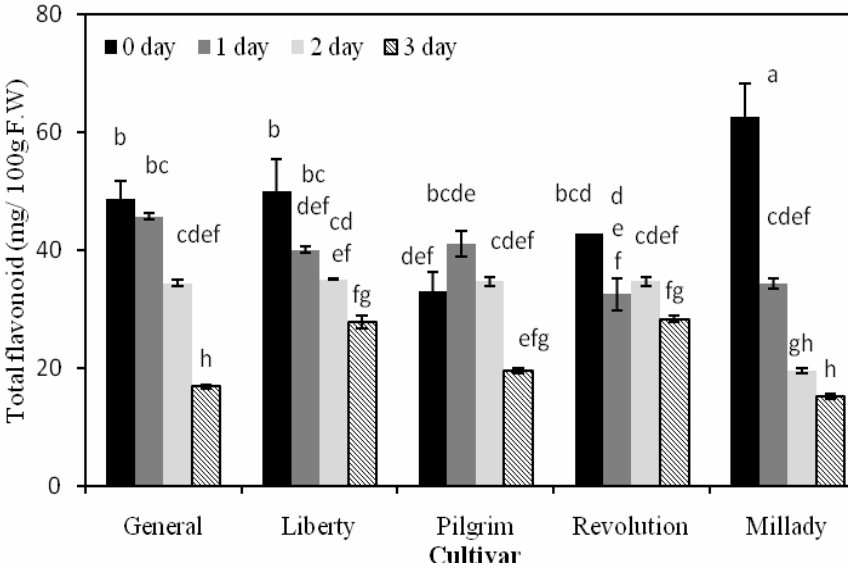

Figure 7. Changes in total flavonoid content of five broccoli cultivars during three days storage at 20°C. Vertical bars represent the average values with ±SE (n = 3).

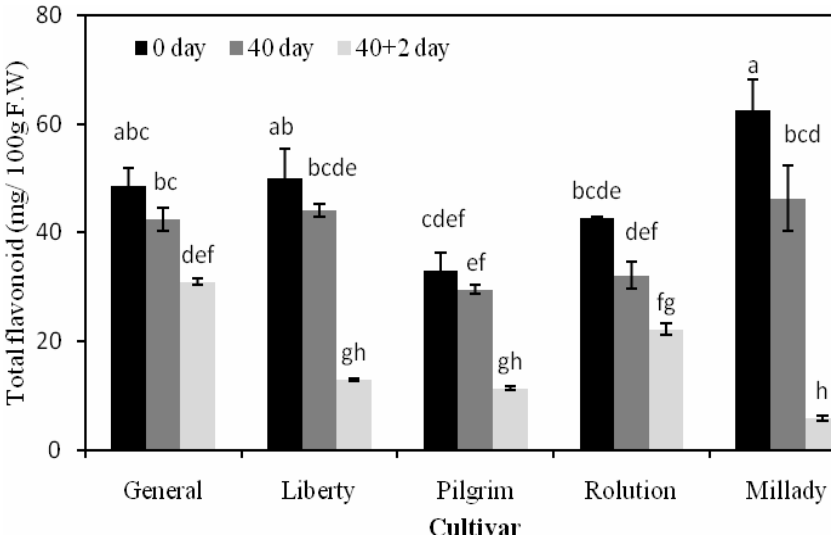

Figure 8. Changes in total flavonoid content of five broccoli cultivars after 40 days storage at 0°C and two additional days at room temperature. Vertical bars represent the average values with ±SE (n = 3).

