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**YIELD AND QUALITY OF GREEN LEAFY
VEGETABLES IN POSTHARVEST**

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INDEX

~ Foreword	pp. 3
~ Abstract	pp. 5
~ Riassunto	pp. 5
~ I. Sulphur fertilization and light storage are critical determinants of the nutritional value of ready-to-eat <i>friariello campano</i> (<i>Brassica rapa</i> L. subsp. <i>Sylvestris</i>)	pp. 6
~ II. Exogenous proline improves quality postharvest of rocket leaves upon salinization	pp. 16
~ III. Effects of saline conditions on postharvest storage of two sweet basil cultivar (<i>Napoletano</i> and <i>Genovese</i>)	pp. 40
~ IV. Profile of bioactive compounds during storage of fresh-cut spinach: the role of endogenous ascorbic acid in the improvement of product quality	pp. 51
~ V. Conclusions	pp. 67
~ References	pp. 68

~ FOREWORD

The evolution of the food styles and, particularly, the increasing application of products with high content of services is determining a progressive increase of consumers' interest for the products of IV range. They represent the most recent answer of the food industry to the demands of a segment of consumers who have the necessity to prepare rapid domestic meals and who want a product with high organoleptical and nutritional prerogatives.

The products of IV range are fresh, washed, cut, manufactured to result ready for the consumption. After their harvest, most fresh vegetables undergo continuous changes of their nutritional profile that begin with physiological alterations associated with wound (cut) responses. They generally activate chemical and enzymatic reactions that lead to a rapid decay of the product quality such as the increase of the respiration, the production of ethylene, the enzymatic browning, the loss of colour and the disidratation that provoke a rapid deterioration of the product. The low temperatures of storage, the packaging in protective atmosphere, are all effective and necessary means to maintain the quality of these products.

The fact that crop deficiencies of sulphur (S) were reported with increasing frequency in the last years caused a greater attention on the importance of this element in plant nutrition. In many countries of the world S deficiency was recognized as a limiting factor for crop production. Especially in Western Europe the incidence of S deficiency increasingly reported in Brassicaceae over the last decade. While in soil sulphate is a direct S source for plants, generally more than 95% of soil S is organic bonded and divided into sulphate ester S and carbon-bonded S. Although not readily plant available, organic S compounds may potentially contribute to the S supply of plants via mineralization. While sulphate esters play an important role in the short term release of sulphate, carbon-bonded S seems

to be responsible for long-term mineralization. Predicting the S requirement of plants has become of increasingly importance in European agriculture.

Salinity is one of the most critical abiotic stresses affecting crop yield and quality in various agricultural systems. Salinization of soils is a natural phenomenon occurring in areas of the world where evaporation exceeds precipitation. Biological systems, however, showed wide adaptation to environmental stresses including salt, and plants can be found growing in saline environments.

Plants exposed to salt stress undergo changes in their environment. The ability of plants to tolerate salt is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions, and maintain ion homeostasis. Essential pathways include those that lead to synthesis of osmotically active metabolites, specific proteins, and certain free radical scavenging enzymes that control ion and water flux and support scavenging of oxygen radicals or chaperones. The ability of plants to detoxify radicals under conditions of salt stress is probably the most critical requirement. Many salt-tolerant species accumulate methylated metabolites, which play crucial dual roles as osmoprotectants and as radical scavengers. Their synthesis is correlated with stress-induced enhancement of photorespiration.

The objective in these experiments was to assist the biochemical pathways on green leaves of plants grown in different environmental conditions, and their maintenance in postharvest.

ABSTRACT

The present PhD Thesis estimates the biochemical answers of 4 green leafy vegetables minimally processed.

The first chapter reports the behaviour of the "friariello campano" grown in a floating system and upon different N:S ratio in the nutrition solution and its preservation in darkness and in a light storage.

The second chapter concerns the biochemical answer to salt stress of rocket (*Diplotaxis tenuifolia* L.) upon exogenous leafy proline applications and its preservation in darkness and in a light storage.

In the third chapter the course in postharvest of two cultivar of sweet basil cultivated upon salinitation was observed.

In the fourth chapter the enzymatic browning in ready-to-eat spinach was estimated.

Riassunto

Il presente lavoro valuta le risposte biochimiche di 4 specie di ortaggi da foglia, conservate in IV gamma.

Il primo capitolo riporta il comportamento di friariello campano allevato in floating system a differenti rapporti di N:S nella soluzione nutritiva e conservato in condizioni di buio e di luce.

Il secondo capitolo stima le risposte biochimiche allo stress salino e all'applicazione fogliare di prolina in rucola (*Diplotaxis tenuifolia* L.) conservata a due diversi regimi luminosi.

Nel terzo capitolo, è stato osservato il comportamento in post-raccolta di due cultivar di basilico cresciute sotto stress salino.

L'ultimo capitolo riguarda una prova di conservazione di spinacio in IV gamma e la valutazione delle attività enzimatiche responsabili dell'imbrunimento.

CHAPTER I

Sulphur fertilization and light storage are critical determinants of the nutritional value of ready-to-eat "friariello campano" (*Brassica rapa* L. subsp. *Sylvestris*)

1. Introduction

The friariello campano (*Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort.) is a short-cycle, cool-season leafy vegetable belonging to the Cruciferae family. It is mostly cultivated in Southern Italy and it has a remarkable commercial importance since consumers tend to associate local and traditional produce to high quality and healthy products, for which there is an increasing interest. The nutritional value of fresh vegetables can be affected at different steps within the field-to-market pipeline. Therefore both pre- and post-harvest effectors should be considered in order to increase the produce quality and to preserve it until the final consumption.

Antioxidant molecules have an important function after harvest since they inhibit or delay the initiation and propagation of oxidative reactions that will eventually lead to general product quality decay (Veliouglu *et al.*, 1998). In Brassica species, antioxidants are mainly represented by glucosinolates, ascorbic acid and flavonoids (Winkler *et al.*, 2007). The biosynthesis of glucosinolates, which are also responsible for the characteristic flavour of Brassica products (Marschner, 1995), has been reported to depend on sulphur availability (Asare and Scarisbrick, 1995). In addition, studies by Clarkson *et al.*, (1999) have also demonstrated an interaction between sulphur and nitrogen assimilation. Since the plant tissue ascorbic acid content is also related to the nitrogen content (Lee and Kader, 2000), we hypothesized that sulphur availability during plant growth could directly or indirectly affect the biosynthesis of several vegetable quality components and, consequently, be a critical determinant of the nutritional value of friariello.

Upon harvest, friariello leaves should be consumed within a few days, since its shelf-life is of approximately 7-10 days (Orsini *et al.*, 2006). In this respect, most fresh vegetables undergo, after harvest, to continuous changes of the nutritional profile that begin with physiological modifications associated with wound (cut) responses, which generally activate chemical and enzymatic reactions that lead to a rapid decay of the product quality. Decreased levels of vitamin C have been reported in brassica species during storage (Lee and Kader 2000). Several reports on post-harvest handling and storage refer of loss of glucosinolates (Rangkadilok *et al.*, 2002). Moreover, the total antioxidant activity in broccoli has been reported to increase during storage because of a significant presence of phenolic antioxidant agents (Leja *et al.*, 2001). Nevertheless, most of the literature data mainly refer to produce stored in the darkness, whereas there are only a few reports on the influence of light during storage (Toledo *et al.*, 2003; Noichinda *et al.*, 2007; Siomos *et al.*, 1995). In addition, while there are several reasons to predict that the presence of light may promote the antioxidant degradation during storage (Lee and Kader, 2000; Noichinda *et al.*, 2007), there is no indication on its influence on nitrate and nitrite concentrations. Since antioxidant activity and nitrate/nitrite contents both contribute to determine the nutritional level of most leafy vegetable, we wanted to assess how a combination of pre-harvest (sulphur fertilization) and post-harvest (storage light conditions) parameters may affect the nutritional value of friariello campano.

2. Material and Methods

The experiment was carried out at the University of Naples Federico II (40°49' N, 14° 15' E, 30 m a.s.l.) in a cold glasshouse during the 2006 spring. Ecotypes of friariello were grown on a floating system. Each growing unit was a 4 m² container filled with 1000 liters of aerated nutrient solution replaced every week. Seeds were germinated on 0.25 m² polystyrene panels filled with peat-moss. Fourteen days after sowing (DAS), on March 26,

the polystyrene panels were moved onto the aerated nutrient solution to obtain a final density of 100 plants m⁻². The standard nutrient solution contained: N 26.2 mM; N-NO₃⁻ 17.6 mM; N-NH₄⁺ 8.6 mM; Cl⁻ 3.0 mM; PO₄⁻ 3.5 mM; Ca 5.0 mM; Mg 3.7 mM; K 10.5 mM; Na 2.2 mM; Bo: 0.02 mM; Fe 0.04 mM (Pimpini *et al.*, 2001). Three sulphur concentrations were obtained by adding elemental S to the nutrient solution to a final concentration of 2.6 mM (S1), 3.7 mM (S2) and 6.5 mM (S3) corresponding to N:S ratios (v/v) of 10, 7 and 4, respectively. EC and pH of the nutrient solutions were kept at 2-2.5 dS m⁻¹ and 6-6.5, respectively.

The experimental design was a split-plot with three replications. The sulphur concentrations in the nutrient solution were assigned to the main plots and the ecotypes were assigned to the sub-plots. Plants were harvested on May16, at 51 DAS. At harvest, the fresh product was manually cleaned, by discarding stems, damaged leaves and yellow inflorescences. Intact leaves were then accurately washed three times with microbiologically pure water, and air flow dried on a strainer. The leaves were subsequently kept in plastic boxes. The containers were wrapped with a multistrata film (PET 12+ COEX/EVOH/PE 95), highly impermeable to gases and water vapour, with permeability to O₂ < 5 dm³ m⁻² d⁻¹ bar⁻¹ at 23 °C and 0% U.R. and to H₂O < 5g m⁻² d⁻¹ bar⁻¹ at 38 °C and 100% U.R. (Gruppo Fabbri S.P.A., Modena, Italy).

The containers were kept at 4 °C for 9 days, under two light regimes: darkness (control) or 8-hour daily low intensity light (16 μmol m⁻² s⁻¹). Leaf samples were collected on day 0, 3, 6 and 9. Leaf dry weights were measured upon oven dehydration at 60°C (until steady weight). Nitrates and nitrites were measured spectrophotometrically (HACH DR/2000) on dried and ground tissue samples after cadmium reduction (Sah, 1994).

Two different cation assays were utilized to measure the antioxidant activity of the hydrophilic (HAA) and lipophilic (LAA) fractions on lyophilized leaf samples. The antioxidant activity was measured on the water-soluble fraction using the DMPD [N, N-

dimethylpphenylenediamine] method and expressed as mmol of Ascorbic Acid (AA) per 100 g of dry weight (Fogliano *et al.*, 1999). The Lipophilic fraction was measured with the ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)] method performed as described by Pellegrini *et al.*, (1999) and expressed as mmol of Trolox per 100 g of dry weight (Re *et al.*, 1999).

The chlorophyll content was assessed upon extraction from fresh leaf discs (Jeffrey and Humphrey, 1975) and subsequent quantification with a Chlorophyll Meter (SPAD 502, Tokyo, Japan). Colour parameters L*, a* and b* were measured on marketable leaves using a portable colorimeter (Minolta Chroma Meter CR-300, Tokyo, Japan) and ranked based on the CIELAB-system [L* = lightness, a*=refers to values ranging from green (-) to red (+) and b*=refers to values ranging from blue (-) to yellow (+)].

Data were analyzed by ANOVA and means were compared with the Least Significant Difference (LSD) test.

3. Results and Discussion

3.1. Fresh Weight

During storage, a leaf weight loss was found for all treatments and this was greater when the containers were kept under light (Figure 1). Similar results were reported by Noichinda *et al.*, (2007), who associated the weight loss to the higher stomatal conductance of light-exposed leaves. This possibility is consistent with a still active photosynthesis during light storage. An absence of fresh weight change during storage has been reported by Barth and Zhuang (1996) and Zhuang *et al.*, (1997), although this has been found at slightly different storage conditions compared to our experiment. These results indicate that fresh weight losses during storage may result from a combination of different environmental parameters.

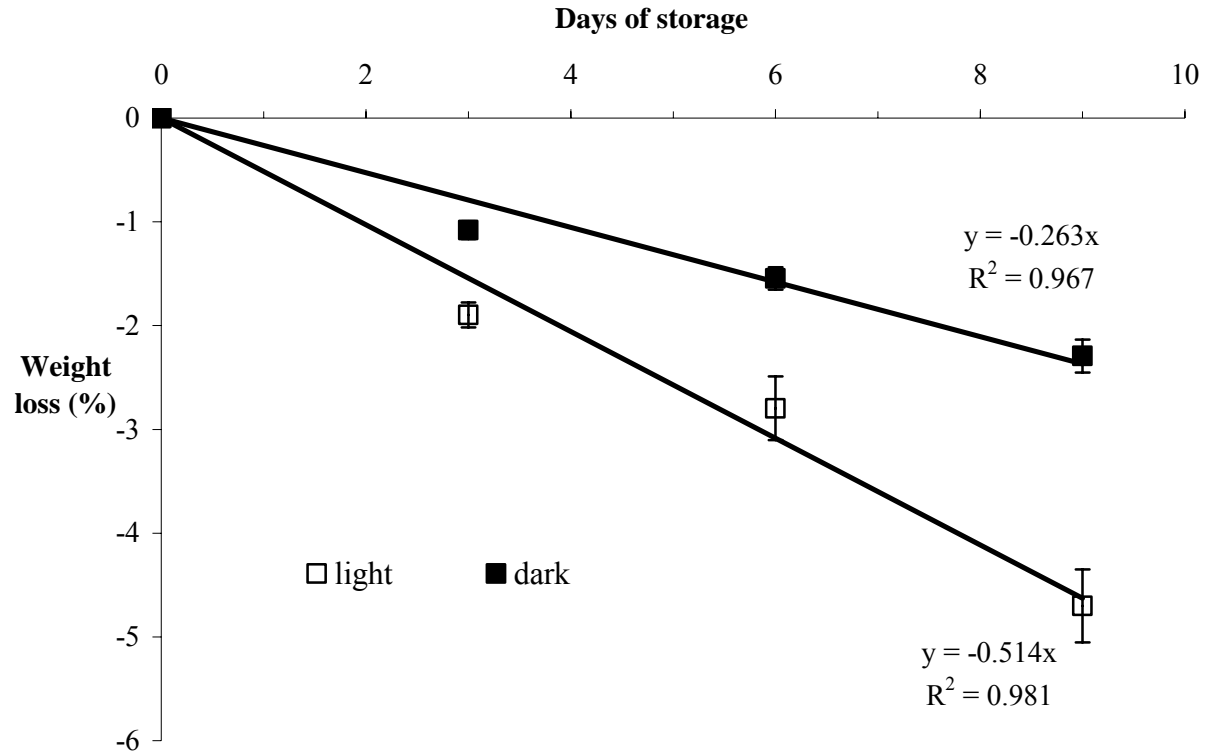


Figure 1 Weight loss of ready-to-eat *Friariello campano* (*Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort.) stored in plastic containers at 4 °C, in darkness (■) or light (□). Data are mean of three replications \pm SE.

3.2. Nitrate and Nitrite Content

Nitrate and nitrite contents in leaves were not affected by sulphur concentration in the nutrient solution. However, leaf nitrate content was reduced during light storage at 4 °C, whereas no modification was recorded at the end of the storage period in darkness (Figure 2). Leaf nitrite content was reduced in both light and dark storage, in measure of the 54% of the initial value after 9 days (1.81 to 0.82 mg NO₂⁻ kg FW⁻¹, respectively). Previous experiments on sweet basil stored in darkness (De Pascale *et al.*, 2006) indicated that the main reduction of nitrate content occurred within the early days of storage, while slight modification could be observed afterward. Nitrate reductase activity (NR) is induced by light (Crawford *et al.*, 1995), then when the produce is stored in the dark, reduction may be only attributed to residual activity of NR. According to our results, reduction in nitrate content was higher in light storage and this could be attributed by the persistent activation

of nitrate reductase (NR), otherwise stopped in absence of light (Figure 2). The nitrite reductase (NiR) does not require light activation (Crawford, 1995; Li and Ann, 1995). Therefore, nitrite reduction was linear for all treatments regardless the presence/absence of light.

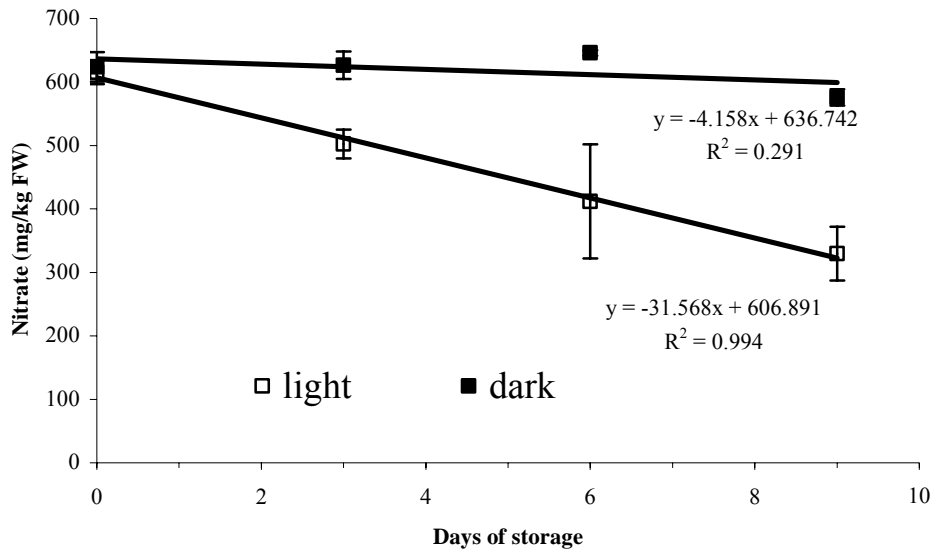


Figure 2. Nitrate contents of ready-to-eat *Brassica rapa* stored in light (□) or darkness (■) at 4 °C. Data are means of three replications ± SE.

