



Qualitative and physiological response of minimally processed garden cress (*Lepidium sativum* L.) to harvest handling and storage conditions

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Abstract

Garden cress (*Lepidium sativum* L.) has become a new leafy vegetable for the fresh-cut sector. The objective of this work was to study the effects of harvest handling practices (packaged immediately after harvest, S_0 ; packaged after 1 h of storage at 28°C, S_1) and storage conditions (light; dark) on the physiology and quality of minimally processed garden cress stored at 4°C for five days. Phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) activity, pigments, ascorbic acid (AA) and dehydroascorbic acid (DHA), total phenolics (TP) content, and antioxidant capacity (AC) were analyzed at pre-processing and at 1, 3 and 5 days after packaging. Fresh weight (FW) loss during shelf-life was measured daily. The S_1 harvest handling practice significantly influenced the pigments content which decreased over time, thus highlighting the importance of starting the cold chain as early as possible. PAL changed significantly over time, increased rapidly by 24.5% one day of shelf-life and then leveled off. At pre-processing, the PPO was higher in the S_1 samples than in the S_0 samples, indicating that the S_1 treatment could have induced PPO activity. PPO increased more over time under light than under dark conditions. POD was significantly influenced by both the harvest handling technique and the storage condition over time. POD was higher in the S_1 samples than in the S_0 samples (4.792 and $3.742 \pm 0.268 \Delta A_{\lambda} \text{ min}^{-1} \text{ g}^{-1} \text{ FW}$, respectively). It increased over time and reached a maximum value earlier under dark than under light conditions. The harvest handling technique significantly influenced the AA and DHA content over time. The S_1 treatment caused ca 13% loss in AA before packaging. AA decreased and DHA increased during shelf-life. The TP content was significantly influenced by the interaction between the harvest handling technique and storage condition over time. The greatest TP content increase was measured in the S_0 samples and in particular in samples stored under light conditions (33.5%). The AC significantly changed over time. Like TP, the AC increased from pre-processing to the first day of shelf-life, and then started to gradually decrease, reaching a reduction of 16.0% of the initial value after five days. The fresh weight loss was minimal (0.21%) at the end of shelf-life. An efficient and rapid harvest handling and storage implementation proved fundamental to reduce quality deterioration during shelf-life.

Key words: Fresh-cut convenience, shelf-life, antioxidant capacity, phenolic compounds, ascorbic acid, freshness, postharvest physiology, PAL, PPO, POD.

Introduction

Garden cress (*Lepidium sativum* L.) is an annual herb that belongs to the Brassicaceae family, which is rich in health promoting phytochemical constituents. Garden cress has recently become a new leafy vegetable for the fresh-cut sector, due to the interest of consumers, producers and processors¹. Therefore, it is necessary to understand the effect of pre-harvest and postharvest factors on the physiological and qualitative changes of garden cress processed for fresh-cut convenience in order to develop technologies that maintain quality and prolong shelf-life. During minimal processing, fruits and vegetables are treated in a series of stages, in which their structure and tissues are generally damaged or removed. Consequently, they have a short shelf-life and may be subjected to tissue browning which occurs during postharvest processing (handling, cutting, packaging, etc.) and subsequent storage². Tissue browning in leafy vegetables is a common and serious disorder, which is easily detected by consumers. Phenylpropanoid metabolism is considered the current biochemical model for plant tissue browning³. Phenylalanine ammonia lyase (PAL, EC 4.3.1.5), the enzyme involved in phenolic biosynthesis, produces phenols which can be oxidized, in the presence of

oxygen, by polyphenol oxidase (PPO, EC 1.14.18.1) and peroxidase (POD, EC 1.11.1.7) to quinones that spontaneously polymerize into browning pigments^{3,4}. PAL has been found to be low in non-stressed plant tissues, while it increases several fold in the presence of stress, such as wounding⁵. Thus, PAL activity could be an index of shelf-life and quality, especially of color and texture in minimally processed vegetables⁶. PPO and POD are very relevant in the subsequent oxidative degradation of phenolic compounds in terms of the production of brown polymers. At present, it is commonly accepted that PPO catalyses two different reactions in the presence of molecular oxygen: the hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinone⁷. The *o*-quinones non-enzymatically polymerise and give rise to heterogeneous black, brown pigments, commonly called melanins^{8,9}. POD is almost ubiquitous in plants and can oxidize both mono- and diphenols in the presence of small amounts of hydrogen peroxide¹⁰. POD involvement in slow processes such as browning could be possible although its role in the enzymatic browning of fruits and vegetables is still under discussion. It has been suggested that PPO could be the promoter of POD activity

because hydrogen peroxide is generated during the oxidation of phenolic compounds in the PPO-catalyzed reaction^{11,12}. Recent results have shown that total phenolic compounds, which are known to be one of the most important groups of natural antioxidants that accumulate in minimally processed vegetables tissues, are responsible for a high antioxidant capacity^{13,14}. The increase in antioxidant capacity has been linearly correlated to the increase in phenolic compounds in wounded iceberg and Romaine lettuce (*Lactuca sativa* L.) tissues¹⁵.