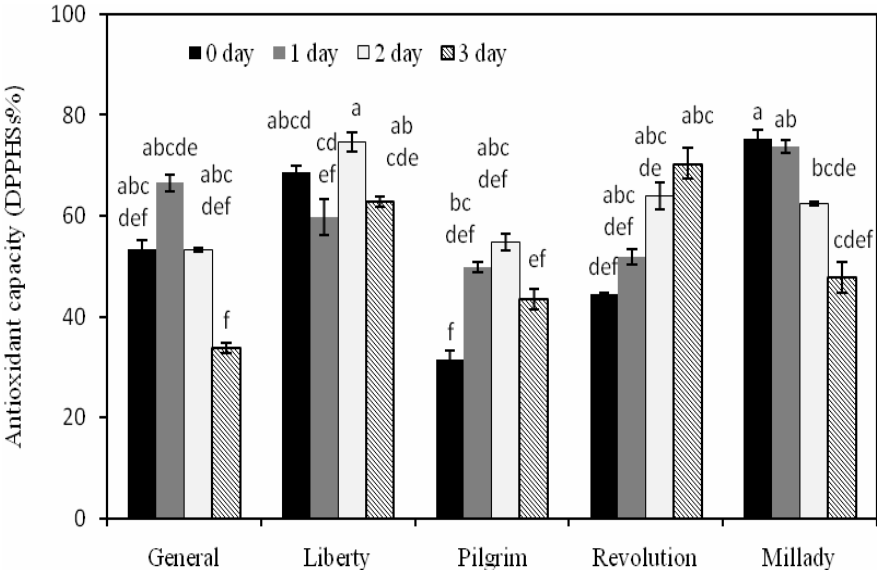

Figure 9. Changes in antioxidant capacity of five broccoli cultivars during three days storage at 20°C. Vertical bars represent the average values with ±SE (n = 3).

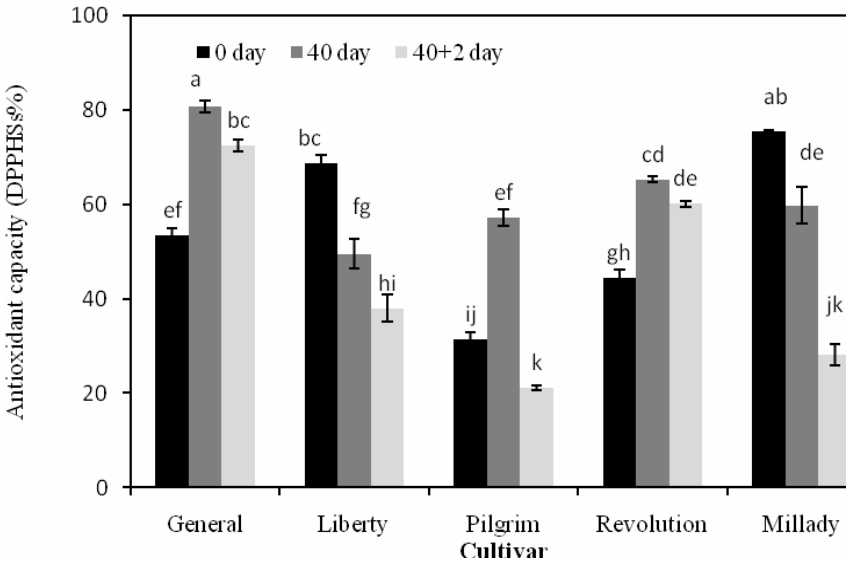

Figure 10. Changes in antioxidant capacity of five broccoli cultivars after 40 days storage at 0°C and two additional days at room temperature. Vertical bars represent the average values with ±SE (n = 3).

Peroxidase (POD)

The results have been showed that storage of broccoli florets both at room temperature (20 °C) and low temperature (0 °C) caused the significant increase of POD activity (Figs 11 and 12). The rapid increase of POD activity was observed after remove to 20 °C. Storage of florets at room temperature caused the increase of POD activity in 'General', 'Pilgrim' and 'Millady' cultivars (Fig 11). The highest activity of this enzyme was found in the 'General' and 'Millady'cultivars in the end storage (Fig 11). In low temperature storage POD activity increased significantly comparison to the initial value in all cultivars exception 'General and 'Revolution' cultivar. No significant differences were recorded in other cultivars in the end storage (Fig 12).