3.3. Antioxidant Capacity

A higher sulphur concentration in the nutrient solution caused an increase of both HAC and LAC (table 1). Since glucosinolates and ascorbic acid are major components of the antioxidant fraction of *Cruciferae* and their concentration is dependent on sulphur availability (Asare and Scarisbrick, 1995), the observed higher HAC was likely associated to an increased level of these compounds (Lee and Kader, 2000; De Pascale *et al.*, 2008). Interestingly, these differences disappeared by the end of the storage period, at which time a general reduction of the HAC was measured for all treatments (from 1,31 to 0,84 mmol Ascorbic acid eq 100 g FW-1). In contrast to previous studies by Albrecht *et al.*, (1990), who reported a remarkable stability of the hydrophilic antioxidant activity during broccoli

and Brussels sprouts storage, we observed a 37% mean reduction of the initial HAC value, and this was higher in S2 and S3 leaves.

Moreover, the LAC was higher at the end of the storage period. Such increase could be associated to 1) the conversion of the hydrophilic to the lipophilic fraction, through an ascorbic acid mediated tocopherol production (Kunert and Ederer, 1985); 2) the wound induced ex-novo synthesis of molecules associated to LHA (Kang and Saltveit, 2002); 3) an enhanced biosynthesis of lipophilic antioxidant molecules, which may continue even after harvest because the responsible enzymes are still active (Rodriguez-Amaya, 1997).

It should be also considered that processes associated to cell wall degradation occurring during storage, may increase the amount of extractable lipophilic antioxidant compounds. Consistently, others reports have documented that cooking (De Sà and Rodriguez-Amaya, 2004) and homogenization (Van Het Hof *et al.*, 2000) may facilitate the antioxidants extraction, because of softening/breaking of the cell walls.

The exposure to light during storage did not affect either the hydrophilic or lipophilic fractions during storage (data not shown). However, it has been suggested that a continuous illumination with white light maintains the photosynthetic capacity of spinach leaves during post-harvest storage. This may increase the availability of soluble carbohydrates such as glucose, which may contribute to the control of the ascorbic acid homeostasis (Lee and Kader, 2000; Toledo *et al.*, 2003).

Table 1. Hydrophylic (HAC) and lipophylic (LAC) antioxidant fractions in ready-to-eat *Brassica rapa* stored in plastic bags at 4 °C for 9 days under low light levels ($16 \text{ mol m}^{-2} \text{ s}^{-1}$) or in the darkness. Mean values of six replications; ns=not significant; *=significant at $P \leq 0.05$; **=significant at $P \leq 0.01$.

	LAC mmol Trolox eq 100 g⁻¹ FW	HAC mmol Asc acid eq 100 g⁻¹ FW
S1	0.255	0,98
S2	0.302	1,13
S3	0.314 **	1,20 **

3.4. Chlorophyll content and colour indexes to assess the dynamic of quality decay

Chlorophyll levels were lower in dark-stored leaves (Figure 3). The dynamic of chlorophyll degradation during storage has been the focus of many food scientists since this parameter is closely associated to the product shelf life (Zhuang *et al.*, 1994 and 1997; Toivonen and Sweeney, 1998; Deeli and Toivonen, 1999). According to Zhuang *et al.*, (1994 and 1997), the post-harvest senescence of broccoli, expressed by the chlorophyll loss, is correlated with lipid peroxidation, which would lead to cell-membranes disruption. SPAD values were higher in light stored leaves and decrease during storage. (Figure 4) No effects were attributable to sulphur nutrition (data not shown).

However a significant correlation between SPAD values and the total chlorophyll was recorded (Figure 5). Thus, values determined by the SPAD technique could provide an indication of the relative amount of chlorophyll present in plant. Either sulphur nutrition (data not shown) or the presence of light during storage (table 2) did not affect a* and L* indexes. Negative values of a* (green intensity) moved toward zero (reduced green intensity) during storage. L* values were increased at the end of the storage. The level of yellowness was more stable in light stored leaves (b* index, table 2) and was higher in S2 and S3, although we did not find significant differences for this index between light and dark storage at the end of the test period.

Overall, the SPAD chlorophyll meter turned out to be a more convenient and reliable methodology to assess the product quality decay based on chlorophyll degradation.

Table 2. Colour Indexes. a* , b* and L* of ready-to-eat *Brassica rapa* stored in plastic bags at 4 °C for 9 days under low light levels ($16 \text{ mol m}^{-2} \text{ s}^{-1}$) or in the darkness. Mean values of six replications; ns=not significant; *=significant at $P \leq 0.05$; **=significant at $P \leq 0.01$.

	L*	a*	b*
Light	41,3	-13,7	40,5
Darkness	41,1 <i>ns</i>	-12,5 <i>ns</i>	33,4 **

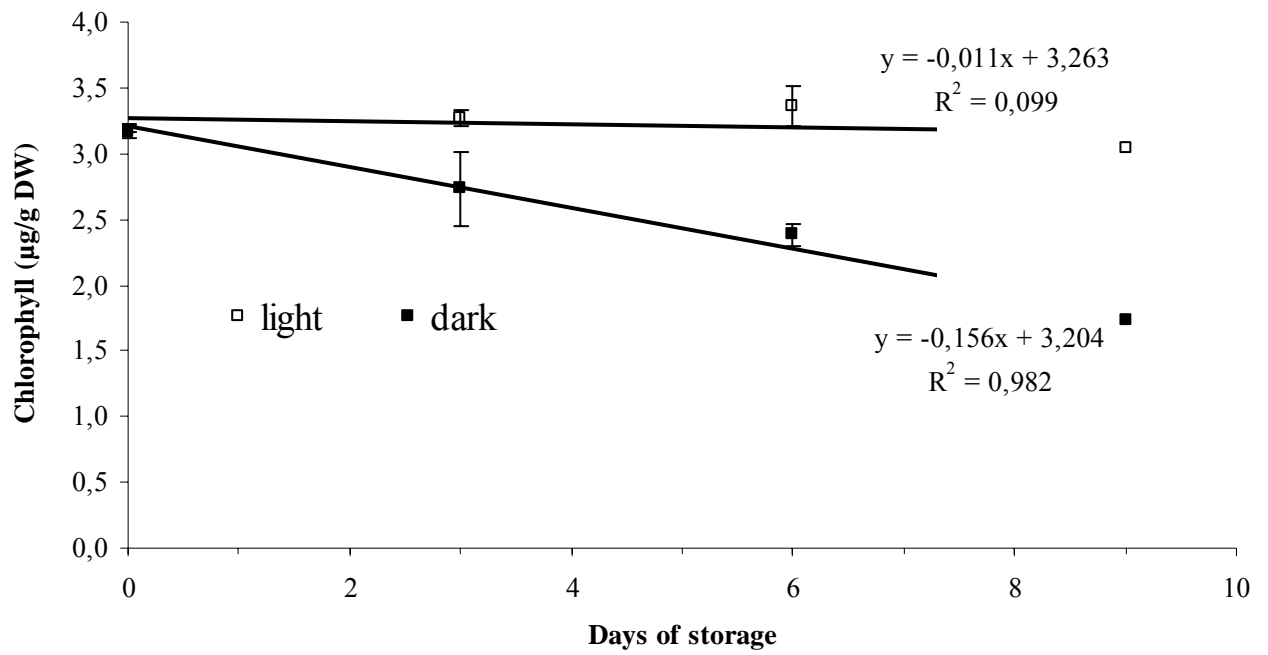


Figure 3. Total chlorophyll of *Brassica rapa* stored in light (□) or darkness (■) at 4 °C. Data are means of three replications \pm SE.

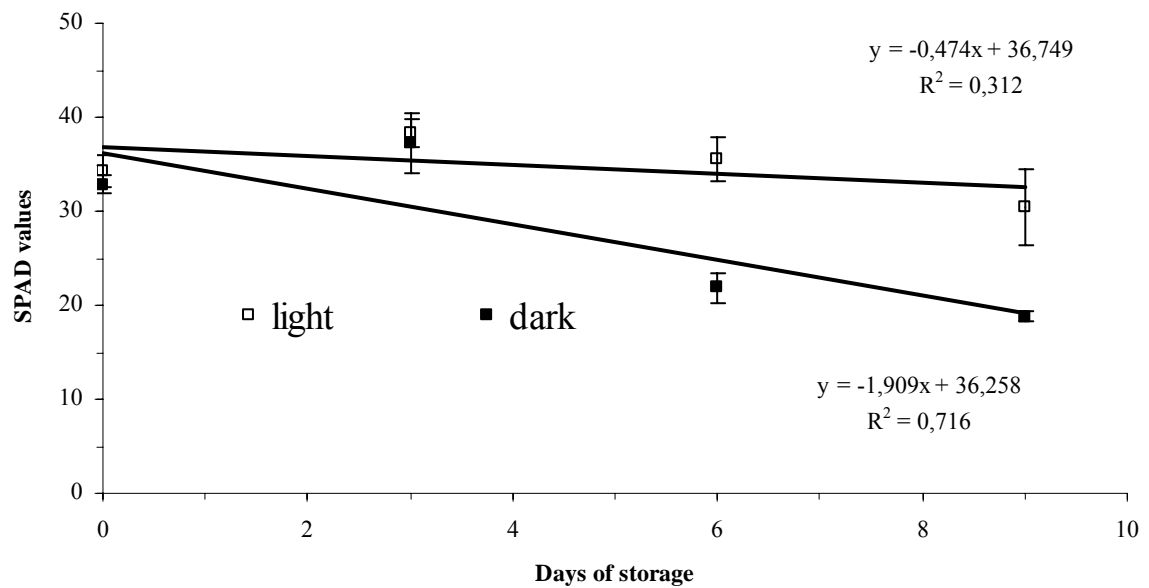


Figure 4. SPAD values of *Brassica rapa* stored in light (□) or darkness (■) at 4 °C. Data are means of nine replications \pm SE.

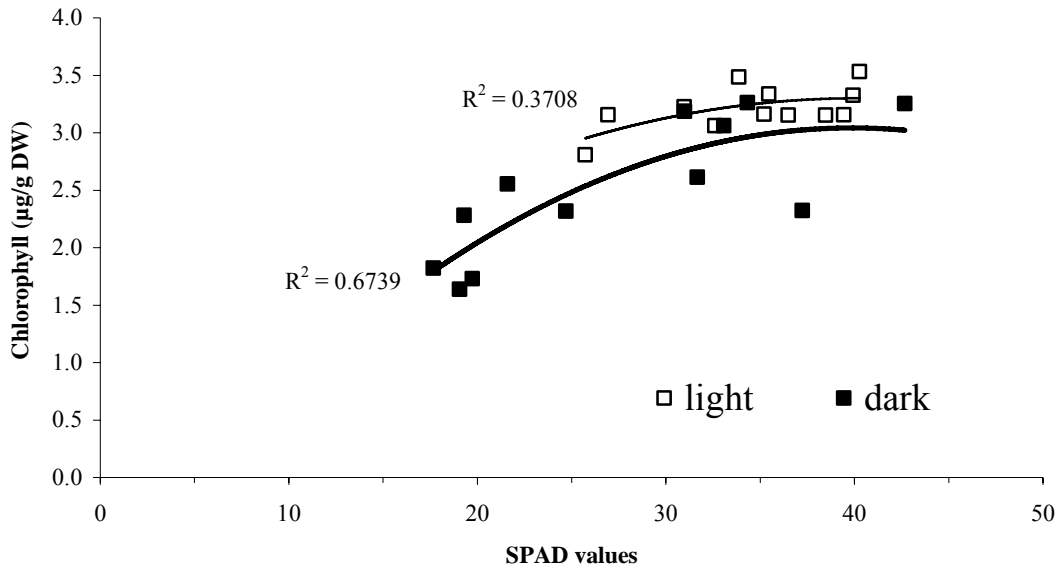


Figure 5. Regression between SPAD values vs. Total Chlorophyll in leaves of *Brassica rapa* stored in light (□) or darkness (■) at 4 °C.

4. Conclusions

Sulphur fertigation affects positively antioxidant capacity and does not influence leaf nitrate content. The light and the time storage reduce the nitrate content and maintain the green colour. The storage is harmful for hydrophilic fraction, but at the same time it improves the lipophilic antioxidant capacity. In conclusion, *friariello campano* grown upon sulphur nutrition and preserved in the light, maintains high the quality parameters of fresh-cut product.

CHAPTER II

Exogenous Proline Improves Quality Postharvest of rocket leaves upon salinization

1. Introduction

Rocket (*Diplomatix tenuifolia* (L.) DC.) is a member of the family Brassicaceae found in most Mediterranean countries. Rocket is a cruciferous crop used extensively in baby leaf salads. Like other cruciferous vegetables, rocket contains a range of health-promoting phytonutrients including vitamin C, fiber, flavonoids, carotenoids and glucosinolates (Barillari *et al.*, 2005; Crozier *et al.*, 1997; Kris-Etherton *et al.*, 2002; Van Poppel *et al.*, 1999). Many phytochemicals, including glucosinolates, phenols, and flavonoids are pungent and bitter tasting, which poses a quandary for a food industry intent on raising the levels of these compounds in their products to boost perceived health attributes (Drewnowski and Gomez-Carneros, 2000).

The discovery of bioactive components in foods is exciting, suggesting the possibility of improved public health through diet. Yet the content of bioactive components in plant food varies, making quality control and intake recommendations problematic. Variation in content of bioactive components in fruits and vegetables depends upon both genetics and environment, including growing conditions, harvest and storage. Cruciferous vegetables, which contain both anticarcinogenic and antioxidant properties, are excellent examples to illustrate the problem in assessing health benefits of foods that vary in content of bioactive components (Jeffrey *et al.*, 2003).

Some nutrients, such as the antioxidant vitamins, carotenoids, tocopherols and ascorbic acid, appear to play a dual role in metabolism. At one dose they are required for normal growth and development, and at a higher dose they appear to provide antioxidant

protection against chronic diseases, including chronic heart disease, arthritis and cancer (Krinsky *et al.*, 2000).

Green leafy vegetables have significantly higher total carotene content and highest total phenols than other vegetables. The major carotenoids in green leafy vegetables are lutein and all-trans- β -carotene (Cho *et al.*, 2007).

Vitamin C is a universal constituent of all green leaf plants, its minimum intake must be of 60mg day for adults. In crop plants, vitamin C content is influenced by seasonal factors such as sowing time and harvesting date and is often used as a 'marker' for postharvest deterioration (Sood Mand Malhotra 2001). It develops an important role in photosynthesis and photoprotection, in defence against ozone and other oxidative stresses (Smirnoff 1996).

Ascorbate occurs in the cytosol, chloroplasts, vacuoles, mitochondria and cell wall (Anderson, *et al.*, 1983; Rautenkranz *et al.*, 1994). The concentration in chloroplasts can be high and is probably related to its central role in photosynthesis (Foyer, 1993).

Consumption of lightly processed vegetables was increased over the last decade. More research was done to identify better cultivation and storage conditions that will minimize nutrient and quality losses in these highly perishable products.

Fresh-cut (minimally-processed) horticultural products are subjected to simple operations soon after harvest, such as cleaning, washing, cutting and packaging, which make them ready-to-use. It is well known that from the time of harvest quality declines in fruit and vegetables and many nutrients are lost rapidly. This quality decline includes visual symptoms, such as loss of turgor and yellowing of green produce, as well as loss of important nutrients, such as sugars and vitamin C (Gil *et al.*, 1999; Lee and Kader, 2000).

Physical damage during preparation causes an increase in respiration rates, biochemical changes and microbial spoilage, which may result in degradation of colour, texture and flavour of the produce (Cantwell, 1996). In other reports, minimal processing increases

phenolic metabolism and therefore, the accumulation of phenolic metabolites (Tomas-Barberan *et al.*, 2000). Moreover, the content of phenols is associated with the sensitivity to enzymatic browning in several vegetables (Brecht *et al.*, 2004).

It is well known that different stresses, location climates, microenvironments and physical and chemical stimulus (often called elicitors) qualitatively and quantitatively alter the content of bioactive secondary metabolites, and whole-plant elicitation increases the amounts of bioactive compounds in foods of plant origin. (Demmig-Adams and Adams 2002; Van Dam *et al.*, 2004).

An elicitor could be considered the proline, which it accumulation in the cell (up to 80% of the amino acid pool under stress and 5% under normal conditions) due to increased synthesis and decreased degradation under a variety of stress conditions such as salt and drought (Delauney and Verma, 1993; Bohnert and Jensen, 1996; Kavi Kishor, 1988; Kohl *et al.*, 1991; Parida and Das, 2005; Sarvesh *et al.*, 1996; Schat *et al.*, 1997; Trotel *et al.*, 1996).

Tolerance to abiotic stress, especially to salt stress, was observed in a variety of transgenics that were engineered for overproduction of proline (Kavi Kishor *et al.*, 1995; Roosens *et al.*, 2002). Proline seems to have diverse roles under osmotic stress conditions, such as stabilization of proteins, membranes and subcellular structures (Vanrensburg *et al.*, 1993), and protecting cellular functions by scavenging reactive oxygen species (Bohnert and Shen, 1999; Samaras *et al.*, 1995; Tsugane *et al.*, 1999; Hong *et al.*, 2000; Okuma *et al.*, 2000, 2002; Heuer, 1994). Among various compatible solutes, proline is the only molecule that has been shown to protect plants against singlet oxygen and free radical induced damages (Alia *et al.*, 1997). Since proline can act as a singlet oxygen quencher (Alia and Pardha Saradhi, 1993), and as a scavenger of OH· radicals, it is able to stabilize proteins, DNA as well as membranes (Matysik *et al.*, 2002).

Salt stress causes significant crop losses, it is the major environmental factor limiting plant growth and productivity (Allakhverdiev *et al.*, 2000), through lowering of the photosynthesis and the production of activated oxygen radicals such hydrogen peroxide, superoxide, singlet oxygen and hydroxyl (Smirnov, 1995).