Ascorbic acid (AA) is another important antioxidant compound considered to directly or indirectly sequester harmful free radicals and inhibit tissue browning. Degl'Innocenti *et al.*³ suggested the possible involvement of the AA metabolism in tissue browning in two lettuce cultivars. The same authors¹⁶ speculated that the main role of AA in fresh-cut rocket (*Eruca sativa* Mill.) is devoted to controlling the radical species formed after cutting and it is also involved in the protection of fresh-cut rocket against enzymatic browning. Reyes *et al.*¹⁷ hypothesized that tissues with high levels of reduced ascorbic acid are prepared to control reactive oxygen species (ROS), thus the phenolics being synthesized are used for other purposes, such as lignin or suberin formation. In tissues with low levels of reduced ascorbic acid, the AA is instead consumed readily and phenolics are possibly synthesized to partly control ROS.

For many years, a great deal of research has been focused on the effects of processing, storage temperature, and modified atmosphere packaging on fresh-cut product quality, physiology and shelf-life¹⁸⁻²⁰. However, very few studies have been carried out on the influence of harvest handling on fresh-cut product behavior. The quality and safety of fresh-cut produce depend not only on cultural techniques and postharvest conditioning, but also on harvesting and handling procedures. Factors that can affect the physiology and quality of produce include the temperature and the air conditions in which vegetables are stored after harvesting²¹. Harvesting and handling methods can determine the extent of maturity and physical injury variability, and consequently influence the nutritional composition of vegetables. Often disregarded phases of the food chain, the harvesting and handling steps should be optimized and the cool chain should be introduced as early as possible to maintain product quality²². However, the refrigeration techniques in the field are not always implemented. In Italy, the cold chain is discontinuous, the farms are not equipped for raw material conditioning in the field, thus there are problems concerning the quality decay and the logistic distribution between growers and processors²³. Fresh produce consumes photosynthates that were stored in the product before harvest. The consumption rate depends on the respiratory activity of a particular commodity and its temperature. A correlation has been found between the respiration rate and shelf-life²⁴. Delays between harvesting and cooling or processing can result in direct losses due to water loss and microbial contamination, and indirect losses, such as flavor and nutritional quality loss²². Pre-processing storage conditions are fundamental to preserve raw material quality; an optimal vegetable storage temperature should be implemented to avoid chilling injury or vegetable thermal shock. Quality decay due to the cooling delays is particularly highlighted in commodities that lose water quickly and show visible symptoms at low levels of water loss, like most leafy vegetables. In Italy, young and tender leafy vegetables, such

as garden cress, rocket, corn salad (*Valerianella olitoria* L.), are used for the fresh-cut sector and their commercial shelf-life reaches at the moment 7 days at most²³.

The influence of light on vegetables during shelf-life is essential for the display of vegetables on sale, although it has not been studied in depth^{25,26}. Minimally processed products respond to light storage conditions in a different way in different vegetables. Light during storage can sometimes prevent green commodity yellowing^{27,28}. Recently, Noichinda *et al.*²⁹ reported that light storage conditions could prevent Chinese kale (*Brassica oleracea* L. var. *alboglabra*) from losing ascorbic acid and increase the soluble sugar level, and that dark storage conditions delayed the leaves from turning yellow. Sanz *et al.*²⁶ also reported that exposure to light lowered the quality of both the green and white parts of chard (*Beta vulgaris* L. var. *vulgaris*), thus significantly reducing its shelf-life.

The objective of the present work was to study the effect of harvest handling practices and light storage conditions on postharvest garden cress quality and physiology.

Materials and Methods

Plant material: The research was conducted in 2007 at the Experimental Center, at the University of Turin, Carmagnola, TO, Italy (45°22' N; 7°65' E) in an automatically temperature controlled glasshouse (35°C/15°C; d/n). The experiment consisted of growing plants in 60-cell styrofoam trays (44 mm top and 25 mm lower diameter, respectively), continuously floating in a nutrient solution (10 litres per tray). The trays were filled with a specific peat-based horticultural medium ('Neuhaus Huminsubstrat N17'; Klasmann-Deilmann, Geeste-Groß Hesepe, Germany). The seeds were obtained from Hortus Sementi srl (Cesena, Italy). Sowing took place on 15 May 2007. The seeded trays were incubated for 2 d at 19°C in a germination chamber, then moved into a glasshouse and arranged according to a randomized complete block design with three replications. Thinning was performed after seedling emergence to reach a final plant density of 360 plants per tray (ca 2,350 plants/m²). During plant growth, the basic nutrient solution consisted of 6 mM N, 6 mM K, 2 mM P, 2 mM Mg and 2.5 mM Ca. Both the pH and the electrical conductivity (EC) of the solution were checked weekly and kept close to 6.0 and 2,000 µS cm⁻¹, respectively.