Lipid peroxidation (MDA)

The lipid peroxidation level increased during storage both at room temperature and low temperature storage, followed by two additional days at room temperature in florets broccoli cultivars (Figs 13 and 14). The highest MDA level was found in 'General'and 'Millady' cultivars in the end storage at room temperature (Fig 13). In low temperature the highest MDA content was observed in 'Liberty , 'Pilgrim' and 'Millady' cultivars (Fig 14).

DISCUSSION

Texture attributes degradation are directly related to water loss, which, in turn, can be evaluated by weight loss measurement (Raffo et al 2008). The rate water and weight loss is mainly dependent of temperature and humidity of the storage (Finger et al 1999) and that storage duration (Javanmardi and Kubota 2006). Cell membrane degradation one of the main physiological changes while senescence were occurred. This physiological changes which results in the lose the integrity of the membrane system, loss of selective permeability properties, change in ability of cell soluble material preservation, increase leakage in content cell and finally loss weight which reason loss of water in cell (Mayak 1987). This result confirmed the observations of Toivonen (1992), Pongson and Morris (1997) and Javanmardi and Kubota (2006) that low temperature markedly delays senescence of broccoli. Changes of chlorophyll level in photosynthetic cells are good indicator of senescence, occurring in green vegetable after harvested. Chlorophyll loss has been associated with lipid peroxidation (Zhuang et al. 1995) and with enhancement of peroxidase activity (Costa et al. 2006). Chlorophyll content degradation was increased

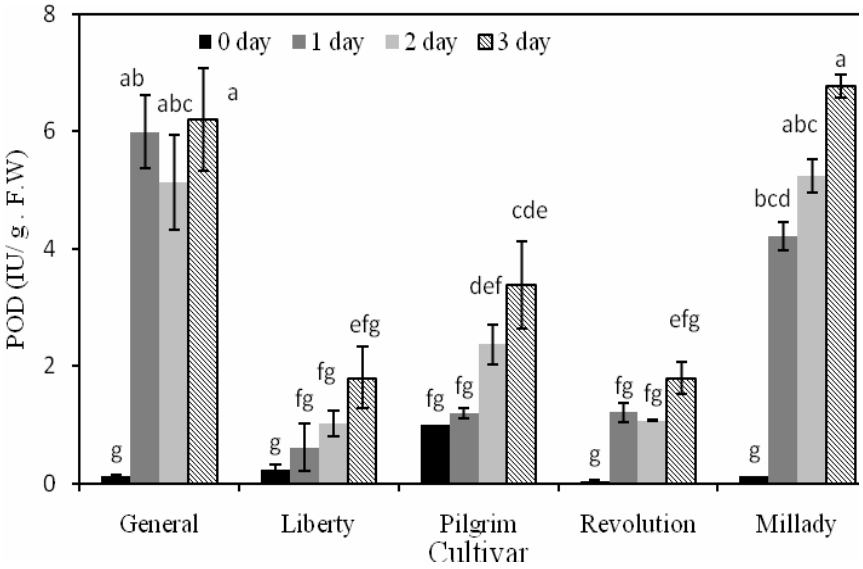

Figure 11. The POD activity of five broccoli cultivars during three days storage at 20°C. Vertical bars represent the average values with ±SE (n = 3). Vertical bars represent the average values with ±SE (n = 3).