The nitrate levels in leafy vegetables can be of considerable importance for their quality owing to its potential harmful effects on human health by leading to methaemoglobinaemia or forming nitrosamines and nitrosamides by reaction with amines and amides after nitrate reduction (Marschner, 1997; Walker, 1990; Bianco *et al.*, 1998; Santamaria *et al.*, 2002). The acceptable daily intake of nitrate was set at 0–3.65 mg/kg body weight by the European Communities' Scientific Committee for Food in 1992. One of the problems in rocket commercialization is the high nitrate content of fresh product, critical for human health. Besides, alternative cultural techniques are required to control mineral nutrition, with the aim of standardizing the qualitative characteristics of Leafy Vegetable Production (Incrocci *et al.*, 2001).

The objective of the work was to set up a Soilless Culture System to grow rocket comparing different growing practices to obtain a fresh-cut product of high quality in terms of marketable standards and shelf life.

2. Materials and methods

The experiment was carried out at the University of Naples Federico II (40°49' N, 14° 15' E, 30 m.a.s.l.) in a cold glasshouse and were implemented two cycle of seeding (spring and summer).

The first was from 18 March to 24 April 2008 (seed-to-harvest time was 39 days), while the second from 5 June to 2 July 2008 (seed-to-harvest time was 27 days). The rocket was sown in 4 m² polystyrene containers filled with sterilised soil and grown in floating systems. Each growing unit was filled with 1.000 L of aerated nutrient solution.

Oxygenation of the solution was maintained with electric pumps. Planting density was 100 plants m^{-2} , as commercially adopted for hydroponic production of rocket. The composition of the standard nutrient solution adopted in all experiments was N-NO_3^- 14 mM; N-NH_4^+ 6 mM; Cl^- 3.0 mM; PO_4^- 3.5 mM; S 6.0 mM; Ca 5.0 mM; Mg 3.7 mM; K 10.5 mM; Na 2.2 mM; Bo: 0.02 mM; Fe 0.04 mM (Pimpini et al., 2001; Marschner, 1995). Starting from 26 DAS (first cycle) and from 19 DAS (second cycle), the leaves of plants were sprayed with solution of proline 20 mM in distilled water and distilled water (control). After 24 hours, the plants were exposed to a salt stress of 0 (control), 25, 50 and 100 mM NaCl. Two days before harvest, the second leafy treatment was applied at plants. The experimental design was a randomized block with two replications.

Determinations and analysis: Plants were harvested on April 24 and on July 2. At harvest, the fresh products were manually cleaned, by discarding stems, damaged leaves and yellow inflorescences. Intact leaves of the first harvest were accurately washed in order of three times with microbiologically pure water (spring crop), while in the second harvest, the leaves were washed with ascorbic acid (150 mg l^{-1}) (summer crop) and both air flow dried on a strainer. The leaves were subsequently kept in plastic boxes. The containers were wrapped with a multistrata film (PET 12+ COEX/EVOH/PE 95), highly impermeable to gases and water vapour, with permeability to $\text{O}_2 < 5 \text{ dm}^3 \text{ m}^{-2} \text{ d}^{-1} \text{ bar}^{-1}$ at $23 \text{ }^\circ\text{C}$ and 0% U.R. and to $\text{H}_2\text{O} < 5 \text{ g m}^{-2} \text{ d}^{-1} \text{ bar}^{-1}$ at $38 \text{ }^\circ\text{C}$ and 100% U.R. (Gruppo Fabbri S.P.A., Modena, Italy).

The containers were kept at $4 \text{ }^\circ\text{C}$ for 6 days, under two storage regimes: 1) dark conditions (control); 2) daily 8-hour exposure to a relatively low light intensity ($\text{PPFD}=16 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$).

Leaf samples were collected on day 0, 2, 4 and 6. During storage, loss weight of the packaging was also evaluated. Leaf dry weights upon oven dehydration at 60°C (until steady weight) and dry matter were measured.

For Nitrates and nitrites contents and antioxidant activity, hydrophilic (HAA) and lipophilic (LAC), see Materials and Methods of previous Chapter Friariello.

The total ascorbic acid defined as ascorbic acid (AsA) and dehydroascorbate (DHA) acid was determined by spectrophotometric detection on fresh plant tissues. The assay was based on reduction of Fe^{3+} to Fe^{2+} by AsA and spectrophotometric detection of Fe^{2+} complexed with 2,2'-dipyridyl method (Kampfenkel *et al.*, 1995). The total carotenoids were measured by Lichtenthaler and Wellburn (1983) by spectrophotometer detection on fresh plant tissues.

Total soluble phenol extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1997).

Colour parameters L^* , a^* and b^* [CIELAB-system, the L^* component represents lightness ranging from 0 = black to 100 = white, the a^* component represents values running from green (-) to red (+), and the b^* component represents values running from blue (-) to yellow (+)] were evaluated on marketable leaves using a portable colorimeter (Minolta Chroma Meter CR-300).

Two derived functions were computed from the lab readings as follows:

Hue angle: $\arctan(b/a)$

Chroma: $(a^2 + b^2)^{1/2}$

The hue angle is the characteristic associated with the conventional perceived colour name; an angle of 90° represents a yellow hue. Objects with higher hue angles are greener. Chroma is an indicator of brightness.

Chlorophyll was determined as described by Lichtenthaler and Wellburn (1983) on fresh leaf. Previously on the same leaf samples greenness measurements were performed using a Chlorophyll Meter (SPAD 502).

Data were analyzed by ANOVA and means were compared with the Difference Middle Significant (DMS) test.

3. Results and discussions

3.1. Experiment I

3.1.1. Fresh weight and Weight Loss

The dry matter (DM) was significantly influenced by salinity; it presented lower values in the control, and increased upon salt concentrations. Proline leafy applications and the light storage did not affect dry matter. During storage, a weight loss was found on 50mM NaCl treatment and this was greater when the containers were kept under light (table 1).

Plants upon salinization tend to compartmentalize most of the absorbed ions in vacuoles and to synthesize compatible organic solutes in the cytoplasm such to maintain the osmotic equilibrium between these two compartments, and following reduction of water (Bradford, 1995; De Lacerdaa *et al.*, 2005; Hare *et al.*, 1998; Hasegawa *et al.*, 2000).

3.1.2. Nitrate and Nitrite Content

Nitrate content was not influenced by salt treatments, while a reduction was observed upon proline applications. (table 1) Leaf nitrate content was reduced in both light and dark storage, in measure of the 45% of the initial value after 6 days. Nitrite content was affected by salt stress (table 1) and decreased of the 12% after 2 days of storage, remained constant until at the end of the storage.

Nitrate content can be of considerable importance for quality of vegetables, due to their harmful effects of these on human health by leading to methaemoglobinaemia or forming nitrosamines and nitrosamides (Meloni *et al.*, 2004; Santamaria *et al.*, 2002).

Table 1. Dry matter percentage (DM %), weight loss (WL %), nitrate and nitrite content of ready-to-eat *Rocket* stored in plastic containers at 4 °C, in darkness or light, as influenced by NaCl treatments (0= control, 25= 25mM; 50=50mM and 100= 100 mM in the nutrient solution) and leafy proline applications (20 mM).

	DM %	WL %	Nitrate mg kg ⁻¹ DW	Nitrite mg kg ⁻¹ DW
NaCl				
0	9,7	1,4	13715	20.02
25	12,4	1,6	11834	16.14
50	12,6	3,4	12455	16.07
100	11,6	1,4	13714	17.34
P=0,05	DMS=0,84	DMS=0,28	ns	DMS=0.68
Proline				
No	11,7	1,4	13653	17.45
Yes	11,5	1,6	12206	17.34
	ns	ns	**	ns
Light				
Dark	11,6	1,3	13073	17.14
Light	11,5	1,7	12787	17.65
	ns	*	ns	*

3.1.3. Antioxidant capacity and antioxidant content

Lipophilic antioxidant capacity did not change upon salinization, while an increase was observed in proline treated leaves (table 2). During the storage a general increment of the LAC was measured for all treatments (from 0,61 to 0,94 mM trolox eq. 100g FW⁻¹). The light storage affected positively LAC in unstressed leaves, while no difference was observed upon salinization (Figure 1).

During the storage, the increase of the LAC could be associated to 1) the conversion of the hydrophilic into the lipophilic fraction, through an ascorbic acid mediated tocopherol production (Kunert and Ederer, 1985); 2) the wound induced ex-novo synthesis of molecules associated to LHA (Kang and Saltveit, 2002); 3) an enhanced biosynthesis of lipophilic antioxidant molecules, which may continue even after harvest because the responsible enzymes are still active (Rodriguez-Amaya, 1997).

Hydrophilic antioxidant fraction decreased upon salinization, and no change was observed upon proline applications (table 2). A general reduction of the HAC was measured in all treatments, assuming a higher value in the dark until at the 4th day (Figure 2). Studies have

previously reported that a reduction in HAC could be due to the degradation of the ascorbic acid pool (Lee and Kader, 2000; Toledo *et al.*, 2003).

Total carotene did not change upon salinization (table 2). During the storage, it decreased in the dark, while in the light, it was preserved (Figure 3). The leaf submitted to higher salt stress and proline applications presented greater carotene content (Figure 4).

Phenolic compounds increased upon salinization (table 2), besides no change was verified in the light and in a dark storage.

Vitamin C (ASA+DHA) increased upon salinization. In proline treated leaf, ASA resulted higher, while DHA did not change (table 2). A retention in Vitamin C was observed in the light storage, while in the darkness, ASA loss was partially compensated by the increase in DHA and the final values of vitamin C remained high (Figure 5). This result was in good agreement with a previous study (Koukounaras *et al.*, 2007; Lee and Kader 2000).

ASA and DHA increased upon proline applications. ASA resulted more, on the contrary DHA was lowest in the light storage (Figure 6).

Vegetables with high AA retention were those with high in total sulfur and glutathione content. Glutathione may be involved in the mechanism responsible for reduction of DHA to AA in crucifers (Albrecht *et al.*, 1990).

According to Noichinda *et al.*, (2007) and Toledo *et al.*, (2003), continuous illumination of white light effectively supports the photosynthetic capacity in spinach leaves during postharvest storage thereby increasing the availability of soluble carbohydrates, especially glucose, enabling them to contribute to the control of the ASA pool size.

Under conditions of environmental stress, for example salinity, and drought, ROS levels increase in plant cells. Therefore, plants have developed a number of antioxidant defense mechanisms to protect themselves against ROS. These mechanisms employ such factors as antioxidant enzymes, including SOD, peroxidase (POD), and catalase (CAT), as well as

low molecular weight antioxidants, including ascorbic acid, GSH, and phenolic compounds (Noctor and Foyer 1998).

Table 2. Lipophylic (LAC) and Hydrophylic (HAC) fractions, total carotene, total phenols and Vitamin C content of ready-to-eat *Rocket* stored in plastic containers at 4 °C, in darkness or light, as influenced by NaCl treatments (0= control, 25= 25mM; 50=50mM and 100= 100 mM in the nutrient solution) and leafy proline applications (20mM).

	LAC (mM trolox eq. 100 g ⁻¹ FW)	HAC (mM Asc.ac. eq. 100g ⁻¹ FW)	total carotene (µg mg ⁻¹ FW)	Total phenols gallic acid (mg*g ⁻¹ FW)	Vitamin C (mg 100g ⁻¹ FW) (ASA + DHA)	
NaCl						
0	0,77	1,26	2,97	2,13	21,05	6,42
25	0,74	1,26	2,95	2,49	27,21	8,72
50	0,75	1,23	3,03	2,99	26,58	10,42
100	0,73	1,21	3,03	2,61	27,93	9,50
P=0,05	DMS=0,02	DMS=0,02	ns	DMS=0,46	DMS=0,69	DMS=0,59
Proline						
No	0,72	1,24	2,89	2,58	22,00	8,83
Yes	0,78 **	1,24 ns	3,09 **	2,53 ns	29,38 **	8,70 ns
Light						
Dark	0,72	1,27	2,74	2,54	21,36	11,22
Light	0,78 **	1,22 **	3,24 **	2,57 ns	30,02 **	6,31 **

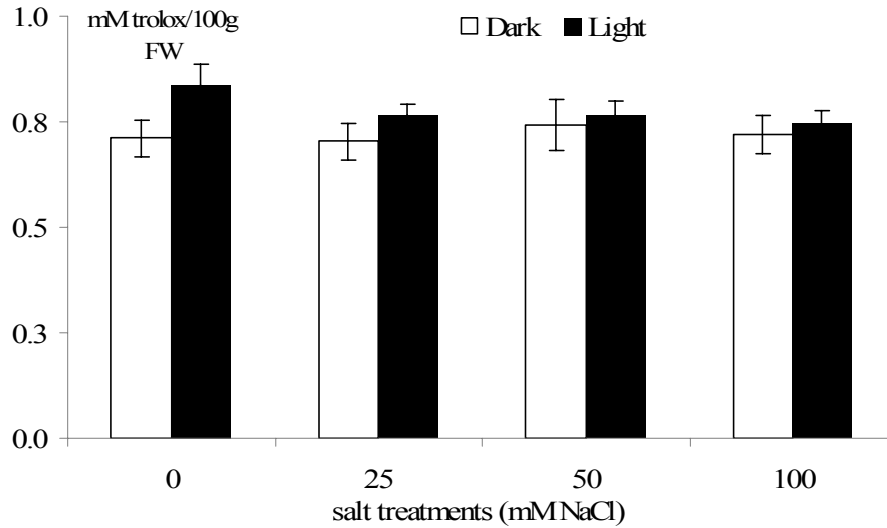


Figure 1 Lipophylic fractions of ready-to-eat *Rocket* stored in plastic containers at 4 °C, in darkness or light, as influenced by NaCl treatments (0= control, 25= 25mM; 50=50mM and 100= 100 mM in the nutrient solution). Data are means of three replications \pm SE.

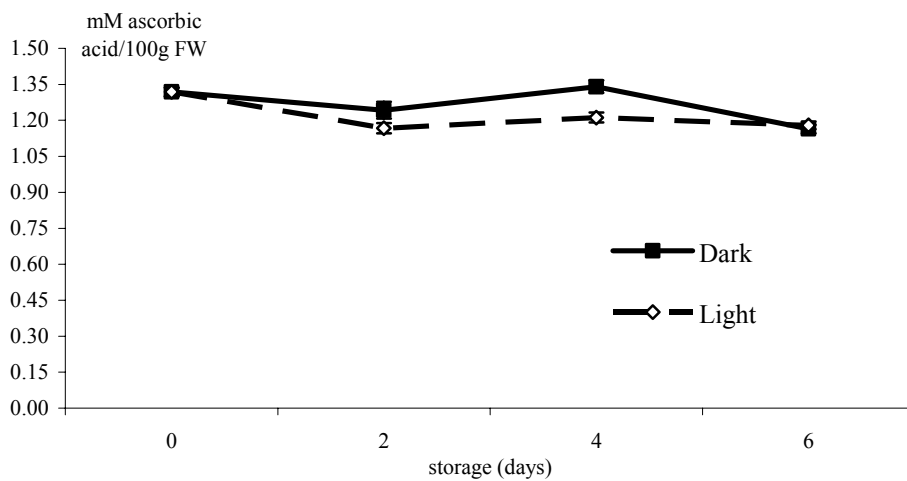


Figure 2 Hydrophylic fractions of ready-to-eat *Rocket* stored in plastic containers at 4 °C, in darkness or light. Data are means of three replications \pm SE.

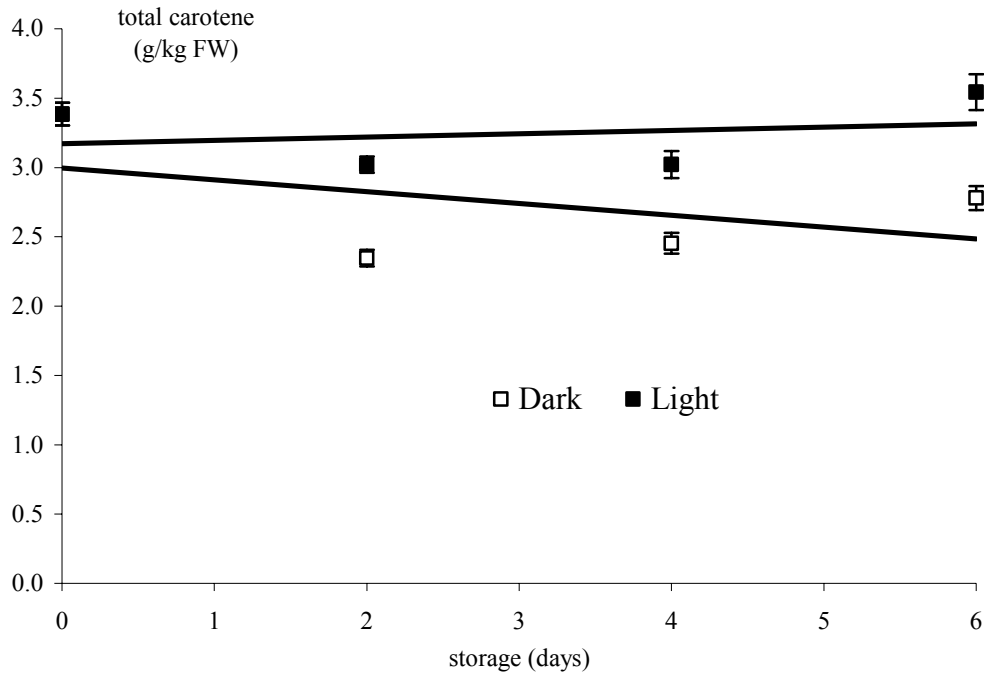


Figure 3 Total carotene content of ready-to-eat *Rocket* stored in plastic containers at 4 °C, in darkness or light. Data are means of three replications \pm SE.