Harvesting took place on 4 June 2007, after 20 d of cultivation. Each replicate consisted of a full tray of 360 plants (ca 240 g of FW). At harvest, the raw material was processed as fresh-cut convenience and two harvest handling practices were compared. The cut aerial parts of the plants were packaged immediately at harvest (S₀) or 1 h after harvest (S₁). The 1 h of lag involved keeping the raw material for 1 hour at ambient temperature (28°C). Samples of 25 g were sealed in polypropylene film with oxygen permeability of 1,330 cm³m⁻²d⁻¹ (Alvapack s.r.l.; Bologna, Italy). The packaged samples were stored under two conditions (light vs dark conditions) in a refrigerator (Medika 600; Fiocchetti Cold Manufacturer, Luzzara, (RE), Italy) at 4°C for 5 days. The packaged samples, stored under light, were continuously exposed to a PAR light of 4.28 µmol m⁻² s⁻¹. The total number of treatments resulted in 2 harvest handling practices x 3 blocks = 6 at harvest and 2 harvest handling practices x 2 light conditions x 3 blocks = 12 during shelf-life.

Parameters related to the sensorial quality decay, such as the

chlorophyll and carotenoids content, PAL, PPO and POD activity, ascorbic acid content, antioxidant power, and the total phenolic content were analyzed both at pre-processing (0) and 1, 3 and 5 days after packaging. The packaged samples were weighed daily from harvest to the end of shelf-life to monitor the fresh weight loss.

Pigment content analysis: Frozen tissue (0.2 g) of each sample was ground in 80% acetone/water (v/v) (Panreac, Barcelona, Spain) and extracted overnight over 12 h at 4°C in the dark. The extract was filtered and the final volume was adjusted to 10 ml with the same acetone/water solution. The filtered solution was used for the spectrophotometric determination of chlorophyll a (*Chl a*), chlorophyll b (*Chl b*) and carotenoids at wavelengths of 662, 645 and 470 nm, respectively. The amount of these pigments was calculated according to the Lichtenthaler and Wellburn formulas³⁰ and expressed as milligrams per gram of fresh weight (mg g⁻¹ FW).

Enzyme activity analysis: PAL activity was measured, as described by Campos *et al.*³¹ and Degl'Innocenti *et al.*³ with some modifications. In brief, 0.5 g of frozen tissue of each sample was homogenized in a 4 ml 50 mM sodium phosphate buffer (PBS, pH 8.0) (Sigma-Aldrich GmbH, Steinheim, Germany) containing 0.01 g polyvinylpyrrolidone (Merck KGaA, Darmstadt, Germany) on ice. The homogenate was centrifuged at 20,000 × g at 4°C for 20 min. The resulting supernatant was collected as enzyme extract. PAL activity was carried out by mixing a 0.1 ml extract, 0.5 ml 50 mM L-phenylalanine (Sigma-Aldrich GmbH, Steinheim, Germany) and a 1.40 ml 50 mM PBS. After 1 h of incubation at 40°C, the absorbance was measured at 290 nm. The enzyme activity was expressed as μmol cinnamic acid (Sigma-Aldrich GmbH, Steinheim, Germany) per hour per gram of fresh weight (μmol cinnamic acid h⁻¹ g⁻¹ FW). PPO and POD activities were measured by homogenizing 0.5 g of tissue from each sample in 4 ml of 50 mM PBS (pH 7.0) on ice. The homogenate was centrifuged at 20,000 × g at 4°C for 20 min and the supernatant was used as the crude enzyme source. PPO activity was determined according to the Degl'Innocenti *et al.*³ method with a slight modification. A 0.1 ml volume of enzyme extract was incubated in PBS containing 2.5 mM of pyrocatechol (Sigma-Aldrich GmbH, Steinheim, Germany) at 25°C for 30 min before absorbance was recorded at 480 nm. One unit of PPO activity was defined as the amount of enzyme that causes a change of 0.01 in absorbance per minute and it was expressed as Unit per gram of fresh weight (Unit g⁻¹ FW). POD activity was measured according to the Nickel and Cunningham procedures³² with a slight modification. Activity was measured as an increase in absorbance at 470 nm due to guaiacol oxidation. The reaction mixture contained 25 mM PBS (pH 7.0), 0.05% guaiacol (Sigma-Aldrich Inc., St Louis, MO, USA), 10 mM H₂O₂ (Sigma-Aldrich GmbH, Seelze, Germany) and enzyme. The increase in absorbance at 470 nm was recorded and POD activity was expressed as ΔA_λ per min per gram of fresh weight (ΔA_λ min⁻¹ g⁻¹ FW).