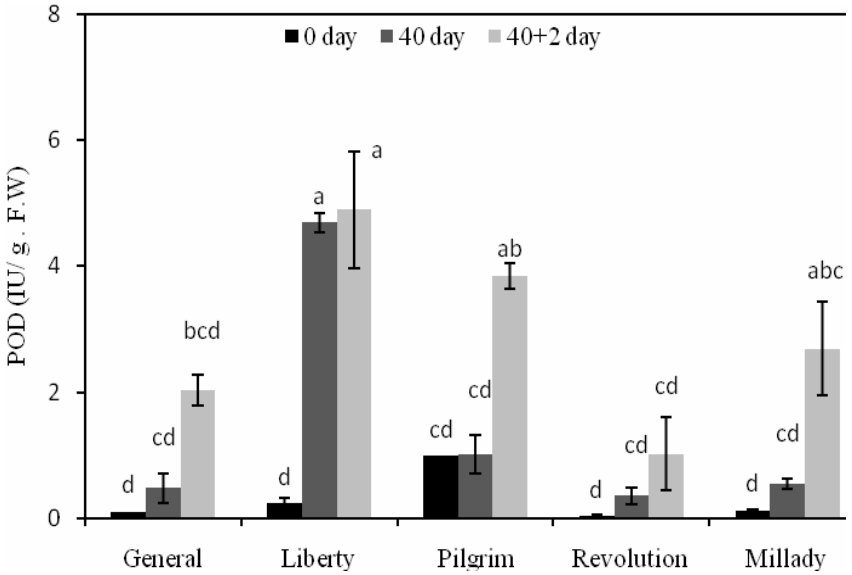

Figure 12. The POD activity of five broccoli cultivars after 40 days storage at 0°C and two additional days at room temperature. Vertical bars represent the average values with ±SE (n = 3). Vertical bars represent the average values with ±SE (n = 3).

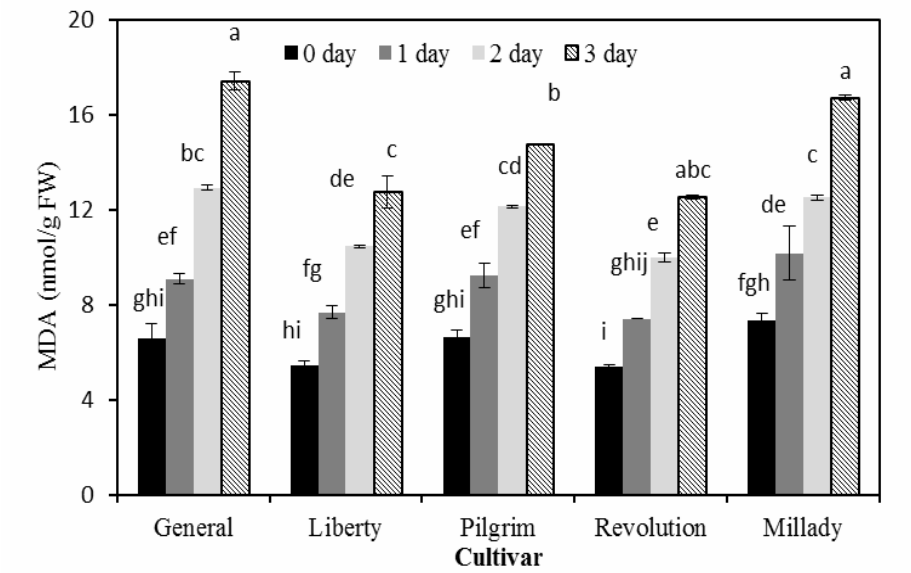

Figure 13. Changes in MDA content of five broccoli cultivars during three days storage at 20°C. Vertical bars represent the average values with ±SE (n = 3).