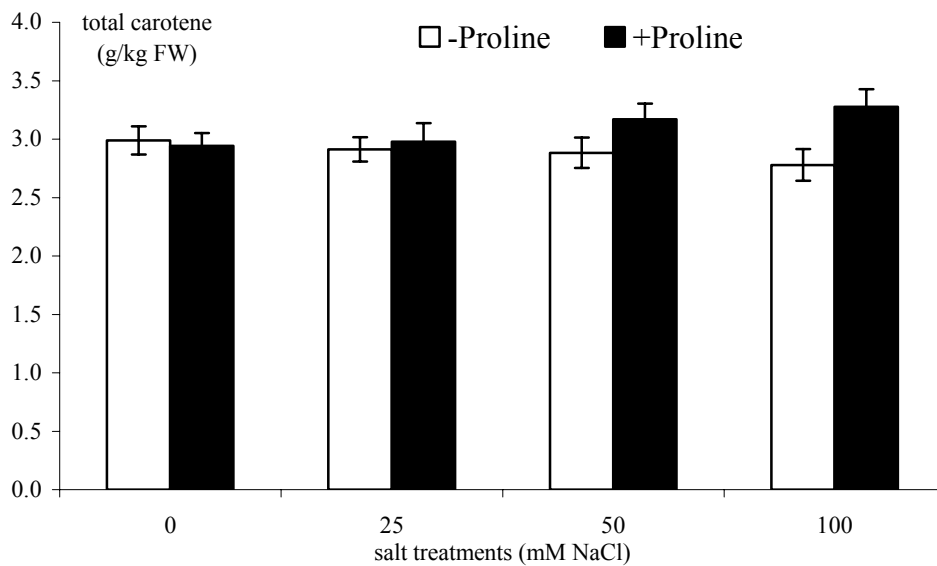


Figure 4 Total carotene content of ready-to-eat *Rocket* stored in plastic containers at 4 °C, in darkness or light, as influenced by NaCl treatments (0= control, 25= 25mM; 50=50mM and 100= 100 mM in the nutrient solution) and leafy proline applications (20mM). Data are means of three replications \pm SE.

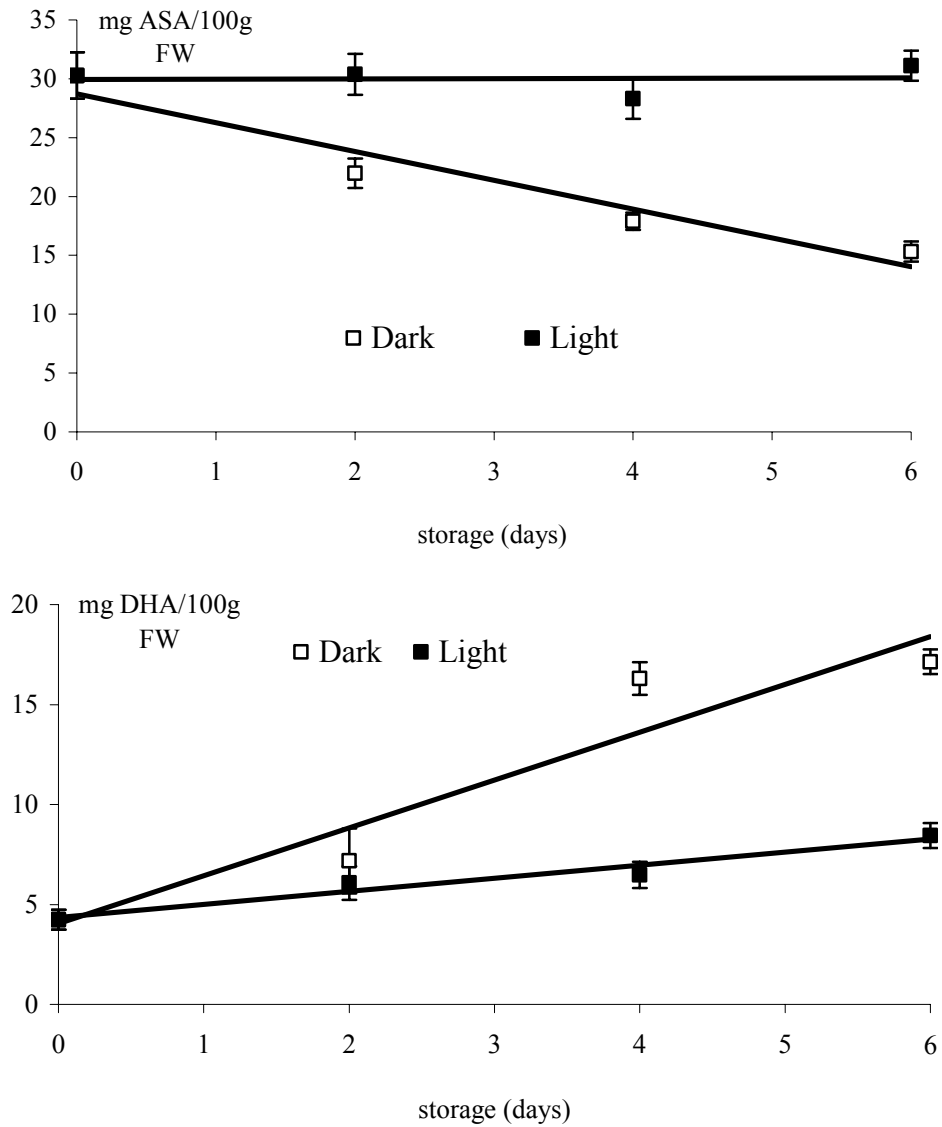


Figure 5. Ascorbic acid (ASA) and dehydroascorbic (DHA) content of ready-to-eat *Rocket* stored in plastic containers at 4 °C, in darkness or light. Data are means of three replications \pm SE.

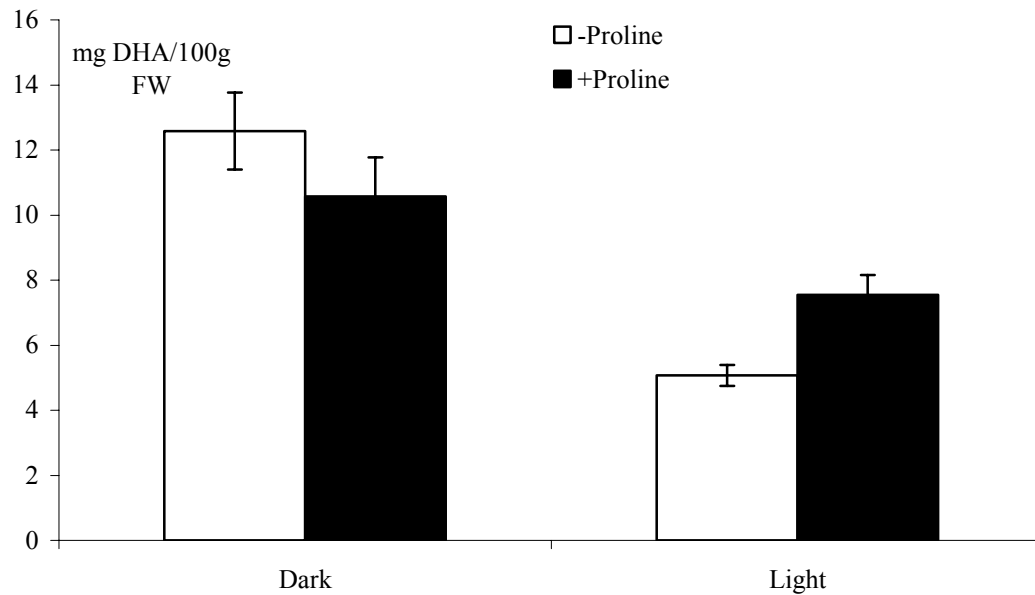
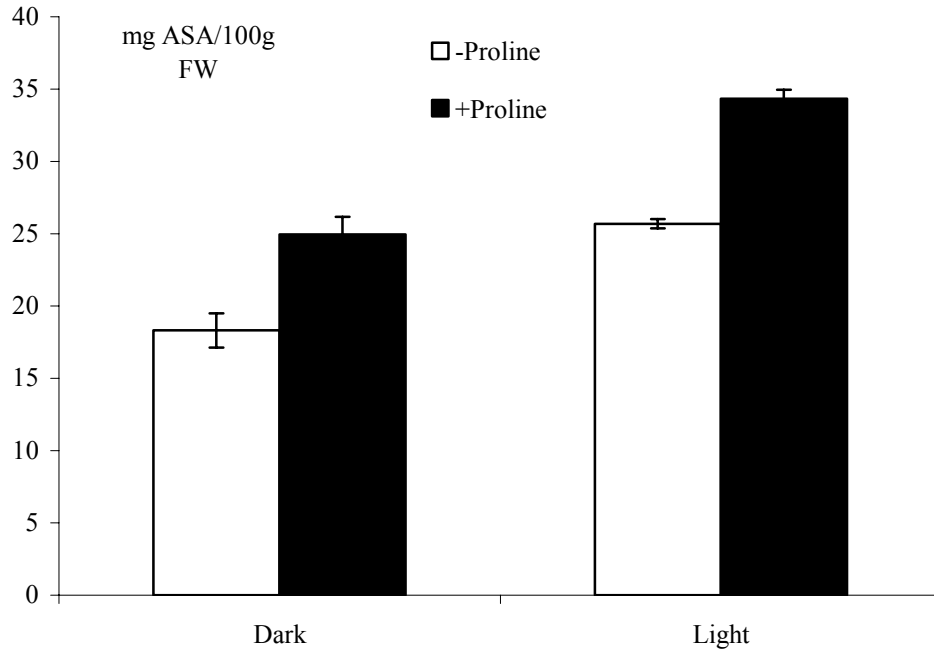


Figure 6 Ascorbic acid (ASA) and dehydroascorbic (DHA) content of ready-to-eat *Rocket* stored in plastic containers at 4 °C, in darkness or light, as influenced by leafy proline applications (20mM in water). Data are means of three replications \pm SE.

3.1.4. Chlorophyll content and colour indexes to assess the dynamic of quality decay

Table 3 reports data on chlorophyll levels and colour indexes affected by salt treatments, proline applications, time and regimes of storage.

The total chlorophyll did not vary under salt stress and increased in proline applications. In storage a reduction was observed. According to Hamilton and Heckathorn (2001), the proline plays a critical role in protecting photosynthetic activity under stress.

Table 3. Total chlorophyll and colour indexes of ready-to-eat Rocket (*Eruca Sativa* Miller) stored in plastic containers at 4 °C, in darkness or light, as influenced by NaCl treatments (0= control, 25= 25mM; 50=50mM and 100= 100 mM in the nutrient solution) and leafy proline applications (20mM in water).

	Total chlorophyll ($\mu\text{g}\cdot\text{mg}^{-1}$ FW)	SPAD	L*	a*	b*	chroma	HUE angle
NaCl							
0	0,23	39,0	38,94	-8,89	18,4	20,5	153,8
25	0,24	41,1	38,19	-8,55	16,6	18,7	152,3
50	0,26	38,8	39,86	-8,78	19,8	21,7	155,1
100	0,24	38,9	39,29	-9,23	20,4	22,4	155,1
P=0,05	ns	DMS=1,30	DMS=0,73	DMS=0,23	DMS=0,98	DMS=0,95	DMS=0,98
Proline							
No	0,22	39,8	38,50	-8,86	18,0	20,1	153,2
Yes	0,26 **	39,1 ns	39,63 **	-8,87 ns	19,6 **	21,6 **	154,9 **
Light							
Dark	0,23	39,0	39,24	-8,76	18,9	20,9	154,2
Light	0,26 ns	39,9 *	38,90 ns	-8,97 **	18,7 ns	20,8 ns	154,0 ns
Storage							
Day 0	0,33	42,0	37,7	-8,3	17,5	19,4	154,2
Day 6	0,25 **	36,6 **	41,4 **	-7,2 **	14,4 **	16,1 **	152,6 **

SPAD values were higher in light stored leaves and decreased during storage. A reduction was attributed to salt stress.

Salt treatments affected L*, a*, b*, chroma and Hue angle indexes. Negative values of a* (green intensity) moved toward zero (reduced green intensity) during storage. L* values increased at the end of the storage.

3.2. Experiment II

3.2.1. Dry matter and Weight Loss

Data presented in table 4 shows dry matter and weight loss in rocket leaves.

During the storage, dry matter did not change, while increased weight loss (data not shown).

3.2.2. Nitrate and Nitrite content

Data presented in table 4 shows the nitrate and nitrite contents in rocket leaves.

The leaf nitrate content was reduced in both light and dark storage, in measure of the 8 % of the initial value after 6 days, while nitrite content increased until the 4th day (data not shown).

Nitrate reductase activity (NRA) of leaves decreases in many plants under salt stress (Abdel Baki *et al.*, 2000; Flores *et al.*, 2000; Rao and Gnham, 1990; Gouia *et al.*, 1994; Meloni *et al.*, 2004). The primary cause of a reduction of NRA in the leaves is a specific effect associated with the presence of Cl⁻ in the external medium. This effect of Cl⁻ seems to be due to a reduction in NO₃⁻ uptake and consequently a lower NO₃⁻ concentration in the leaves, although a direct effect of Cl⁻ on the activity of the enzyme cannot be discarded (Cram, 1973; Flores *et al.*, 2000; Smith, 1973;). In our experiment, this occurred in rocket grown on 100 mM NaCl in the nutrient solution.

Table 4. Dry matter percentage (DM %), weight loss (WL %), nitrate and nitrite content of ready-to-eat *Rocket* stored in plastic containers at 4 °C, in darkness or light, as influenced by NaCl treatments (0= control, 25= 25mM; 50=50mM and 100= 100 mM in the nutrient solution) and leafy proline applications (20 mM).

	DM	LW	Nitrate mg kg ⁻¹ DW	Nitrite mg kg ⁻¹ DW
NaCl				
0	9,7	1,8	24450	21,39
25	10,7	1,8	25467	18,98
50	11,4	1,9	24025	17,42
100	10,8	1,8	22153	18,03
P=0,05	DMS=0,85	ns	DMS=1751	DMS=0,78
Proline				
No	10,7	1,8	23408	19,02
Yes	10,6	1,9	25139	18,89
	ns	ns	**	ns
Light				
Dark	10,6	1,7	24979	19,11
Light	10,7	2,0	23568	18,80
	ns	ns	*	ns

3.2.3. Antioxidant capacity and antioxidant content

Lipophilic and hydrophilic fractions were affected by salt treatments. LAC was higher in proline sprayed leaves (table 5). During the storage, lipophilic fraction decreased in plants grown on 100mM NaCl in measure of the 25% and remained constant in other treatments (data not shown).

HAC was reduced in both dark and light storage, in measure of the 27%, 14%, 17% and 36% in plants grown upon 0, 25, 50 and 100mM NaCl, respectively. Hydrophylic fraction decreases in postharvest as reported by Lee and Kader, (2000).

Total carotene did not change under salt stress (table 5): the content was higher under light conditions until the fourth day; later this takes the same values under dark conditions (Figure 7). A similar course was reported by Barth and Zhuang (1996). Proline sprayed-plants grown on 50mM and 100mM NaCl presented greater total carotene content (Figure 8). Total carotene loss was of 41%, 52%, 67% and 66% after 6 day of storage, on 0, 25, 50 and 100mM NaCl, respectively.

Total phenols content resulted to be higher on 25mM NaCl treatments and in light stored leaves. Proline affected on his content (table 5). Total phenols increased in the control in measure of the 57% after 6 days of storage, while decreased of the 18%, 51% and 37% on 25, 50 and 100mM NaCl treatments, respectively. Total phenols were reduced during the storage such as reported in rocket and lettuce leaves (Koukounaras *et al.*, 2007; Sanchez *et al.*, 2006; Tavarini *et al.*, 2006).

Vitamin C (ASA + DHA) increased upon salinization and proline treated leaves assuming a higher ASA and a lower DHA content (table 5). Moreno's reports of *et al.*, (2008) presented an increase in ASA under salt stress.

A general reduction was obtained during the storage in all treatments. ASA loss was of the 23%, 30%, 29% and 51% in the control, 25, 50 and 100mM NaCl solutions, respectively.

DHA increased after 6 days of the 50%, 39%, 41% and 33% in the control, 25, 50 and 100mM NaCl solution, respectively.

A decrease in ASA was compensated by an increase in DHA (Figure 9),so that the vitamin C content remained constant at the end of the storage, maintaining a higher value in the light, such as reported by Lee and Kader (2000), Koukounaras et al (2007). Salt stress causes water deficit as a result of osmotic effects on a wide variety of metabolic activities of plants and this water deficit results in oxidative stress (Parida and Das, 2005). Therefore, the plants defend themselves from these reactive oxygen species through the induction of activities of certain antioxidative enzymes (Hernandez *et al.*, 2000).

Table 5. Liphophylic (LAC) and Hydrophylic (HAC) fractions, total carotene, total phenols and Vitamin C content of ready-to-eat Rocket stored in plastic containers at 4 °C, in darkness or light, as influenced by NaCl treatments (0= control, 25= 25mM; 50=50mM and 100= 100 mM in the nutrient solution) and leafy proline applications (20mM).