Ascorbic acid and dehydroascorbic acid content analysis: AA and dehydroascorbic acid (DHA) were determined spectrophotometrically according to the Kampfenkel *et al.* methodology³³. One gram of fresh material was ground in 6% of freezing trichloroacetic acid/water (w/v) (Sigma-Aldrich GmbH, Steinheim, Germany) on ice. The homogenate was centrifuged at

15,000 × g at 4°C for 10 min and the supernatant was immediately used for the AA and DHA analyses. The results were expressed as milligram per 100 g of fresh weight (mg 100 g⁻¹ FW) based on the calibrations that were compared with standard curves produced by freshly prepared L-ascorbic acid (Sigma-Aldrich Inc, St Louis, MO, USA) (10-250 mg l⁻¹) and dehydroascorbic acid (Sigma-Aldrich GmbH, Steinheim, Germany) (1-100 mg l⁻¹). The DHA content was computed from the difference between the total AA and AA.

Total phenolic content and antioxidant capacity analysis: One gram of frozen tissue was homogenized, for each sample, with pure methanol (assay ≥ 99.9% (GC), Fluka GmbH, Buchs, Switzerland) and the homogenates were centrifuged at 15,000 × g at 4°C for 20 min. The total phenolic content was measured using the Folin-Ciocalteu procedure³⁴ which involved mixing a 100 μl aliquot of the methanol extract with 500 μl of Folin-Ciocalteu reagent (Sigma-Aldrich GmbH, Buchs, Switzerland). After standing for 3 min, 400 μl of 7.5% sodium carbonate/water (w/v) (BDH Ltd, Poole, England, UK) were added and the contents of the tubes were thoroughly mixed before incubation at 20°C for 30 min. The absorbance at 760 nm was read and the result was expressed as milligram gallic acid (Sigma-Aldrich Inc, St Louis, MO, USA) per gram of fresh weight (mg gallic acid g⁻¹ FW).

The ferric-reducing antioxidant power (FRAP) assay was based on the Benzie and Strain method.³⁵ The fresh FRAP reagent was prepared by mixing 25 ml of 300 mM sodium acetate (Sigma-Aldrich Inc., St Louis, MO, USA) -acetic acid (Fluka Chemie, Buchs, Switzerland) buffer (pH 3.6), 2.5 ml of 20 mM FeCl₃ (Sigma-Aldrich GmbH, Buchs, Switzerland) and 2.5 ml of 10 mM TPTZ (2, 4, 6-tripyridyl-s-tiazine) (Sigma-Aldrich, Buchs, Switzerland). For this analysis, a 20 μl aliquot of methanol extract, prepared as described above, was mixed with 980 μl of the FRAP reagent. The mixture was kept at 20°C for 4 min before absorbance was determined at 593 nm. Antioxidant capacity, expressed as micromole ferrous ion per gram of fresh weight (μmol Fe²⁺ g⁻¹FW), was calculated compared with the standard curve produced by the fresh ammonium ferrous sulfate (Aldrich Inc., St Louis, MO, USA) (100-1000 μM).

All the spectrophotometric analyses were conducted using a Beckman DU®-65 spectrophotometer (Beckman Instruments, Inc.-Fullerton, CA, USA).

Statistical analysis: All the data concerning pigments, enzymatic activity, ascorbic acid, antioxidant power capacity and total phenolics content were submitted to the analysis of variance with repeated measurements³⁶ using a Mixed Model to analyze the trends over time using the Statistical Package for Social Science (SPSS Version 13.0, SPSS Inc., Chicago, IL, USA). The fresh weight loss data were submitted to the analysis of variance with repeated measurements using the Greenhouse-Geisser F-adjustments³⁷⁻⁴⁰.

Results and Discussion

Pigment content: The harvest handling significantly influenced the content of the overall total chlorophyll and carotenoids and, over time, of *Chl a*, *Chl b*, total chlorophyll and carotenoid (Table 1). The storage condition, its interaction with harvest handling and over time had no significant effect on these pigments.

The pigment content decreased from pre-processing time

Table 1. Probability values obtained from the analysis of variance on treatment and over time bases for chlorophyll a (*Chl a*), chlorophyll b (*Chl b*), total chlorophyll and carotenoids content in minimally processed garden cress from harvest to 5 days of shelf-life at 4°C.