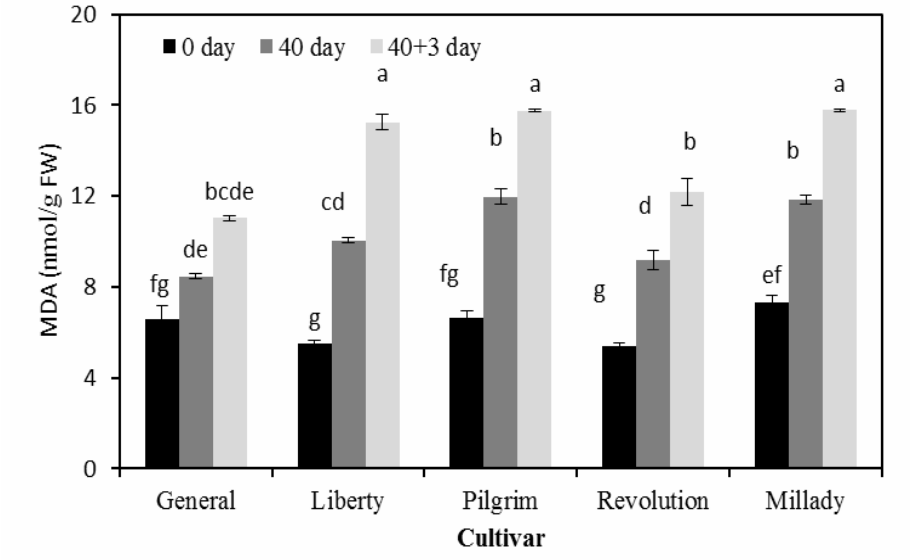

Figure 14. Changes in MDA content of five broccoli cultivars after 40 days storage at 0°C and two additional days at room temperature. Vertical bars represent the average values with ±SE (n = 3).

with temperature in broccoli florets (Starzyinska et al. 2003) and detected pakchoy leaves (Able et al. 2005). These results demonstrated that the temperature has an important role in florets senescence in broccoli (King and Morris 1994, Finger et al. 1999) and in some instances, influence the rate of senescence as measured by chlorophyll loss (Hodges 2003).. Starzyinska et al. (2003) reported that chlorophyll was degraded at a slower rate in the broccoli stored at 0°C, when compared with the material stored at room temperature. Low storage temperature delayed yellowing and reduced loss in chlorophyll concentration (Starzyinska et al. 2003).

Chain carotenoids are very powerful quenchers of ROS and at relatively low concentration can effectively protect membrane lipids from oxidation (Hodges 2003) and reducing oxidative damage (Hanson et al. 2009). Therefore, they serve as antioxidants in plants (Griffiths et al. 2007). The cell membrane deterioration process occurred by enzymes and ethylene production during senescence that lead to carotenoid degradation (Thompson et al. 1987). The data of the present experiment were in agreement with some previous studies, which also reported that low temperature was beneficial to maintain the carotenoids content (Griffiths et al. 2007, Yang et al. 2010). Also already research has been showed a decrease in total carotenoid content in a *S. phureja* line and in the *S. tuberosum* cultivar, Desiree, after 9 months of storage at 4 ◦C (Griffiths et al. 2007).

Phenolic compounds degradation associated with a loss of cellular compartmentation membrane integrity and enzymatic activities because of naturally synthesized enzymes by fruits and vegetables. Especially, polyphenoloxidase (PPO) and POD were reported as main agents responsible for the degradation of phenolic compounds in plants (Baltacig et al. 2011). With the long time during of storage, cells gradually lose the integrity of the membrane system, which results in the loss of compartmentation and allowing enzymes to act on their substrates (Zhang et al. 2000). The dversity stresses, such as mechanical and gas injuries, would do harm to the membrane structures of fruits and vegetables and the areal distribution might be destroyed (Yang et al. 2011). The phenolic compouns and related enzymes could interact with each other (Yang et al. 2011). Therefore, one reason for the phenolic contents increasing first might be induced to synthesize phenolic compounds due to mechanical and injury when florets were harvested (Yang et al. 2011). When membrane structures are still in a healthy condition, phenolic compounds and enzymes could not interact with each other because being located in different parts of cells. The synthesis of phenolic compounds is predominant and the contents of phenolic compounds increase (Yang et al.

2011). However, when the membrane structures are damaged, with the storage time being prolonged, the oxidization of phenolics compounds becomes the major trend and phenolic compounds were decreased in cell (Yang et al. 2011, Toor and Savage 2006). Accumulation of phenolic compounds, as an index of postharvest senescence, was observed during short-term storage of lettuce at 20°C and 5°C (Leja et al. 1994), although a few studies report constant or decreasing levels during storage (Kalt 2005).

Flavonoids are important secondary plant metabolites (Koh et al. 2009) and possess strong antioxidant activity due scavenging reactive oxygen species and inhibitation of oxidative stress (Hounsome et al. 2009). Flavonoid oxidation also plays a role in defending the plant against various stresses (Pourcel et al. 2006). The enzymatic oxidation of polyphenols, particularly flavonoids occurs during storage, when cell integrity is affected. Furteremore, POD, one of the enzymes are known to be involved in flavonoid oxidation. POD catalyze the oxidation of phenolic substrates through the associated reduction of hydrogen peroxide in the peroxidative cycle. Thus, Low temperature could decrease the membrane lipid degradation and POD activity, and, as a consequence, could slow down the flavonoid oxidation. Similar results were observed in lettuce (DuPont et al. 2000) and broccoli (Yuan et al. 2010).