	LAC (mM trolox eq. 100 g ⁻¹ FW)	HAC (mM Asc.ac. eq. 100g ⁻¹ FW)	total carotene (µg mg ⁻¹ FW)	Total phenols gallic acid (mg*g ⁻¹ FW)	Vitamin C (mg 100g ⁻¹ FW) (ASA + DHA)	
NaCl						
0	0,79	0,96	3,12	1,68	21,89	13,60
25	0,73	0,92	2,85	1,83	26,03	16,52
50	0,75	0,91	2,98	1,65	27,46	19,59
100	0,66	0,81	3,00	1,60	30,06	19,28
P=0,05	DMS=0,04	DMS=0,05	ns	DMS=0,15	DMS=0,69	DMS=0,41
Proline						
No	0,72	0,89	2,81	1,68	21,13	18,42
Yes	0,75 *	0,91 ns	3,16 **	1,70 ns	31,59 **	16,07 **
Light						
Dark	0,73	0,92	2,87	1,57	25,29	17,99
Light	0,73 ns	0,88 ns	3,11 *	1,81 **	27,43 **	16,51 **

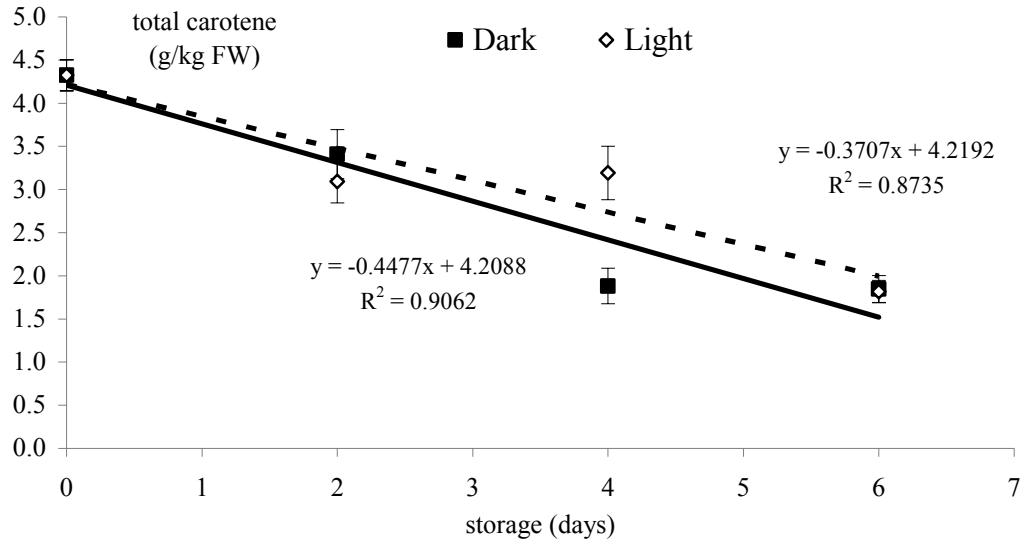


Figure 7 Total carotene content of ready-to-eat *Rocket* stored in plastic containers at 4 °C, in darkness or light. Data are means of three replications \pm SE.

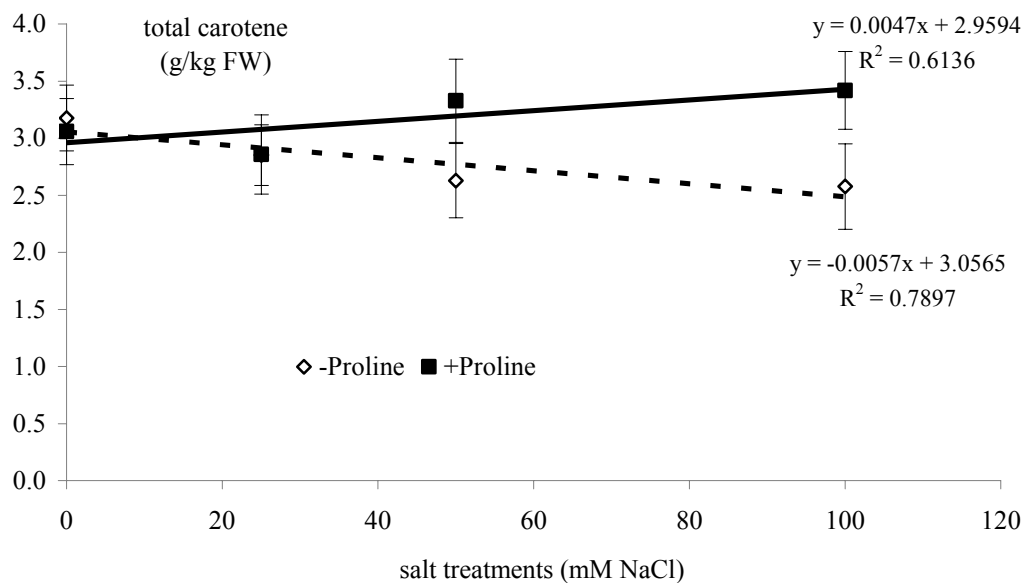


Figure 8 Total carotene content of ready-to-eat *Rocket* stored in plastic containers at 4 °C, in darkness or light, as influenced by by NaCl treatments (0= control, 25= 25mM; 50=50mM and 100= 100 mM in the nutrient solution) and leafy proline applications (20mM). Data are means of three replications \pm SE.

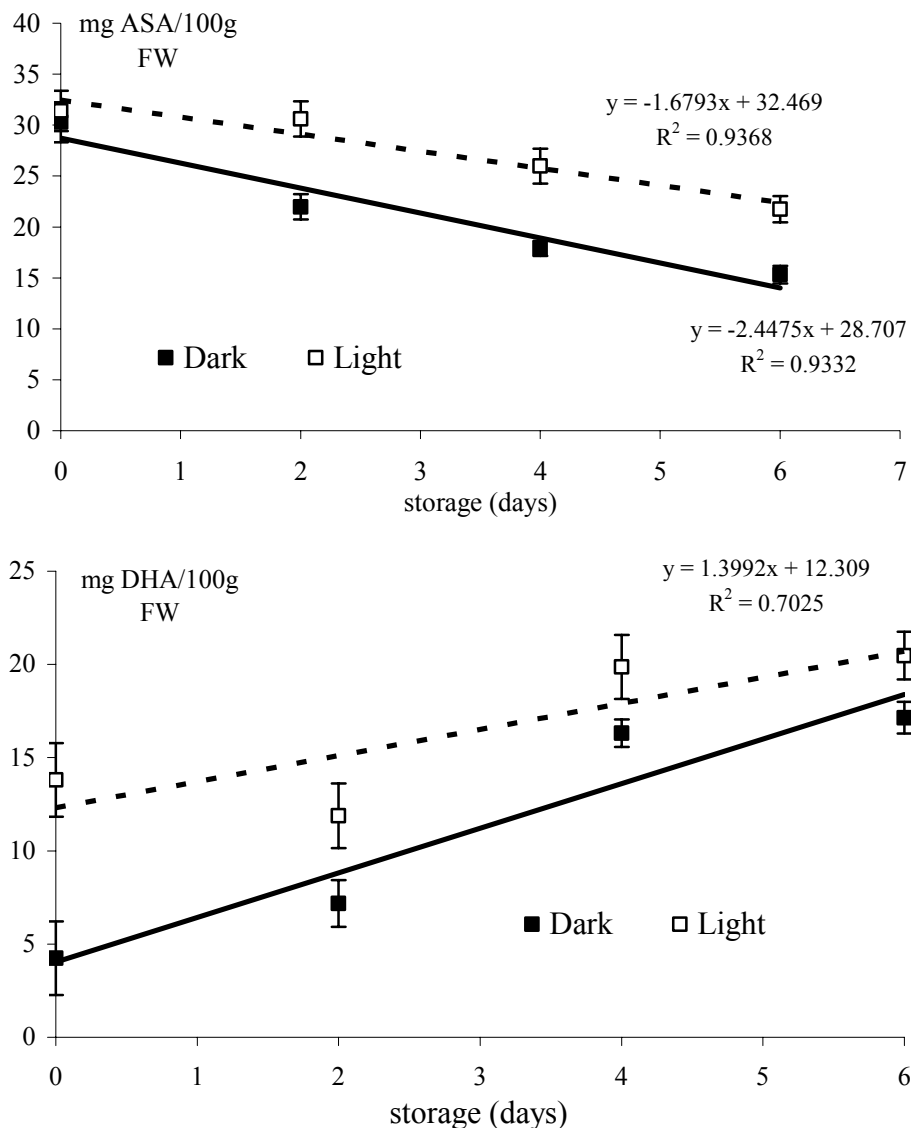


Figure 9. Ascorbic acid (ASA) and dehydroascorbic (DHA) content of ready-to-eat *Rocket* stored in plastic containers at 4 °C, in darkness or light. Data are means of three replications \pm SE.

3.2.4. Chlorophyll content and colour indexes to assess the dynamic of quality decay

Colour indexes and total chlorophyll content were presented in table 6 respect the salt stress, proline applications, time and regimes of storage.

Chlorophyll content was not affected by salt treatments and proline applications, while the storage engraved on his content. It decreased in the time, showing a higher value in the light until forth day, to assume later the same value in the dark at the end of the storage (Figure 10). It was suggested (Toledo *et al.*, 2003), that a continuous illumination with

white light maintains the photosynthetic capacity of spinach leaves during post-harvest storage.

Total chlorophyll decreased after 6 days of storage, in measure of the 44%, 59%, 67% and 66% on 0, 25, 50 and 100mM NaCl, respectively.

Although there are reports of photosynthesis suppression upon salt stress (Soussi *et al.*, 1998; AliDinar *et al.*, 1999; Romeroaranda *et al.*, 2001), there are also reports that photosynthesis is not slowed down by salinity and is even stimulated by low salt concentration (Rajesh *et al.*, 1998; Kurban *et al.*, 1999). On the contrary, in the our results, the salt stress had no direct effect on Chlorophyll content.

A reduction of the spad values was attributed to salt stress. Salt treatments affected a*, b* and chroma indexes. Negative values of a* (green intensity) moved towards more negative values (increased green intensity) during the storage, such as spad values. L* values and the yellowing (b*) were increased at the end of the storage.

Tab 6. Total chlorophyll and colour indexes of ready-to-eat Rocket stored in plastic containers at 4 °C, in darkness or light, as influenced by NaCl treatments (0= control, 25= 25mM; 50=50mM and 100= 100 mM in the nutrient solution) and leafy proline applications (20mM). Data are means of three replications ± SE.

	Total chlorophyll ($\mu\text{g}\cdot\text{mg}^{-1}$ FW)	SPAD	L*	a*	b*	chroma	HUE angle
NaCl							
0	0,22	43,5	41,6	-9,3	21,1	23,1	2,71
25	0,19	43,4	41,2	-8,7	19,6	21,5	2,71
50	0,21	48,3	41,6	-8,5	18,9	20,8	2,70
100	0,20	47,4	41,4	-8,6	19,3	21,2	2,70
P=0,05	ns	DMS=2,43	ns	DMS=0,42	DMS=1,54	DMS=1,55	ns
Proline							
No	0,20	44,3	42,4	-8,9	20,7	22,6	2,72
Yes	0,21	47,1	40,5	-8,7	18,7	20,7	2,69
	ns	**	**	ns	**	**	**
Light							
Dark	0,19	45,6	42,2	-8,8	19,9	21,8	2,71
Light	0,21	45,8	40,7	-8,9	19,6	21,5	2,70
	ns	ns	**	ns	ns	ns	ns
Storage							
Day 0	0,25	45,3	41	-7,7	16,8	18,5	2,70
Day 6	0,10	47,7	41,5	-9,1	20,3	22,3	2,71
	**	**	**	**	**	**	ns

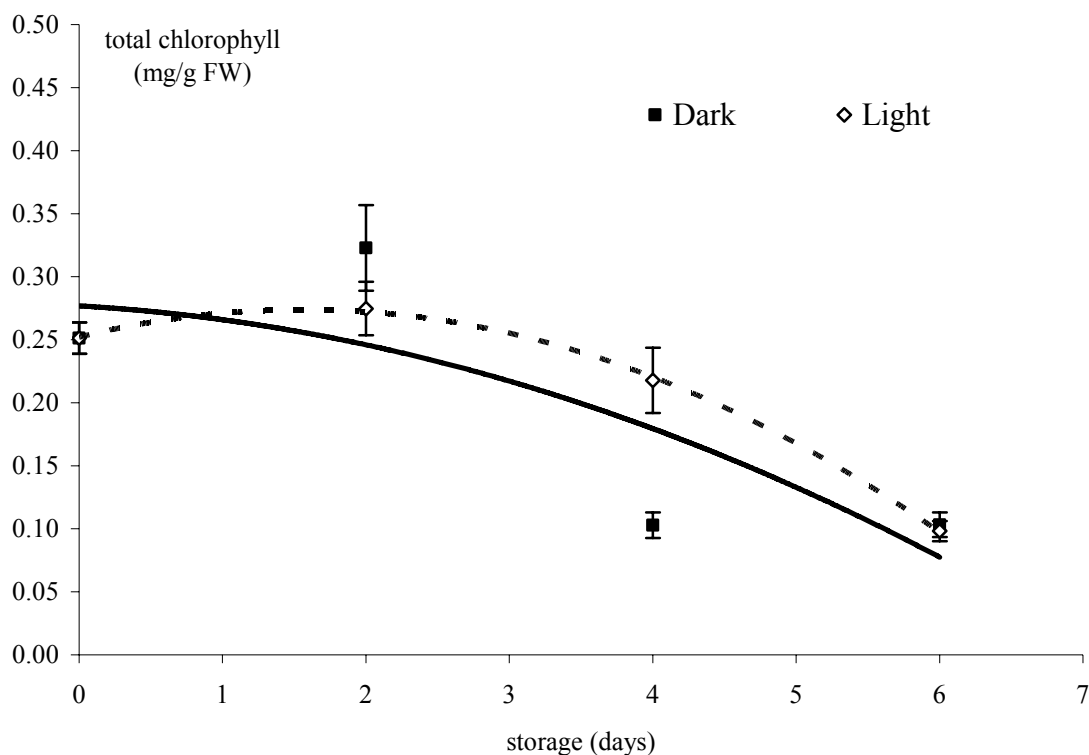


Figure 10. Total chlorophyll content of ready-to-eat *Rocket* stored in plastic containers at 4 °C, in darkness or light. Data are means of three replications \pm SE.

4. Conclusions

Salt stress bring a water reduction in the cells and therefore this causes an increase in dry matter. Moreover, salt stress does not involve a decrease in nitrate and nitrite content, but the time of storage favored their diminution. In the spring crops observes about half of the nitrate content than at the summer crops. Proline affects positively nitrates amount in spring crops.

Salt stress affects negatively liphophylic and hydrophylic antioxidant capacity, while an increment observes in vitamin C and total phenols in spring crops and vitamin C in summer crops. While spring rocket presents more dehydroascorbic acid, in the summer one this is reduced of half. Therefore, the summer product results be less oxidized. Proline applications improve liphophylic antioxidant capacity, total carotene and ascorbic acid contents in both crops.

The time storage influences positively LAC, while HAC loss observes. The light storage affected negatively HAC in spring crop. The light storage affected more positively the total carotene and ascorbic acid content than the dark storage in both products, and in summer rocket also total phenols are resulted higher in the light conditions.

The chlorophyll content and the colour are very important parameters because they compromise the consumer's choice. Chlorophyll degradation is the most serious alteration postharvest in rocket leaves, what involves the yellowing. Salinity does not influence the total chlorophyll, but the time of storage compromises its content.

Then, rocket leaves can be stored at 4°C with a maximum of shelf life of 4 days and under light regimes. Moreover, rocket growth upon salinitation and upon exogenously leafy proline applications improves in quality.

CHAPTER III

Effects of Saline Conditions on postharvest storage of two Sweet Basil Cultivar (*Napoletano* and *Genovese*)

1. Introduction

Basil (*Ocimum basilicum* L.) is an aromatic herb, members of the Lamiaceae family, used extensively to add a distinctive aroma and flavour to food. The leaves can be used fresh or dried to use them as a spice. The cultivar displayed a wide diversity in growth habit, flower, leaf and stem colors, and aromas. Many of the cultivar belong to the “Sweet” basil group, as ‘Genovese’, ‘Italian large leaf’, ‘Napoletano’, and ‘Sweet’ dominating fresh and dry culinary herb markets (Simon *et al.*, 1999).

Basil contains a wide range of essential oils rich in phenolic compounds (Simon *et al.*, 1990; Phippen and Simon 2000) and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins (Nakatani, 1997; Phippen and Simon 1998; Kahkonen *et al.*, 1999).

In the sweet basil group are many green basil varieties, rich in a combination of linalool, methylchavicol and 1,8-cineole (Simon *et al.*, 1990).

The main antioxidant activity from basil plants does not arise from their essential oils, but rather from other phenolics such as flavonoids in green basils and anthocyanins in purple basils (Juliani and Simon 2002).

Basil can not be kept for long periods after harvest, and the essential oil content and quality can be reduced during postharvest. The main cause of losses is endogenous, though external factors as chilling injury may contribute (Lange and Cameron, 1994, 1997). The cutting of vegetable provokes wound-induced physiological and biochemical reactions that shorten product storage life (Salveit, 1997).

These changes facilitate the water loss, and the enzymatic degradation of chlorophyll associated with tissue senescence or tissue browning (Cantwell and Reid, 1994; Philosoph-Hadas *et al.*, 1994; Meir *et al.*, 1997). Water loss in fresh vegetables results in wilted, dull appearance that reduces eye appeal and freshness. Prevent water loss means improves shelf life, appearance and desirability. It is prevented storing the product at low temperatures and at the high relative humidity. Furthermore, an increased peroxidase and lipoxygenase activities are responsible for tissue yellowing (Yamauchi and Watada, 1991).

Physiological changes following wounding include increased respiration and ethylene production, and accumulation of phenol compounds, natural substrates of oxidative enzymes (Brecht *et al.*, 2004; Couture *et al.*, 1993; Castaner *et al.*, 1999).

Moreover, other indicator of nutritional quality of the vegetables is the ascorbic acid (AA). AA is a molecule of dietary importance to humans. A wide range of factors such as genotype, preharvest and postharvest conditions influence his content (Lee and Kader 2000).

It plays an important role in photosynthesis (Foyer, 1993; Smirnoff, 1996), provides significant biochemical functions as an antioxidant, and acts as a defence against oxidative stress (Smirnoff, 1996). Under conditions of environmental stress, e.g. salinity and drought, ROS levels increase in plant cells. Therefore, plants have developed a number of antioxidant defense mechanisms to protect themselves against ROS. These mechanisms employ such factors as antioxidant enzymes, including SOD, peroxidase (POD), and catalase (CAT), as well as low molecular weight antioxidants, including ascorbic acid, GSH, and phenolic compounds (Noctor and Foyer, 1998).