Source of variation	<i>Chl a</i>	<i>Chl b</i>	Total chlorophyll	Carotenoids
Harvest handling	0.068	0.103	0.016	0.008
Storage condition	0.750	0.924	0.752	0.844
Harvest handling x Storage condition	0.975	0.936	0.970	0.913
Block	0.963	0.885	0.903	0.822
Analysis over time				
Time	<0.001	<0.001	<0.001	<0.001
Harvest handling x Time	<0.001	<0.001	<0.001	<0.001
Storage condition x Time	0.781	0.837	0.782	0.804
Harvest handling x Storage condition x Time	0.743	0.959	0.797	0.942

(day 0) to the end of shelf-life (Day 5) for all significant treatments (Table 2). The *Chl a*, *Chl b*, total chlorophyll and carotenoids content decreased by 18.5 and 45.5%, 7.9 and 49.0%, 16.0 and 46.5% and by 11.3 and 44.8%, respectively, in the S_0 and S_1 samples. The *Chl a* content decreased more than *Chl b* in the S_0 samples, while a slightly opposite trend was found in the S_1 samples. In the S_0 samples, the pigment deterioration reached a peak after one day of shelf-life, then it slowed down, while in the S_1 samples, the degradation was gradual over time, although of greater magnitude. Field temperature affects turgidity and influences the susceptibility of leafy vegetable crops to physical damage during harvest and handling⁴¹. It could be hypothesized that the greater pigment degradation in the S_1 samples was related to a quality deterioration that occurred during the 1 hour pre-processing storage at 28°C. The high air temperature increased the field temperature, canopy transpiration and respiration rates and turgor loss, all factors that are more responsible for quality loss, than packaging the raw material at harvest (S_0 samples). Turgor loss could also explain the greater pigment content in the S_1 samples than the S_0 samples, which was probably due to a concentration effect.

It has been established that postharvest chlorophyll degradation is an indicator of green leafy vegetables quality loss, while there is a lack of supporting data to indicate a trend of chlorophyll degradation from harvesting and onward and which early storage conditions can accelerate this physiological process, thus quantifying the detrimental effect. The greater decrease that occurred in the S_1 garden cress samples than in the S_0 samples during the five days of shelf-life could clarify our understanding regarding the effective loss of quality that occurs in the very early stages after harvest, highlighting the importance of starting the cold chain as soon as possible.

Table 2. Effect of harvest handling over time on chlorophyll a (*Chl a*), chlorophyll b (*Chl b*), total chlorophyll and carotenoids content in minimally processed garden cress from harvest to 5 days of shelf-life at 4°C.

Harvest handling	Days	<i>Chl a</i> (mg g ⁻¹ FW)	<i>Chl b</i> (mg g ⁻¹ FW)	Total chlorophyll (mg g ⁻¹ FW)	Carotenoids (mg g ⁻¹ FW)
S_0	0	0.721±0.019	0.229±0.015	0.950±0.006	0.248±0.002
	1	0.509±0.033	0.187±0.030	0.696±0.045	0.175±0.013
	3	0.613±0.033	0.229±0.019	0.842±0.046	0.234±0.014
	5	0.587±0.026	0.211±0.011	0.798±0.037	0.220±0.009
S_1	0	0.777±0.013	0.304±0.008	1.081±0.019	0.288±0.004
	1	0.594±0.025	0.218±0.009	0.813±0.034	0.221±0.009
	3	0.557±0.061	0.202±0.029	0.759±0.086	0.211±0.025
	5	0.423±0.009	0.155±0.006	0.578±0.013	0.159±0.015

Each value is the mean of three replicates ±SE. S_0 = processed and packaged at harvest; S_1 = processed and packaged after 1 h of storage at ambient temperature (28°C).

Enzyme activities: The tested factors and their interaction had no significant effect on the overall PAL activity, which significantly changed over time (Table 3). PAL activity rapidly increased by 24.5% after one day of shelf-life regardless of the treatments, and then leveled off, reaching 0.187 μmol of cinnamic acid h⁻¹ g⁻¹ FW at the end of shelf-life (Table 4). PAL activity depends on the species and its phenolic compound content, cutting intensity and storage temperature^{42,43}. Tavarini *et al.*¹⁴ noted that PAL activity can be cultivar dependent. The authors used two lettuce cultivars (*Lactuca sativa* L. var. *capitata* cv. Verpia and *Lactuca sativa* L. var. *acephala* cv. Lollo Rossa) provided by a local supermarket; after processing as fresh-cut product, the lettuces were stored at 4°C in the dark. It was found that the PAL activity increase in cv. Verpia reached a maximum level after 1 day of storage and then decreased marginally until the end of shelf-life, while the PAL activity in cv. Lollo Rossa decreased upon storage and reached the minimum level after two days. Degl'Innocenti *et al.*¹⁶ processed and stored different vegetables, obtained from a local market, in a 1.5-liter plastic box at 4°C for 72 hours. The authors found that PAL activity in lettuce (*Lactuca sativa* L. var. *capitata*) significantly increased in the first hours after cutting with a peak observed after 6 hours; PAL activity in escarole (*Cichorium endivia* var. *latifolium*), did not change in the first 48 h of storage, but it then increased significantly and reached the highest value after 72 h; PAL activity in rocket reached a maximum value 12 h after cutting. It can be concluded that, regardless of the species variability, PAL is an early start enzyme after stress and that it then rapidly increases.

