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# Bioactive Compounds during Storage of Fresh-Cut Spinach: The Role of Endogenous Ascorbic Acid in the Improvement of Product Quality

ANTONELLA BOTTINO,[†] ELENA DEGL'INNOCENTI,[‡] LUCIA GUIDI,*[,‡] GIULIA GRAZIANI,[§] AND VINCENZO FOGLIANO[§]

Dipartimento di Ingegneria Agraria e Agronomia del Territorio, University of Napoli "Federico II", 80055 Portici (NA), Italy, Dipartimento di Chimica e Biotecnologie Agrarie, University of Pisa, 56124 Pisa, Italy, and Dipartimento di Scienza degli Alimenti, University of Napoli "Federico II", 80055 Portici (NA), Italy

Spinach is rich in bioactive constituents such as vitamin C, flavonoids and phenolic acids. In this work, the biochemical modifications occurring during one week of storage at 4 °C were evaluated both in intact and in fresh-cut spinach. Results showed that vitamin C concentration is less affected by storage in fresh-cut spinach with respect to intact spinach. MS/MS analysis showed that the main flavonoids are not modified during storage in intact leaves, while some of them increased significantly during storage in the fresh-cut samples. Fresh-cut spinach did not show color alteration even if PPO activity increased significantly during storage. This finding was related to the high ascorbic acid content, which delays the subsequent polymerization events. This finding was confirmed by the unaltered concentration of phenolic compounds in fresh-cut spinach during storage. In conclusion, data about nutritional content and visual performance concurrently suggest that spinach is a suitable species for marketing as a fresh-cut product.

**KEYWORDS: Antioxidant capacity; flavonoid; fresh-cut; polyphenol oxidase; *Spinacia oleracea*; vitamin C**

## INTRODUCTION

It is well-known that fruits and vegetables play a key role against numerous chronic diseases, as cancer, cardio- and cerebrovascular and neurological diseases (*1*). This protective effect has generally been attributed to different antioxidant constituents, such as vitamins C and E, carotenoids, flavonoids and phenolic acids.

This evidence prompted health bodies to suggest an increase of the dietary intake of fruits and vegetables. However, nowadays many people do not want to spend much time preparing vegetable foods every day, and so they frequently use fresh-cut vegetables, which are horticultural products subjected to minimal processing which was described as "cleaning, washing, cutting and packaging". The key criteria to consider a vegetable or fruit as a fresh-cut product include that the tissue is in a living, respiring physiological state (*2*). Fresh-cut products are subject to rapid deterioration and short shelf life as compared to intact products. 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 (*3*). An important alteration induced by cutting is the browning of the tissues that is a direct consequence of polyphenol oxidase (PPO) action on endogenous polyphenols in the presence of oxygen (*4, 5*). Different species are differently subjected to the enzymatic browning induced by cutting operations during fresh-cut vegetables preparation (*5, 6*).

In a previous work we found that the ascorbic acid (ASA) content is the main factor determining the protection against enzymatic browning (*5*). It is well-known that ASA is the key component of the commercial antibrowning formulations available on the market (*7*). It was hypothesized that ASA controls PPO activity through its ability to reduce quinones to the native diphenols (*8*) but ASA can inhibit PPO also by decreasing the pH of the cytosol (*9*).

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 (*10*). However, there is contrasting evidence in the literature due to the simultaneous accumulation of phenolic compounds induced by the cutting process through the activation of enzyme phenylalanine ammonia lyase (*11*). In a recent work (*12*) it is reported that the antioxidant capacity of some fruits and vegetables was positively influenced by

* Corresponding author. Phone: +39 050 2216613. Fax: +39 050 2216630. E-mail: guidilu@agr.unipi.it.

[†] Dipartimento di Ingegneria Agraria e Agronomia del Territorio, University of Napoli "Federico II".

[‡] University of Pisa.

[§] Dipartimento di Scienza degli Alimenti, University of Napoli "Federico II".

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cutting, the concentrations of reduced ascorbic acid and phenolic compounds being much higher in cut tissue.