Nitrate and nitrite amounts of foods are of great importance regarding the consumers' health excessive nitrate accumulation can occur in some leaf and root vegetables.

The presence of anti-nutritional compound such as nitrates in fresh leaves depends on the cultivar (Wang *et al.*, 2003), light exposure (Li and Ann, 1995), and nutrient availability (Crawford, 1995).

In the present work, we have quantified some nutritional modifications in leaves of sweet basil in ready to eat cultivated in hydroponics systems in different salt concentrations in the nutrient solution.

2. Material and Methods

The experiment was carried out at the University of Naples Federico II (40°49' N, 14° 15' E, 30 m.a.s.l.) in a cold glasshouse, in summer 2007. Two cultivars of sweet basil (*Ocimum basilicum*), respectively NAP and GEN, were sown in polystyrene containers filled with sterilized soil and grown in floating systems. Each growing unit was a 4 m² container, filled with 1.000 litres of aerated nutrient solution replaced every week. Oxygenation of the solution was maintained with electric pumps. Planting density was of 100 plants m⁻², as commercially adopted in the region for hydroponic production of basil. The cycle was conducted in September (seed-to-harvest time 30 days). The composition of the standard nutrient solution adopted in all experiments was: N-NO³⁻ 14 mM; N-NH⁴⁺ 6 mM; Cl⁻ 3.0 mM; PO₄³⁻ 3.5 mM; S 6.0 mM; Ca 5.0 mM; Mg 3.7 mM; K 10.5 mM; Na 2.2 mM; Bo: 0.02 mM; Fe 0.04 mM (Marschner, 1995, Pimpini *et al.*, 2001;).

Plant stress was performed as follows: starting from 10 DAS, plants were maintained under salt stress of 0, 100, 200 mM NaCl. The experimental design was a randomized block, with 200 plants for block and four blocks for treatment.

Determinations and analysis: Plants were harvested on October 9. At harvest, the fresh products were manually cleaned, by discarding stems, damaged leaves and yellow inflorescences. Intact leaves were accurately washed in order of three times with microbiologically pure water, and air flow dried on a strainer. The leaves were

subsequently kept in plastic boxes. The containers were wrapped with a multistrata film (PET 12+ COEX/EVOH/PE 95), highly impermeable to gases and water vapor, with permeability to O₂ < 5 dm³ m⁻² d⁻¹ bar⁻¹ at 23 °C and 0% U.R. and to H₂O < 5g m⁻² d⁻¹ bar⁻¹ at 38 °C and 100% U.R. (Gruppo Fabbri S.P.A., Modena, Italy).

The containers were kept at 4 °C for 9 days, under darkness storage. Leaf samples were collected on day 0, 3, 6 and 9. Leaf dry weights upon oven dehydration at 60°C (until steady weight) and dry matter were measured.

For Nitrates and nitrites contents and antioxidant activity, hydrophilic (HAA) and lipophilic (LAC), see Materials and Methods of previous Chapter Friariello.

For total ascorbic acid, total carotenoids, total phenols and total chlorophyll, see Materials and Methods of previous Chapter Rocket.

Colour parameters L*, a* and b* [CIELAB-system, the L* component represents lightness ranging from 0 = black to 100 = white, the a* component represents values running from green (-) to red (+), and the b* component represents values running from blue (-) to yellow (+)] were evaluated on marketable leaves using a portable colorimeter (Minolta Chroma Meter CR-300).

Two derived functions were computed from the lab readings as follows:

Hue angle: $\arctan (b/a)$

Chroma: $(a^2 + b^2)^{1/2}$

The hue angle is the characteristic associated with the conventional perceived colour name; an angle of 90° represents a yellow hue. Objects with higher hue angles are greener.

Chroma is an indicator of brightness.

Previously on the same leaf samples greenness measurements were performed using a Chlorophyll Meter (SPAD 502).

Data were analyzed by ANOVA and means were compared with the Least Significant Difference (LSD) test.

3. Results and discussion

3.1. Fresh Weight

During storage, a leaf weight did not change, such as in Barth and Zhuang (1996) and Zhuang *et al.*, (1997), while the dry matter was greater in leaves exposed to the NaCl, and in GEN cv. (Figure 1).

It was widely recognized that during osmotic adjustment afterward salt stress, the cells tend to compartmentalize many of the absorbed ions in vacuoles and at the same time to synthesize and to accumulate compatible organic solutes in the cytoplasm, in order to maintain the osmotic equilibrium between these two compartments and such osmotic change results in reduced water uptake (Bradford 1995; De Lacerda *et al.*, 2005; Hare *et al.*, 1998; Hasegawa *et al.*, 2000).

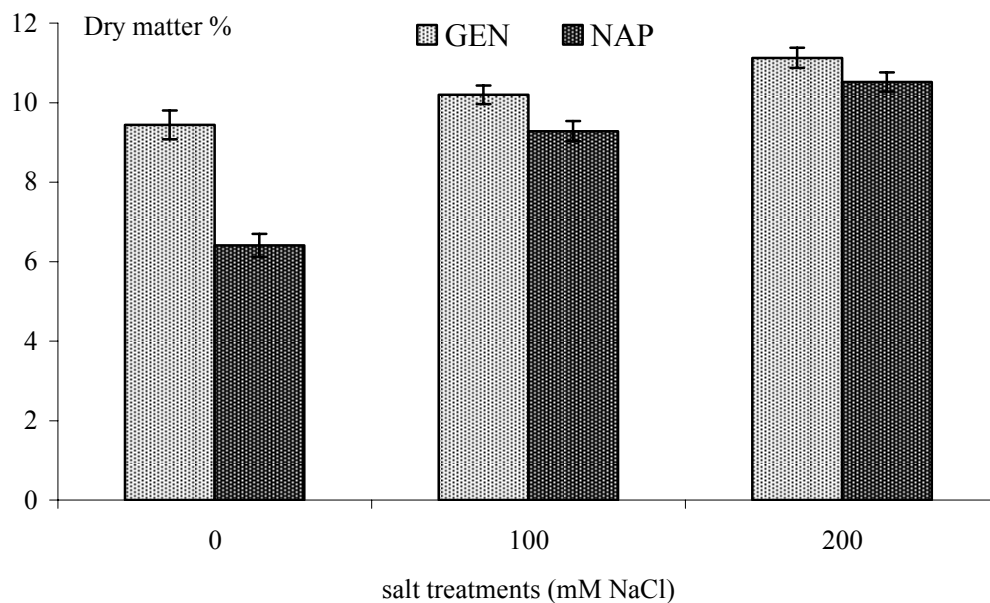


Figure 1. Dry matter of ready-to-eat *Basil* stored in plastic containers at 4 °C, in darkness, as influenced by NaCl treatments (0= control, 100= 100mM; and 200= 200 mM in the nutrient solution). Data are means of three replications \pm SE.

3.2. Nitrate and Nitrite Content

The nitrate content was greater in NAP, while the nitrite amount was higher in GEN. Both contents were affected by salt concentrations in the nutrient solution. Plants grown on 200mM NaCl in the nutrient solution, presented a lower nitrate and a higher nitrite amount. However, leaf nitrate content was reduced during storage at 4 °C, while leaf nitrite content increased (table 1; table 2).

Previous experiments on sweet basil stored in darkness (De Pascale *et al.*, 2006), indicated a reduction of nitrate content in storage. Such reduction may be attributed to residual activity of NR (Crawford *et al.*, 1995).

Several studies reported that nitrate uptake and nitrate reductase activity (NRA) decreased in plants under salt stress (Rao and Gnham, 1990; Gouia *et al.*, 1994; Meloni *et al.*, 2004; Flores *et al.*, 2000). Such reduction in NO₃⁻ uptake could be due to the increasing of Cl⁻ ions in the leaves (Cram, 1973; Smith, 1973; Flores *et al.*, 2000).

Table 1. Nitrate content in ready-to-eat sweet basil stored in plastic bags at 4 °C in the darkness as influenced by cultivar and salt treatments; 0= control; 100= 100mM NaCl in nutrient solution; 200= 200mM NaCl in nutrient solution.

mMol NaCl	GEN (mg kg FW ⁻¹)		NAP (mg kg FW ⁻¹)	
	Day 0	Day 9	Day 0	Day 9
0	1387	702	2583	807
100	1422	1351	2286	1586
200	1273	1017	1692	1080
DMS=222 at P=0,05				

Table 2. Nitrite content in ready-to-eat sweet basil stored in plastic bags at 4 °C in the darkness as influenced by cultivar and salt treatments; 0= control; 100= 100mM NaCl in nutrient solution; 200= 200mM NaCl in nutrient solution.

mMol NaCl	GEN (mg Kg FW ⁻¹)		NAP (mg kg FW ⁻¹)	
	Day 0	Day 9	Day 0	Day 9
0	1,21	3,72	1,05	2,59
100	1,33	4,13	0,98	3,49
200	2,29	4,27	2,67	5,13
DMS=0,32 at P=0,05				

3.3. Total Antioxidant Capacity and Antioxidant Content

Salt stress affected both HAC and LAC. Hydrophylic fraction resulted to be cultivar-dependent. At the end of the storage period, a general reduction of both hydrophilic and lipophilic fractions were measured for all treatments (table 3). In Sweet basil, the course in the time was confirmed from De Pascale *et al.*, (2006).

Data presented in table 4 shows the total carotene, total phenols and total ascorbic acid contents in basil leaves than to the cultivar, salt treatments and time of storage.

During the storage was observed a development in ascorbic acid of the 60% and 38% in the control, 75% and 75% in 100mM and 60% and 70% in 200mM NaCl, respectively in GEN and in NAP leaves.

Previously results reported a reduction of ascorbic acid in storage as a consequence of the ascorbic acid pool degradation (Lee and Kader, 2000).

Salt stress and the storage postharvest did not affect total carotene content. Generally, carotenoids levels declined under salt stress, (Hernandez *et al.*, 1995; Khavarinejad and Mostofi, 1998; Negi and Roi, 2004), on the contrary, in leaves of alfalfa plants were not significant changed (Khavarinejad and Chaparzadeh, 1998).

The phenolic compounds increased upon salinization and were reduced significantly in storage as reported in rocket and lettuce leaves. (Koukounaras *et al.*, 2007; Tavarini *et al.*, 2006). Strong positive correlation between phenols content and lipophilic antioxidant activity in sweet basil, was reported by Juliani and Simon (2002).

Table 3. Hydrophylic (HAC) and lipophylic (LAC) antioxidant in ready-to-eat sweet basil stored in plastic bags at 4 °C in darkness as influenced by cultivar and NaCl treatments; 0= control; 100= 100mM NaCl in nutrient solution; 200= 200mM NaCl in nutrient solution. ns=not significant; *=significant at P=0.05; **=significant at P=0.01.

	HAC mMol asc.ac. 100g⁻¹ DW	LAC mMol trolox 100g⁻¹ DW
GEN	5,67	3,82
NAP	6,28 **	3,92 ns
0mM	5,16	3,47
100mM	5,41	3,96
200mM	7,35	4,18
P=0,05	DMS=0,41	DMS=0,27
0	6,87	6,01
3	5,93	3,51
6	5,89	3,37
9	5,20	2,60
P=0,05	DMS=0,47	DMS=0,31

Table 4. Total carotene, total phenols and total ascorbic acid content in ready-to-eat sweet basil stored in plastic bags at 4 °C in darkness as influenced by cultivar and NaCl treatments; 0= control; 100= 100mM NaCl in nutrient solution; 200= 200mM NaCl in nutrient solution. ns=not significant; *=significant at P=0.05; **=significant at P=0.01.

	Total Carotene (µg*mg FW⁻¹)	Total Phenols gallic acid (mg 100g FW⁻¹)	ASA tot (mg*100g FW⁻¹)
Gen	7,42	122,20	34,09
Nap	7,22 ns	80,70 **	28,34 **
0mM	7,36	65,31	25,21
100mM	7,38	91,09	35,35
200mM	7,24	147,95	33,08
P=0,05	ns	DMS=15,92	DMS=1,18
0	7,35	131,07	54,77
3	7,55	96,60	32,02
6	7,12	91,48	19,76
9	7,27	86,65	18,31
P=0,05	ns	DMS=18,39	DMS=1,36

3.4. Chlorophyll content and colour indexes to assess the dynamic of quality decay

A reduction in chlorophyll levels was observed upon salinization such as Khavarinejad and Mostolfi (1998) and in postharvest such as reported in stored lettuce salads. (Tavarini *et al.*, 2006)

In Figure 2a and Figure 2b the course in the time of the total chlorophyll content as influenced by salt treatments and cultivar was reported.

During storage, the dynamic of chlorophyll degradation has been the focus of many food scientists since this parameter is closely associated to the product shelf life (Zhuang *et al.*, 1994 and 1997; Toivonen and Sweeney, 1998; Deeli and Toivonen, 1999). The decrease in the chlorophyll content may be due to an increase of the degradation or to a decrease of chlorophyll synthesis (Santos, 2004).

The process of photosynthesis and photosynthesis rates are usually lower in plants exposed to the salinity and especially to the NaCl (AliDinar *et al.*, 1999; Downton *et al.*, 1985; Kao *et al.*, 2001; Khavarinejad and Mostofi, 1998; Marschner 1995; Romeroaranda *et al.*, 2001; Soussi *et al.*, 1998). It is previously been reported that salt stress induces a reduction of the number of chloroplasts (Marchner and Possingham, 1975).

Moreover, the chlorophyll loss is responsible of the yellowing of fresh-cut products and is the result of destruction of compartments that occurs when cells are broken, allowing substrates and oxidases to come in contact (Martinez and Whitaker, 1995; Heaton and Marangoni, 1996). It is also well known that endogenous ethylene accelerates senescence in leaves of some species (Mattoo and Aharoni, 1988; Able *et al.*, 2003; 2005), and that senescence primarily was expressed by yellowing, which is generally associated with degradation of chlorophyll.

The colour indexes resulted cultivar-dependent. The higher *b, *L and chroma values were observed in NAP. On the contrary, Spad and *a values were greater in GEN. A slight variation of hue angle from green to blue-green was observed under salt stress and during

storage (De Pascale *et al.*, 2006). At the end of the storage, Spad and *L values decreased (Da Silvia *et al.*, 2005). The *a absolute value increased in stored basil leaves. The yellowness (*b value) and chroma were highest under salt stress and increased during the storage time (table 5).

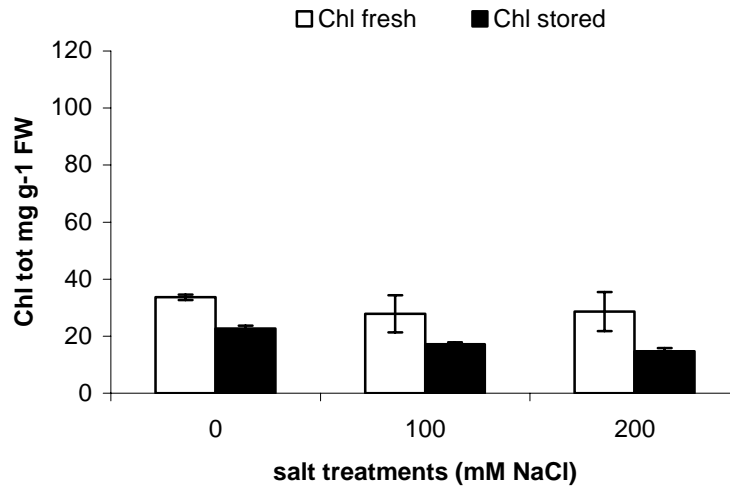


Figure 2a. Total chlorophyll in ready-to-eat Genovese Sweet Basil stored in plastic bags at 4 °C in darkness as influenced by cultivar and NaCl treatments; 0= control; 100= 100mM NaCl in nutrient solution; 200= 200mM NaCl in nutrient solution. Data are means of three replications ± SE. Mean values; ns=not significant; *=significant at P=0.05; **=significant at P=0.01.

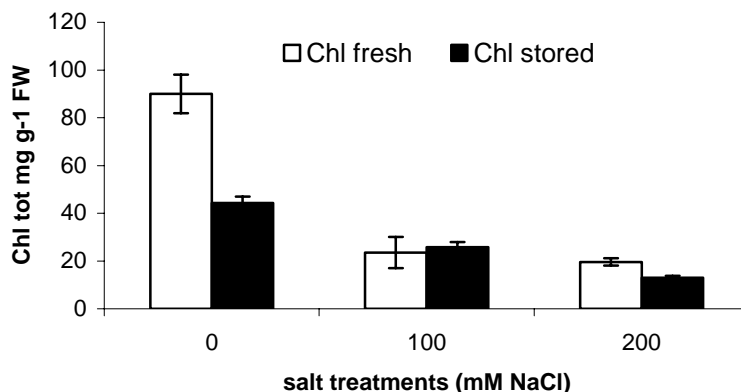


Figure 2b. Total chlorophyll in ready-to-eat Neapolitano Sweet Basil stored in plastic bags at 4 °C in darkness as influenced by cultivar and NaCl treatments; 0= control; 100= 100mM NaCl in nutrient solution; 200= 200mM NaCl in nutrient solution. Data are means of three replications ± SE. Mean values; ns=not significant; *=significant at P=0.05; **=significant at P=0.01.