Wounds occurring during fresh-cut processing induce synthesis of the PAL protein that increases PAL activity and an accumulation of the phenolic compounds that are responsible for tissue

Table 3. Probability values obtained from the analysis of variance on treatment and over time bases for PAL, PPO and POD activity in minimally processed garden cress from harvest to 5 days of shelf-life at 4°C.

Source of variation	PAL activity	PPO activity	POD activity
Harvest handling	0.767	0.813	0.030
Storage condition	0.965	0.673	0.126
Harvest handling x Storage condition	0.957	0.808	0.912
Block	0.816	1.000	0.831
Analysis over time			
Time	0.004	<0.001	<0.001
Harvest handling x Time	0.807	0.418	0.943
Storage condition x Time	0.530	0.142	0.019
Harvest handling x Storage condition x Time	0.777	0.043	0.055

Table 4. Effect of the time on PAL enzyme activity in minimally processed garden cress from harvest to 5 days of shelf-life at 4°C.

Days	PAL activity ($\mu\text{mol cinnamic acid h}^{-1} \text{g}^{-1} \text{FW}$)
0	0.155±0.006
1	0.193±0.008
3	0.192±0.005
5	0.187±0.005

Each value is the mean of three replicates ±SE.

discoloration⁴⁴. An important and linear correlation was found between PAL activity and phenolic content in excised iceberg lettuce mid-rib tissues during the first 36 hours of shelf-life at 5°C. In our study, harvest cutting was the only wounding that occurred and it could have limited PAL activity, which increased during shelf-life and thus kept values low. These values in fact ranged from 0.155 to 0.187 $\mu\text{mol of cinnamic acid h}^{-1} \text{g}^{-1} \text{FW}$, whilst in literature PAL values range from 0.2 to 0.6 $\mu\text{mol of cinnamic acid h}^{-1} \text{g}^{-1} \text{FW}$ in rocket,¹⁶ or in lettuce^{14,16}. This could also be related to the low concentration of phenolic compounds that was found (see over).

As shown in Table 3, PPO activity was significantly affected by the interaction between harvest handling and storage condition over time. At harvest, the PPO activity value was higher in the S₁ samples than in the S₀ samples, indicating that leaving the harvested produce for 1 h at 28°C before packaging could have induced PPO activity (Table 5). The PPO activity increased during shelf-life and this increase occurred more under light than under dark conditions. At the end of shelf-life, the highest increase (49.9%) and the lowest increase (19.7%) were measured in the S₀ samples stored under light and dark conditions, respectively. The PPO increase measured in the S₁ samples was 29.7% under light and 25.7% under dark. Immediate packaging at harvest could have emphasized the light effect during shelf-life on PPO, while packaging 1 h after harvest could have shortened the gap between dark and light storage conditions in terms of PPO activity.

The commercial value of these results, should they be confirmed, could be of practical use, considering that at present the majority of fresh-cut produce is placed in illuminated display units; eliminating this practice could lead to a slowing down of the decay process.

The PPO activity reached a maximum value after 5 days of shelf-life under light, regardless of the harvest handling treatment, and after 1 day for S₀ and 3 days for S₁, respectively, under dark conditions (Table 5). The PPO activity was slower in reaching its

Table 5. Effect of interaction between harvest handling and storage condition over time on PPO enzyme activity in minimally processed garden cress from harvest to 5 days of shelf-life at 4°C.

Storage condition	Days	Harvest handling	
		S ₀	S ₁
PPO activity (unit g ⁻¹ FW)			
Light	0	5.867±0.533	6.578±0.641
	1	7.378±0.387	7.200±0.671
	3	7.556±0.694	7.289±0.641
	5	8.800±0.308	8.533±0.308
	0	5.867±0.533	6.578±0.641
Dark	1	8.089±0.470	6.933±0.407
	3	6.489±0.320	9.244±0.641
	5	7.022±0.235	8.267±0.671

Each value is the mean of three replicates ±SE. S₀= processed and packaged at harvest; S₁= processed and packaged after 1 h of storage at ambient temperature (28°C).

peak compared to PAL activity. Similar results were obtained by Degl'Innocenti *et al.*¹⁶ who found that PPO activity was quite low in lettuce and increased significantly only after 48 h, one day later than PAL activity.

The POD activity was significantly influenced by the overall harvest handling and the storage condition over time (Table 3). The overall POD activity was higher in the S₁ samples (4.792±0.268 $\Delta A_{\lambda} \text{ min}^{-1} \text{g}^{-1} \text{FW}$) than in the S₀ samples (3.742±0.268 $\Delta A_{\lambda} \text{ min}^{-1} \text{g}^{-1} \text{FW}$), suggesting that leaving the harvested raw material at ambient temperature for 1 h could have accelerated the metabolism that induces POD activity, in the same way as for PPO activity. The POD activity, like the PAL and PPO activities, increased over time and reached a maximum value earlier under dark than under light; however, the maximum increase values were similar for both light and dark conditions, 102 and 105%, respectively (Table 6). Like PPO, the POD activity was slower than the PAL activity in reaching its peak, which occurred after three days under dark and five days under light.