Antioxidants are involved in scavenging ROS produced during senescence, and therefore their levels decrease during early day of storage (Hounsome et al. 2009) but under various abiotic stresses and storage of vegetables the extent of ROS production exceeds the antioxidant defense capability of the cell, resulting in cellular damages and therefore reduction antioxidant compounds (Lemoin et al. 2010). Storage of vegetables and fruits is often associated with loss of antioxidant compounds (Hounsome et al. 2009). In some studies, however, an increase in antioxidant levels was found during short-term storage of broccoli and tomatoes (Leja et al. 2001, Toor and Savage 2006). On the other hand there is a consensus that the antioxidant capacity is directly correlated with phenolic compounds (Tavarini et al. 2008, Koh et al. 2009). Therefore, Plants with high levels of antioxidants, either constitutive or induced, have been reported as having greater resistance to this oxidative damage (Navarro et al. 2006).

An increases in the activity POD was generally a consequence of the system ability to delay senescence (Toivonen and Sweeney 1998).These results and ours indicated that POD is involved in the senescence of products because it catalyzes the decomposition H_2O_2 (Hodges 2003). Therefore, POD could be involved in the degradation of chlorophyll (Dong et al. 2004) Thus, induction of peroxidase activity is a well known indicator of stage of senescence and intense stress (Starzyinska et al. 2003).

Increments of POD activity during Cantaloupe Melon (Lamikanra and Watson, 2001) senescence were previously reported. Funamoto et al. (2002) reported that POD activity increased markedly during yellowing of broccoli, the lowest POD activity associated with the highest chlorophyll content and the highest POD activity determined with the lowest chlorophyll content. Starzyinska et al. (2003) reported that storage of broccoli at room temperature caused the significant increase of POD activity, while at low temperature (5 °C) activity of the enzyme rose after 10 days.

Malondialdehyde (MDA) is an end product of lipid peroxidation and has been used as a direct indicator of membrane injury (Yuan et al. 2010). It has been reported that the membrane permeability and the level of MDA content increase during fruit and vegetable senescence (Dhindsa et al. 1981, Yuan et al. 2010). Zhuang et al. (1995) also showed that postharvest senescence of broccoli is correlated with lipid peroxidation (MDA content), leading to cell-membrane disintegration. Broccoli florets are known to produce ethylene as they senescence and ethylene is known to enhance memberane lipid degradation in other senescing system (Deschene et al. 1991). Thus, low temperature may inhibit membrane lipid degradation indirectly by inhibiting the action and synthesis of ethylene (Deschene et al. 1991). The rapid increase of MDA content in flower bud tissue indicates the importance of the influence of storage temperature in induction senescence in broccoli florets tissues.

CONCLUSIONS

Results demonstrated that the importance of storage temperature and cultivar on antioxidant change and the rate of senescence as measured by chlorophyll loss. The ‘General’ and ‘Revolution’ cultivars with maintenance a stable chlorophyll content and low POD activity and high phenolic antioxidant content, during 40 days storage at 0°C, followed by two additional days at 20 °C that was suitable for the long-term storage. while during short-term storage at room temperatures, the lowest and highest POD and phenolic antioxidant content, respectively, was observed in the ‘Liberty’ and ‘Revolution’ cultivars, that were showed ability to maintain higher chlorophyll content. Furthermore, POD and MDA can be an important factor antioxidant degradation that cause yellowing florets of broccoli. Finally low temperature could decrease biochemical processes rate of flower buds, thus could maintenance antioxidant components and slow destroy lipid peroxidation and POD activity.

ACKNOWLEDGMENT. The authors are grateful from University of Guilan for financial and technical support.

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