Table 5. Colorimetric indexes in ready-to-eat sweet basil stored in plastic bags at 4 °C in darkness as influenced by cultivar and NaCl treatments; 0= control; 100= 100mM NaCl in nutrient solution; 200= 200mM NaCl in nutrient solution. ns=not significant; *=significant at P=0.05; **=significant at P=0.01.

	a	b	L	Hue Angle	Chroma	Spad
Cultivar						
Gen	-8,73	25,63	44,57	160,69	27,12	31,48
Nap	-9,35 **	27,86 **	46,60 **	160,90 ns	29,42 *	25,12 **
NaCl						
0 mM	-9,70	26,85	44,93	159,32	28,59	30,74
100 mM	-8,33	23,57	43,70	160,26	25,02	30,40
200 mM	-9,09	29,82	48,13	162,81	31,20	23,77
P=0,05	DMS=0,36	DMS=2,22	DMS=1,08	DMS=0,02	DMS=2,18	DMS=1,47
day						
0	-7,93	20,77	45,92	158,87	22,28	30,01
3	-8,18	22,74	47,72	159,93	24,19	27,68
6	-9,09	27,88	46,21	161,72	29,34	28,79
9	-10,96	35,60	42,50	162,67	37,26	26,71
P=0,05	DMS=0,42	DMS=2,56	DMS=1,24	DMS=0,02	DMS=2,52	DMS=1,69

4. Conclusions

In this work, the two cultivar have a different behaviour upon salinization. An improvement in the antioxidant capacity and antioxidant content observes upon salt treatments. The colour is a very important parameter, and from it depend the choice of the final consumer. Unfortunately, the salt stress conditions the green colour and increases the yellowing. In conclusion salt stress has a significant impact on quality of fresh cut basil leaves, while packaging involves a decline of the nutritional status in basil tissues.

CHAPTER IV

Profile of bioactive compounds during storage of fresh-cut spinach: the role of endogenous ascorbic acid in the improvement of product quality

1. Introduction

It is well known as fruits and vegetables play a key role against numerous chronic diseases, as cancer, cardio- and cerebro-vascular and neurological disease (Ness & Powles, 1997). This protective effect has generally been attributed to the antioxidant constituents, such as vitamin C and E, carotenoids, flavonoids and phenolic acids. These antioxidant compounds protect against chronic diseases through their effects on free radical damage to protein, lipids and DNA.

This fact suggests a growing consumption in dietary use of fruit and vegetables. However, nowadays many people do not spend much time to prepare vegetable foods every day and so they frequently use fresh cut vegetables, which are horticultural products subjected to minimally processing which was described as “cleaning, washing, cutting and packaging”. The key criteria to consider a vegetables or fruit as fresh-cut product includes that the tissue is in a living respiring physiological state (Brecht, *et al.*, 2004). This determines that fresh-cut products are subjected to rapid deterioration and short shelf life as compared to intact ones. The alterations are the direct result of the wounding associated with processing, which leads to physical and physiological changes that strongly affect the quality of the product (Saltveit, 1997). An important alteration induced by cutting is the browning of the cut tissues that is a direct consequence of polyphenol oxidase (PPO) action on endogenous polyphenols in presence of oxygen (Tomas-Barberan *et al.*, 1997; Degl’Innocenti, *et al.*, 2007). Different species showed a different sensitivity to enzymatic browning induced by cutting operates to produce fresh-cut vegetables (Degl’Innocenti *et al.*, 2005; Degl’Innocenti *et al.*, 2007) and in a previous work the endogenous ascorbic acid

(ASA) content determined protection against enzymatic browning (Degl'Innocenti *et al.*, 2007). It is well known that ASA is the key component of the commercial anti-browning formulations available on the market (Rupasinghe, *et al.*, 2005). It was hypothesized that ASA controls PPO activity through its ability to reduce quinones to the native diphenols (Nicolas *et al.*, 1994) but ASA can inhibit PPO also by decreasing the cytosol pH (Vamos-Vigyazo, 1981).

It is known that fresh-cut operations and storage lead to the destruction of phytochemical compounds even if the extent of nutritional changes is dependent on different factors. Antioxidant phytochemicals might be degraded upon cutting and exposure of tissues to light and oxygen (Gil, *et al.*, 1999). However, there are contrasting evidences in the literature due to the simultaneous accumulation of phenolic compounds induced by cutting process through the activation of enzyme phenylalanine ammonia lyase (Kays, 1997). In a recent work Reyes, *et al.*, (2007) reported that the antioxidant capacity of some fruit and vegetables was positively influenced by cutting being the initial levels of reduced ascorbic acid and phenolic compounds much higher in cut tissue.

Among different leaf vegetables, spinach is usually used after cooking but also fresh in salad. This vegetable has a large nutritional value, being a rich source of vitamin A (and lutein), vitamin C, vitamin E, vitamin K, magnesium and several vital antioxidants such as flavonoids. Bergquist, *et al.* (2005) identified twelve flavonoid compounds in spinach and they found that the flavonoid content was relatively stable during normal retail storage conditions. Also Gil *et al.* (1999) found that flavonoid content of fresh-cut spinach to be rather stable during storage.

Spinach is a rich source of glycosilated flavonoids: these are glucuronides and acylated di- and triglycosides of methylated and methylenedioxyderivatives of 6-oxygenated flavonols (Aritomi, *et al.*, 1986; Ferreres, *et al.*, 1997). The qualitative and quantitative modifications

occurring in these compounds during postharvest storage and particularly for minimally processed spinach have never been investigated.

Spinach showed another important characteristic as compared to other leaf vegetables used for fresh-cut salad. This vegetable does not show alteration attributable to the enzymatic browning that, as reported above, is often the factor limiting shelf-life and marketability of fresh-cut products. This characteristic was shared also with other species, as rocket salad, which as spinach contains high levels of vitamin C (Degl'Innocenti *et al.*, 2007).

The aim of this research was to study the mechanisms of the spinach resistance to the enzymatic browning when stored as fresh-cut product. In addition, the quantitative and qualitative variation in flavonoids and antioxidant capacity upon storage was studied.

2. Material and Methods

2.1. Plant Material

Spinach (*Spinacia oleracea* cultivar RS 3411) was taken from a local market and processed in the laboratory within few minutes. The experiments were carried out in the winter 2007. The leaves were gently washed with chlorinated drinking water and excess water was removed with a manual salad spinner. Afterwards, leaves were cut (approximately 1 cm x 2 cm) perpendicular to the midrib with stainless steel scissor and stored up to seven days at 4°C in quality clear oval PET (Polyethylene Terephthalate) hinged containers (1.5 L) (Elipack EL390, BonSai Plastics, London, UK). Intact spinach leaves were stored under the same conditions as the fresh-cuts. Determinations were made of both fresh-cut and intact leaves at the moment of the cutting and after 72 and 162 h of cold storage.

2.2. Enzyme assay

PPO activity was determined according to the method reported by Cantos, Espin & Tomàs-Barberà (2001). The assay method measures the accumulation of the adduct formed

between the enzymatically generated *o*-quinones and the nucleophile MBTH (3-methyl-2-benzothiazolinone hydrazone). This adduct is reddish in colour, is stable and had high molar absorptivity. Adduct accumulation was followed at 467 nm ($\epsilon = 22300 \text{ M}^{-1}\text{cm}^{-1}$ at pH 5.5). To discriminate between latent and active PPO, 0.1% sodium dodecyl sulphate (SDS) was added to the reaction medium (Espin & Wichers, 1999) Data are expressed as nmol *o*-quinones adduct formed per minute per mg soluble protein. Protein determinations were performed using the Protein Assay Kit II (Bio Rad).

2.3. Phenolic compounds

The extraction of phenolic compounds was performed as described by Degl'Innocenti *et al.*, (2005). The total phenolic content in the methanol extracts was determined according to Folin-Ciocalteu by Singleton and Rossi (1965). A 100 μL aliquot of the supernatant was combined with 500 μL of Folin-Ciocalteu's reagent and 400 μL of sodium carbonate (7.5%). The tubes were mixed for 15 s and then allowed to stand for 30 min at 20°C. Absorption was measured at 765 nm using a UV-Vis spectrophotometer (Ultrospec 2100, Pharmacia). Phenols are expressed as mg gallic acid per g fresh weight.

2.4. Flavonoid extraction

A 0.5 g of freeze dried sample was extracted with 10 ml of MeOH/H₂O (70:30 v/v) using an ultrasonic bath. Then was centrifuged at 4000rpm at 4°C. Extract was filtered through a 0.45- μm -pore filter prior to HPLC analysis.

2.5. HPLC analysis of flavonoids

Filtered extract (20 μL) was injected into an HPLC (Shimadzu LC 10, Shimadzu, Kyoto, Japan) with photodiode array detector. Separations were achieved on a phenomenex column (C18 prodigy 5 μ ODS3 100A size 250x 4,60 nm). Elution was performed using water and formic acid 0.2% (A) and HPLC-grade methanol/Acetonitril (40/60 v/v) (B) as the mobile phases, on a gradient starting with 15% B in A to reach 25% B at 6 min, 30% B at 16 min, 40% B at 20 min, 50% B at 24 min, 60% B at 32 min, 80% B at 35 min and

15% B at 38 min. The flow rate was 0.8 mL/min, and chromatograms were recorded at 365 nm. Flavonoids were identified and confirmed by an LC/MS/MS analysis and quantified using quercetin as external standard and expressed as quercetin content (mg/100g fresh weight).

2.6. LC/MS/MS analysis

Flavonoids were identified by LC-MS/MS. For this purpose, chromatographic separation was performed using an HPLC apparatus equipped with two Series 200 micropumps (Perkin Elmer, Wellesley, MA, USA), an UV/VIS series 200 (Perkin Elmer, Wellesley, MA, USA) detector set at 365 nm, and a Prodigy 5 μ ODS3 100 Å column (250 x 4.6 mm, particle size 5 μ m) (Phenomenex, Torrance, CA, USA). The eluents were: A water 0.2% formic acid; B acetonitrile/methanol (60:40 v/v). The gradient program was the same as described above and the flow was set at 0.8 mL/min. The LC flow was split and 0.2 mL/min was sent to the mass spectrometry. Injection volume was 20 μ L. MS and MS/MS analyses of spinach extracts were performed on an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Canada) equipped with a TurboIonSpray source working in the negative ion mode.

Information Dependent Acquisition (IDA) (14) was used to identify the metabolites: this acquisition method generates a survey scan, single MS spectra with molecular mass information, product ion spectra (MS²) and extracted ion fragment grams (XICs).

2.7. Ascorbic acid content

ASA was determined spectrophotometrically as described by Kampfenkel, *et al.*, (1995). The assay is based on the reduction of Fe³⁺ to Fe²⁺ by ASA and the spectrophotometric detection of Fe²⁺ complexed with 2,2'-dipyridyl. DHA is reduced to ASA by preincubation of the sample with dithiothreitol (DTT). Data are expressed as mg ascorbic acid on 100 g fresh weight.

2.8. Antioxidant capacity

Ferric-Reducing Antioxidant Power (FRAP) assay was based upon the methodology reported by Benzie and Strain (1996). FRAP reagent contained 1 mM 2,4,6-tripyridyl-2-triazine (TPTZ) and 2 mM ferric chloride in 0.25 M sodium acetate at pH 3.6. Newly-prepared reagent was used in each occasion. Ten g of leaf tissue was homogenized with 20 mL of HPLC grade methanol, the homogenate was filtered and centrifuged at 15000g at 20°C for 15 min. An aliquot (0.1 mL) of the methanol extract was added to 0.9 mL of FRAP reagent, and then mixed. The mixture was incubated at 20°C for 4 min and the absorbance at 593 nm was determined against water blank. Calibration was against a standard curve (50-1000 µM ferrous ion) produced by the addition of freshly prepared ammonium ferrous sulphate. FRAP was expressed as mmol ferrous ion g⁻¹ FW.

2.9. Statistical treatment

The experiment was repeated three times with similar results. Two-way ANOVA was used to assess the influence of storage and cutting on the measured quantities. Mean comparison was conducted using LSD test. To correlate the antioxidant capacity and phenols or vitamin C content the regression analysis was carried out.

3. Results

The activity of latent and activated forms of PPO is shown in Figure 1. Both forms increased significantly in spinach leaf when cold stored as fresh-cut product, while no changes were observed in intact leaves. The increase was typically observed at the end of the storage. The presence in spinach of a latent form of PPO enzyme which is activated by SDS was already reported (Sanchez-Ferrer, *et al.*, 1989).

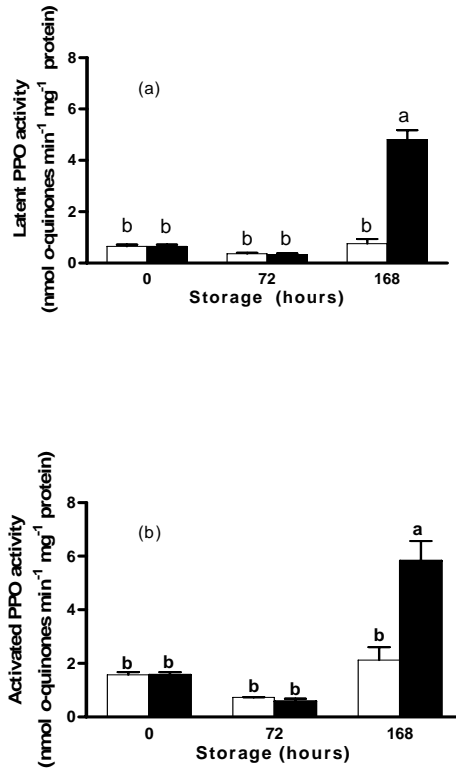


Figure 1 Latent (a) and SDS activated (b) with SDS activity of PPO in intact (white) and fresh-cut (black) tissues of *Spinacia oleracea* during cold storage. The bars represent the mean of 3 replicates with standard deviation. Means of PPO activity followed by the same letter are not significantly different according to LSD test (P=0.05).

As showed in Figure 2, an increase of total phenols content were observed after 72 h of storage in intact leaves, while no modifications were detectable in spinach cut leaves.

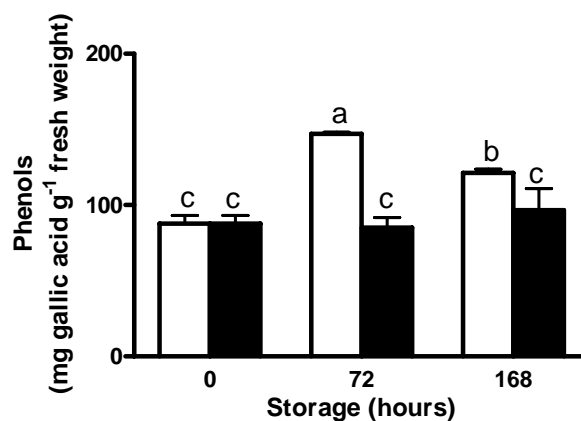


Figure 2 Phenols compounds in intact (white) and fresh-cut (black) tissues of *Spinacia oleracea* during cold storage. The bars represent the mean of 3 replicates with standard deviation. Means of phenols content followed by the same letter are not significantly different according to LSD test (P=0.05).

The main flavonoids identified of spinach by LC-MS-MS were listed in Table 1, while their quantification in fresh-cut and intact spinach tissues was obtained by HPLC analysis (see chromatogram in Figure 3). Data about the effect of cold storage and cut processes were summarised in Figure 4.

The most abundant flavonoid compound by far was: 5-3'-4'-trihydroxy-3-methoxy-6:7-methylenedioxi-flavone-4'-glucuronide which comprised, on average, 25% of the total flavonoids in the fresh material. Spinacetin-glucuronide and jaceidin glucuronide each account for on average of 10-20% of the total flavonoids. Even if the interaction between storage and cutting process was not significant, storage and processing alone significantly influenced total flavonoids content, which did not change in tissue of intact spinach upon storage while a significant increase was observed in fresh-cut leaf (Figure 4).

Table 1. LC/MS/MS characteristics of the main flavonoids present in the extracts of spinach.

Compound	Peak ID Nr.	Precursor ion [M-H] ⁻ (m/z)	Product ions (m/z)
patuletin-3-glucosyl- (1-6) [apiosyl(1-2)]-glucoside	1	787	331
spinacetin- 3-glucosyl- (1-6) [apiosyl(1-2)]-glucoside	2	801	655 345
spinacetin-3-(2''-feroylglucosyl)(1-6)[apiosyl(1-2)]-glucoside	3	977	669 345
spinacetin glucuronide	5	521	345 330
jaceidin glucuronide	6	535	359
5,3'-4'-trihydroxy-3-methoxy-6:7-methylenedioxi-flavone-4'-glucuronide	7	519	343 328
5,4'-dihydroxy-3,3'-dimethoxy-6:7-methylenedioxi-flavone-4'-glucuronide	8	533	357 342

Patuletin = 3,5,7,3',4'-pentahydroxy-6-methoxyflavone
 Spinacetin = 3,5,7,4'-trihydroxy-3,6,3'-trimethoxyflavone
 Jaceidin = 3,7,4'-trihydroxy-3,6,3'-trimethoxyflavone

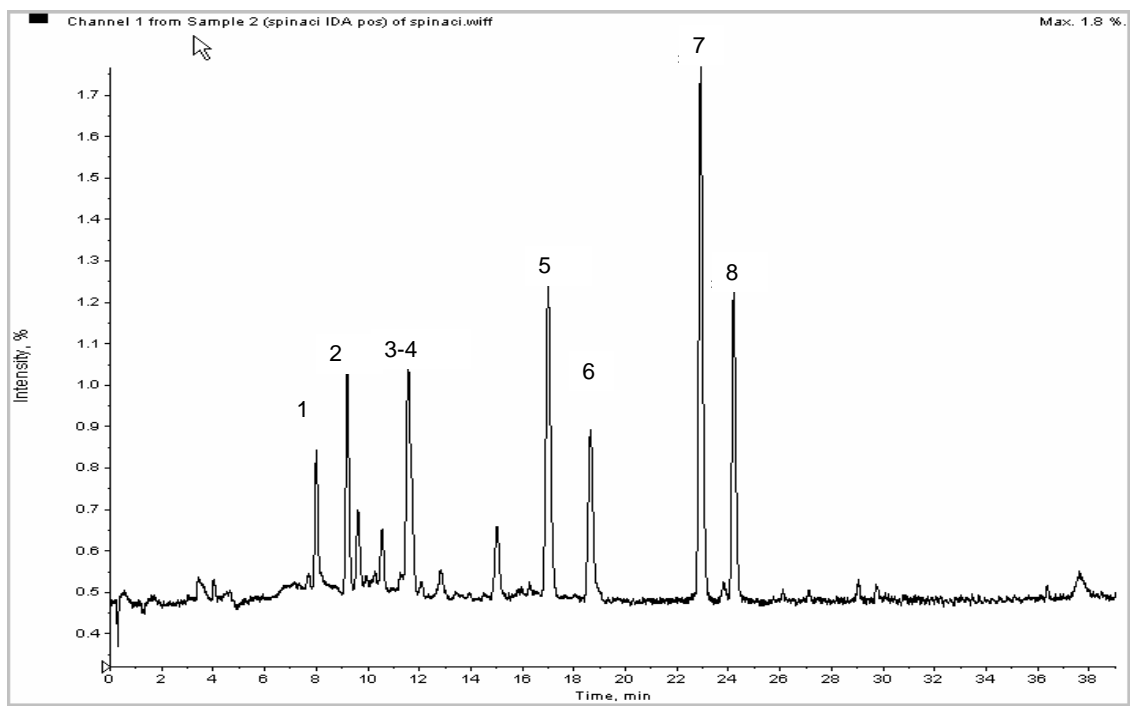


Figure 3 HPLC-chromatogram of *Spinacia oleracea* flavonoids. Peak identification was based on Ms-MS analysis reported in Table 1. 1: patuletin-3-glucosyl-(1-6)[apiosyl(1-2)]-glucoside; 2: spinacetin-3-glucosyl-(1-6) [apiosyl(1-2)]-glucoside; 3: spinacetin-3-(2''-feroylglucosyl)(1-6-)[apiosyl(1-2)]-glucoside; 5: spinacetin glucuronide; 6: jaceidin glucuronide; 7: 5,3'-4'-trihydroxy-3-methoxy-6:7-methylendioxyflavone-4'-glucuronide; 8: 5,4'-dihydroxy-3,3'-dimethoxy-6:7-methylendioxyflavone-4'-glucuronide.