In minimally processed vegetables, particularly in leafy vegetables, handling, cutting, and temperature storage operations induce PAL activity, the first enzyme involved in phenolics synthesis, as well as PPO and POD, producing a brown-colored product due to oxidation phenolics in the presence of oxygen^{43,45}. However, in our experiment, no visible tissue browning was observed at the end of shelf-life, although these enzymes increased by different degrees during storage. Garcia and Barrett⁴⁶ reported that susceptibility to browning can differ from cultivar to cultivar. Garden cress is not processed like lettuce, celery (*Apium graveolens* L.) or others, in which the raw material is cut during processing. Garden cress is packaged and stored as whole canopy,

Table 6. Effect of storage condition over time on POD enzyme activity in minimally processed garden cress from harvest to 5 days of shelf-life at 4°C.

Storage condition	Days	POD activity ($\Delta A_{410} \text{ min}^{-1} \text{ g}^{-1} \text{ FW}$)
Light	0	2.819±0.363
	1	3.323±0.270
	3	5.016±0.396
	5	5.705±0.286
Dark	0	2.819±0.363
	1	4.316±0.244
	3	5.787±0.770
	5	4.351±0.361

Each value is the mean of three replicates ±SE.

like rocket, corn salad and others, in which the only wounding that can occur at the canopy level is at harvesting. Thus, the reduced cut surface could limit the risk of tissue browning.

Ascorbic acid and dehydroascorbic acid content: The harvest handling method significantly influenced the AA and DHA content over time (Table 7). At harvesting, the AA content was higher in the S_0 samples than in the S_1 samples, however it decreased over time in the former and was stable in the latter (Table 8). Keeping the harvested raw material for 1 h at 28°C before packaging caused a loss of about 13% in AA. Lee and Kader⁴⁷ have reported many examples in which the delays between harvest and processing, where the vegetables are left at high temperatures, accelerate AA losses. The highest AA decrease (23.8%) was measured in the S_0 samples after five days of shelf-life, probably due to the DHA increase, which was greater in the S_0 samples than in the S_1 samples. AA is converted to DHA through a non-enzymatic spontaneous reaction. The reaction in the opposite direction, which would regenerate AA, is not thermodynamically the most favored, therefore AA content tends to decrease during shelf-life¹⁶.

The DHA content increased over time, even though the content was higher in the S_1 samples than in the S_0 samples at harvest. The DHA increase over time was greater in the S_0 samples (Table 8). Apart from the treatment, the highest increase was measured after one day of shelf-life, then the DHA started to decrease again but with a high degree of variability. The DHA results confirmed literature data according to which DHA tends to increase in fresh-cut vegetables during storage. Since we used permeable films, the oxygen presence in the packages could have favored the ascorbate oxidase enzyme responsible for AA degradation and DHA increase. Barry-Ryan and O'Beirne⁴⁸

Table 7. Probability values obtained from the analysis of variance on treatment and over time bases for AA (ascorbic acid), DHA (dehydroascorbic acid), total phenolic compounds and antioxidant capacity in minimally processed garden cress from harvest to 5 days of shelf-life at 4°C.

Source of variation	AA	DHA	Total phenolics	Antioxidant capacity
Harvest handling	0.264	0.066	0.879	0.889
Storage condition	0.212	0.523	0.893	0.935
Harvest handling x Storage condition	0.930	0.338	0.521	0.859
Block	0.253	1.000	0.317	0.667
Analysis over time				
Time	0.001	0.049	0.600	<0.001
Harvest handling x Time	<0.001	0.012	0.086	0.094
Storage condition x Time	0.955	0.726	0.950	0.362
Harvest handling x Storage condition x Time	0.402	0.822	0.025	0.285

Table 8. Effect of harvest handling over time on AA (ascorbic acid), DHA (dehydroascorbic acid) in minimally processed garden cress from harvest to 5 days of shelf-life at 4°C.

Harvest handling	Days	AA ($\text{mg } 100 \text{ g}^{-1} \text{ FW}$)	DHA ($\text{mg } 100 \text{ g}^{-1} \text{ FW}$)
S_0	0	13.861±0.418	13.100±0.573
	1	11.623±0.655	29.361±0.911
	3	11.124±0.326	25.623±2.405
	5	10.560±0.341	26.608±1.547
S_1	0	12.058±0.433	21.462±0.466
	1	12.702±0.482	31.994±2.703
	3	12.299±0.583	28.115±1.741
	5	12.476±0.503	23.231±1.944

Each value is the mean of three replicates ±SE. S_0 = processed and packaged at harvest; S_1 = processed and packaged after 1 h of storage at ambient temperature (28°C).

compared three packaging methods (nitrogen flushed before sealing; sealed in air and unsealed oriented polypropylene film) on iceberg lettuce stored at 3 and 8°C for 10 days. The authors found that large amount of AA was converted into DHA due to the increase in oxygen availability, which rose from nitrogen flushed packages to unsealed packages.

Total phenolics content and antioxidant capacity: The total phenolics content was significantly influenced by the interaction between the harvest handling and storage condition over time (Table 7). The total phenolics content increased until it reached a maximum value after one day of shelf-life, then it started to gradually decrease in the following days (Table 9). After five days, the total phenolics content was higher than at harvesting, except for the S_1 samples under light conditions which had a lower content. The greatest content increase was measured in the S_0 samples and, in particular, in samples under light conditions (+33.5%). The total phenolics content is prone to increase during storage, although in literature the extent of the absolute values varies, according to the considered species, produce, raw material supply, processing, storage and extraction methods, therefore a comparison with other results cannot be made.

The antioxidant capacity was not significantly affected by either the harvest handling, or storage conditions, or by their interactions, while it significantly changed over time (Table 7). The antioxidant capacity increased from harvest to one day of shelf-life, and then it started to gradually decrease. After five days, the antioxidant capacity was reduced by 16.0% of the initial value (Table 10). For lettuce, zucchini (*Cucurbita pepo* L.), cabbage (*Brassica oleracea* L.), radish (*Raphanus sativus* L.), celery and carrots (*Daucus*

Table 9. Effect of interaction between harvest handling and storage condition over time on total phenolic compounds in minimally processed garden cress from harvest to 5 days of shelf-life at 4°C.

Storage condition	Days	Harvest handling	
		S ₀	S ₁
Total phenolics (mg gallic acid g ⁻¹ FW)			
Light	0	0.179±0.015	0.187±0.005
	1	0.236±0.003	0.234±0.023
	3	0.208±0.011	0.188±0.017
	5	0.239±0.006	0.177±0.003
Dark	0	0.179±0.015	0.187±0.005
	1	0.249±0.016	0.221±0.024
	3	0.211±0.011	0.217±0.015
	5	0.201±0.004	0.204±0.004

Each value is the mean of three replicates ±SE. S₀= processed and packaged at harvest; S₁= processed and packaged after 1 h of storage at ambient temperature (28°C).

carota L.), it has been reported that the increase of the antioxidant capacity is linearly correlated to the increase of the phenolics content^{14, 15, 17}. The antioxidant capacity of vegetables is known to depend on a wide number of compounds. Wounding can increase the antioxidant capacity; this increase has been correlated to the measured phenolics content, suggesting that phenolic compounds are the main contributors to the change in antioxidant activity^{15, 17, 49, 50}. In our experiment, the limited wounding and the low content of phenolics content could explain the reduced antioxidant capacity.

Fresh weight loss: The harvest handling, storage conditions and their interaction did not influence the fresh weight loss (data not shown), which significantly decreased in time only ($P = 0.003$). After 5 days of shelf-life, the fresh weight decreased by 0.21% and this loss was considered minimal, highlighting that the shelf-

Table 10. Effect of time on antioxidant power capacity in minimally processed garden cress from harvest to 5 days of shelf-life at 4°C.

Days	Antioxidant Capacity (μmol Fe ²⁺ g ⁻¹ FW)
0	4.130±0.129
1	4.449±0.079
3	3.641±0.124
5	3.469±0.092

life of garden cress could be extended some more days. The results are in line with other studies on garden cress shelf-life¹, in which it has been reported that the fresh weight loss was < 1.5% after 10 days of shelf-life at 4°C under dark conditions.

Conclusions

The investigation on the qualitative and physiological response of minimally processed garden cress to harvest handling practices and storage conditions has shown that garden cress still satisfied inherent, visual and commercial quality parameters after five days.

Leaving the harvested raw material at 28°C for 1 hour (S₁ treatment) before packaging increased the deterioration of the pigments and AA contents, which resulted to be very sensitive to the harvest handling conditions. The reduced wounding of

garden cress limited the enzymatic activity of PAL, PPO and POD, even though it increased over time. Moreover, for PPO activity, the harvest handling had a greater effect than the storage conditions, which has proved to be fundamental for POD activity. An efficient and rapid harvest handling and storage implementation after the cultivation phase are fundamental factors that favor the quality of the raw material, thus improving the processing and reducing the quality deterioration during shelf-life.

Exposure to light during shelf-life does not seem to be beneficial to minimally processed garden cress. Even though it is difficult to avoid light during the commercial distribution process, further studies are essential to investigate the influence of light conditions on minimally processed vegetables in order to develop proper protective techniques.

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