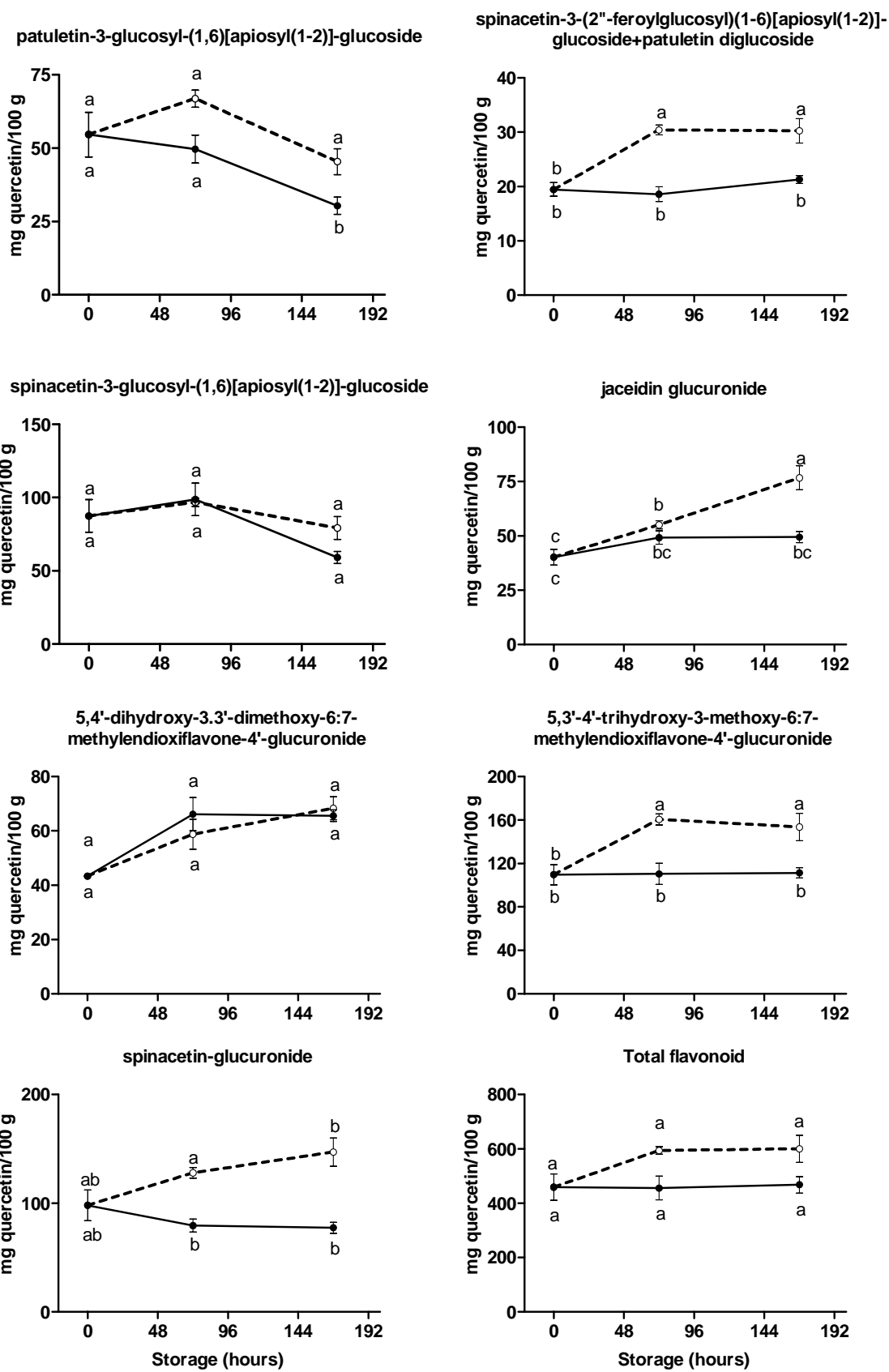


Figure 4 Flavonoid content expressed as mg quercetin/100 g fresh weight in intact (closed circle) and fresh-cut (open circle) tissues of *Spinacia oleracea* during cold storage.

Vitamin C (ASA+DHA) decreased significantly and rapidly upon storage in intact and fresh-cut leaf spinach (Figure 5) and the lowest values was recorded in intact leaves at the end of storage (4.83 mg Vitamin C/100 g fresh weight) (Figure 5A). No changes in DHA content were observed in intact spinach leaves upon storage while the behaviour was different in fresh-cut products. In these samples vitamin C decreased significantly upon storage, but the loss in AA was partially compensated by the increase in DHA values and the final values of vitamin C remained higher at the end of the storage as compared to intact leaves (Figure 5B).

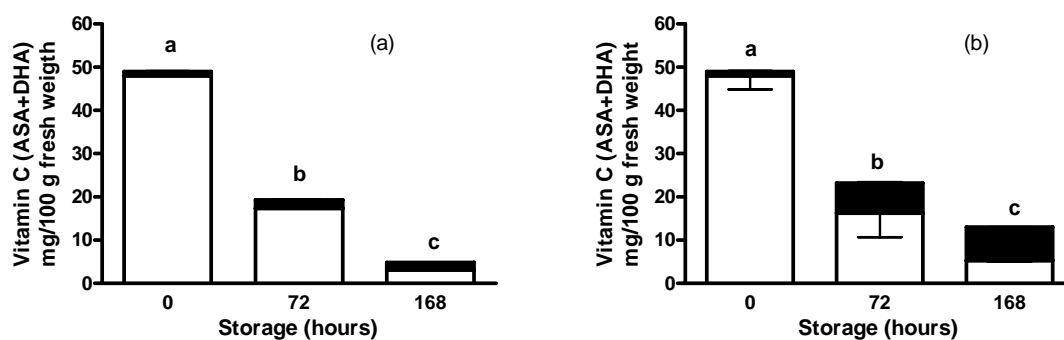


Figure 5. Ascorbic acid (white), dehydroascorbic (black) and Vitamin C (white plus black) content in intact (a) and fresh-cut (b) tissues of *Spinacia oleracea* during cold storage. The bars represent the mean of 3 replicates. Means of vitamin C content followed by the same letter are not significantly different according to LSD test ($P=0.05$).

Values of antioxidant capacity in intact and fresh-cut leaves of spinach are presented in Figure 6. In intact tissues antioxidant capacity did not change for the first 72 h of storage, but significantly decreased of 71% at the end of storage (Figure 6). In fresh-cut leaves the decrease in antioxidant capacity was recorded already after 72 h but at the end of storage the values was reduced of 54% respect to the initial values.

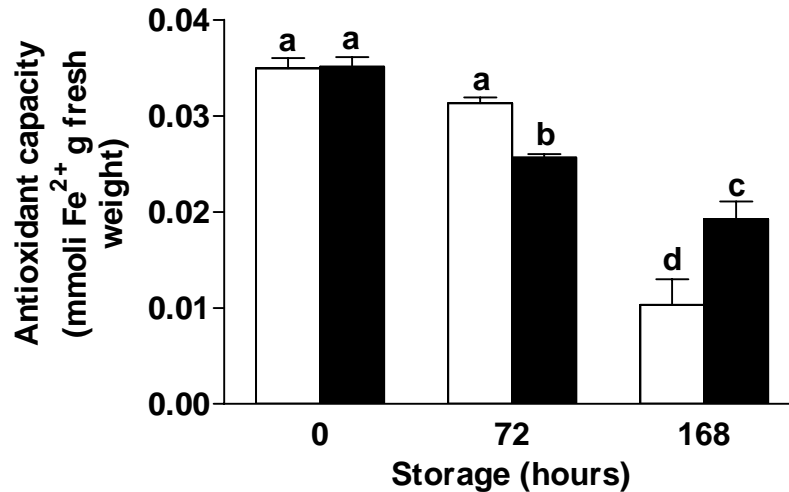


Figure 6 Antioxidant capacity determined by FRAP assay in intact (white) and fresh-cut (black) tissues of *Spinacia oleracea* during cold storage. The bars represent the mean of 3 replicates. Means of antioxidant capacity followed by the same letter are not significantly different according to LSD test ($P=0.05$).

No correlation between antioxidant capacity, phenols and total flavonoids content was found in intact and fresh-cut spinach, whereas significant correlation was observed between FRAP values and vitamin C (ASA+DHA) ($r=0.788$, $P<0.05$ and $r=0.911$, $P<0.01$, respectively in intact and cut spinach leaves) and ASA content ($r=0.868$, $P<0.01$ and $r=0.915$, $P<0.001$). Therefore the decrease of vitamin C and ASA in particularly, observed in intact and cut spinach tissues is the major responsible of the decrease in antioxidant capacity of these tissues. When we determined the correlation between specific flavonoids content and FRAP assay results, a significant correlation was found between patuletin-3-glucosyl-(1,6)[apiosyl(1-2)]-glucoside and antioxidant activity ($r=0.802$, $P<0.05$ and $r=0.899$, $P<0.01$, respectively in intact and fresh-cut spinach tissues).

4. Discussion and conclusions

At the end of one week (168 h) of cold storage as fresh-cut products, spinach did not show any symptoms of colour alteration that are instead typical of other leaf vegetables such as lettuce (Saltveit, 2000; Degl'Innocenti *et al.*, 2007). Colour alteration in fresh-cut fruits and vegetables is the direct consequence of PPO action on polyphenols (Ke & Saltveit,

1989; Tomas-Barberan *et al.*, 1997). Our data showed that in fresh-cut spinach tissues PPO increased significantly only after 168 h of storage. The enzyme PPO is typically located in chloroplast thylakoid membranes and one of its intriguing characteristics is the ability to exist in an inactive or latent status (Mayer & Harel, 1979) particularly in spinach leaves (Sanchez-Ferrer *et al.*, 1989). When latent PPO was treated by SDS its activity increased 2-fold at the various storage times both in intact and fresh-cut spinach tissues.

The fact that total PPO activity increased at the end of the storage in fresh-cut tissues indicated a *de novo* PPO synthesis upon storage due to initial wounding. The decompartmentalization of cellular components with subsequent release of proteases caused a cascade of events leading to the activation of latent PPO (Espin, *et al.*, 1999).

Although PPO activity increased significantly in fresh-cut tissues, they did not show any alteration in colour attributable to enzymatic browning and also the amount of phenol compounds, which are the substrates of PPO activity, did not change upon storage. This indicates that in spinach tissues wounding enhanced the activity of PPO, but its catalytic action did not induce an accumulation of quinones that can polymerize to form brown pigment. The reason for this behaviour of spinach could be related to the high endogenous ascorbic acid content. In fresh cut spinach tissues, as well as in intact leaves, ascorbic acid decreased upon storage. However, in fresh-cut tissues DHA significantly increased because of the conversion of ASA into DHA. As a consequence ASA+DHA content in fresh tissues decreased less than in intact leaves (-73 and -90% respectively in fresh-cut and intact tissues). It is well known that ASA reduced oxidized substrate molecules back to their original state, therefore impairing their polymerization to brown pigment (Arias, *et al.*, 2007). The availability of endogenous ASA in spinach tissues probably reduced a great part of PPO enzymatic reaction products so inhibiting enzymatic browning. In other words, ASA did not stop the enzymatic reaction itself, but it delays the subsequent polymerization events; in fact, no decrease in PPO activity was observed. This phenomenon was already

described in other leaf vegetables characterized by a high endogenous ASA content such as rocket salad (Degl'Innocenti *et al.*, 2007).

The role of ASA in preventing enzymatic browning is an important feature to consider spinach as a suitable species used for fresh-cut product. On the other hand, the decrease of vitamin C content led to a reduction of spinach nutritional values.

Few MS-MS data on spinach flavonoids are present in the literature. After the pivotal paper by Ferreres and co-workers (1997), where the main compounds were isolated and structures determined by NMR. Bergquist *et al.*, (2005) investigated by MS-MS baby spinach, having a flavonoid profile quite different from that here reported. Therefore the MS-MS data summarised in Table 1 represented a useful tool for the comparison of flavonoids profile of different spinach varieties.

Cold storage and cutting had some effects on flavonoids concentration and composition. Along storage total flavonoids content remained constant in intact leaves, while it increased in fresh-cut spinach. The increase was attributable to the increase of the concentration of some of individual flavonoids such as the spinacetin-glucuronide, jaceidin-glucuronide, the 5,4'-dihydroxy-3,3'-dimethoxy-6:7-methylenedioxyflavone-4'-glucuronide or the 5,3',4'-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4'-glucuronide in agreement with previous reports dealing with other species (Ferreres, *et al.*, 1997). The effect of cold storage is limited to some specific compounds: patuletin-3-glucosyl- (1-6) [apiosyl(1-2)]-glucoside and spinacetin-glucuronide decreased significantly also in intact spinach leaves whereas the other flavonoids remained rather stable or showed no response to storage as already reported in literature (Gil *et al.*, 1999). In particular Bergquist *et al.*, (2005) found that in baby spinach the 5,3',4'-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4'-glucuronide represents more than 40% of the total flavonoids and it was unaffected by 9 days of storage at 10°C. In this respect, our data confirm this

behavior in intact leaves and they also show for the first time an increase of about 50% of the amount of this compound upon storage of fresh-cut material.

The marked antioxidant capacity of spinach is due to the high amount of ascorbic acid and to the phenolic compounds particularly to the flavonoid moiety. The high antioxidant activity of flavonoids is due to the position and the degree of hydroxylation especially to the orthodihydroxylation moiety of the B ring, to the carbonyl in position 4 and to the free hydroxyl group in position 3 and/or 5 in the C and A rings, respectively (Rice-Evans & Miller, 1998). In accordance to the structure-activity relationship reported by Rice-Evans & Miller (1998) the 5,3',4'-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4'-glucuronide and patuletin-3-glucosyl-(1,6)[apiosyl(1-2)]-glucoside would exhibit a high antioxidant activity. When the correlation between antioxidant activity and the content of single flavonoids was investigated, a significant correlation was found between patuletin-3-glucosyl-(1,6)[apiosyl(1-2)]-glucoside and FRAP assay results both in intact and fresh-cut spinach leaves. Instead, no correlation was found between antioxidant capacity and the 5,3',4'-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4'-glucuronide.

Antioxidant activity was linearly correlated also with ascorbic acid content both in intact and fresh-cut spinach tissues. On the other hand, cold storage determined a decrease in ascorbic content as already reported in literature for spinach (Kevers, *et al.*, 2007).

Actually the literature reports contrasting evidence about the relationship between phenols and antioxidant activity; some authors have observed a positive relationship (Howard, *et al.*, 2003), while others reported a weak one (Imeh & Khokhar, 2002). The lack of correlation between phenols and antioxidant activity in this work could be due to the assay used (FRAP give a measure of reducing activity) as well as to the lack of specificity of the Folin–Ciocalteu phenol reagent that was used in the determination of total phenolic compounds, as described earlier.

In conclusion, spinach represents a very suitable leaf vegetable for the use as fresh-cut products. It does not show the phenomenon of enzymatic browning probably for its high endogenous content of ascorbic acid. Besides the good visual appearance of this leaf vegetable, spinach is rich of bioactive constituents such as vitamin C and flavonoids. Vitamin C changed at a lower extent in fresh-cut spinach as compared to the intact one even if the antioxidant capacity decreased significantly both in fresh-cut and intact leaf probably because of the cold storage. On the other hand, the most important flavonoids did not change upon storage in intact leaves and some of them increased significantly during storage in the fresh-cut samples.

CHAPTER V

Conclusions

In the present PhD Thesis was observed the course of green leafy vegetables in postharvest grown upon different nutrient solutions using floating system.

In *friariello campano*, the sulphur fertigation affected positively the antioxidant capacity and the light storage maintained high the quality parameters of fresh-cut product.

In *rocket*, the salt stress caused a reduction in the quality parameters, on the contrary an improvement was observed on proline applications. The light preserved very well the quality of rocket in postharvest.

On the contrary, in *basil*, an increment of the antioxidant capacity and in antioxidant content was observed upon salt treatments. The time storage had a negatively impact on nutritional status of fresh cut product.

Finally, the *spinach* does not show the phenomenon of enzymatic browning probably for its high endogenous content of ascorbic acid and then it preserves well as IV range product.

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