Among different leafy vegetables, spinach is usually consumed after cooking, however fresh leaves can also be used in salad. This vegetable has good nutritional value, being a rich source of carotenoids ($\beta$-carotene and lutein), vitamin C, vitamin E, vitamin K, magnesium, and several antioxidants such as flavonoids. Twelve main flavonoids have been previously identified in spinach (*13*). Spinach is a rich source of glycosylated flavonoids: these are glucuronides and acylated di- and triglycosides of methylated and methylenedioxy derivatives of 6-oxygenated flavonols (*14, 15*). Gil et al. (*10*), in a previous report, found that flavonoid content of fresh-cut spinach was stable during storage, and this observation was confirmed during retail storage conditions (*13*).

Spinach showed another important characteristic when compared to other leafy vegetables used for fresh-cut salad as it does not show a significant enzymatic browning, which is the factor limiting shelf life and marketability of fresh-cut products. This characteristic was shared also with other species, such as rocket salad, which also contains high levels of vitamin C (*5*).

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

## MATERIAL AND METHODS

**Plant Material.** Spinach (*Spinacia oleracea* cultivar RS 3411*)* was taken from a local market and processed in the laboratory. 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. Afterward, leaves were cut (approximately 1 cm × 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, U.K.). The containers were maintained closed upon storage. Intact spinach leaves were stored under the same conditions of temperature 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. At the same moment to monitor water loss of fresh-cut and intact leaves of spinach, fresh weight was determined.

**Enzyme Assay.** PPO activity was determined according to the method reported by Cantos, Espin and Tomàs-Barberàn (*16*). 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 color, is stable and had high molar absorptivity. Adduct accumulation was followed at 467 nm ($\varepsilon = 22300\ M^{-1}\ cm^{-1}$ at pH 5.5). To discriminate between basal and active PPO, 0.1% sodium dodecyl sulfate (SDS) was added to the reaction medium (*17*). Data are expressed as nanomoles of *o*-quinone adduct formed per minute per milligram of soluble protein. Protein determinations were performed using the Protein Assay Kit II (Bio Rad).

**Phenolic Compounds.** The extraction of phenolic compounds was performed as described by Degl'Innocenti et al. (*6*). The total phenolic content in the methanol extracts was determined according to Folin−Ciocalteau by Singleton and Rossi (*18*). A 100 $\mu$L aliquot of the supernatant was combined with 500 $\mu$L of Folin−Ciocalteau's reagent and 400 $\mu$L 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 milligrams of gallic acid per 100 g fresh weight.

**Flavonoid Extraction.** A 0.5 g freeze-dried sample was extracted with 10 mL of MeOH/$H_2O$ (70/30; v/v) using an ultrasonic bath. Then the mixture was centrifuged at 4000 rpm at 4 °C. Extract was filtered through a 0.45 $\mu$m pore filter prior to HPLC analysis.

**HPLC Analysis of Flavonoids.** Filtered extract (20 $\mu$L) 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 $\mu$ ODS3 100 Å size 250 × 4.60 mm). Elution was performed using water and formic acid 0.2% (A) and HPLC-grade methanol/acetonitrile (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 $min^{-1}$, 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/100 g fresh weight).

**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), a UV/vis series 200 (Perkin-Elmer, Wellesley, MA) detector set at 365 nm, and a Prodigy 5 $\mu$ ODS3 100 Å column (250 × 4.6 mm, particle size 5 $\mu$m) (Phenomenex, Torrance, CA). 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 setted at 0.8 mL $min^{-1}$. The LC flow was split, and 0.2 mL $min^{-1}$ was sent to the mass spectrometry. Injection volume was 20 $\mu$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) was used to identify the metabolites: this acquisition method generates a survey scan, single MS spectra with molecular mass information, product ion spectra ($MS^2$) and extracted ion fragment chromatograms (XICs).

Acquisition (IDA) was carried out using the range *m*/*z* 150−1200 with a cycle time of 0.5 s and a step size of *m*/*z* 0.2; collision energy was set at −60 V. The analyses were performed using the following settings: drying gas (air) was heated to 400 °C, the capillary voltage (IS) was set to −4000 V, nebulizer gas (air) 12 (arbitrary units), curtain gas (N2) 14 (arbitrary units), collision gas (N2) 4 (arbitrary units), declustering potential (DP) −60 V.

**Ascorbic Acid Content.** ASA was determined spectrophotometrically as described by Kampfenkel, Montagu and Inzè (*19*). The assay is based on the reduction of $Fe^{3+}$ to $Fe^{2+}$ by ASA and the spectrophotometric detection of $Fe^{2+}$ complexed with 2,2′-dipyridyl. DHA is reduced to ASA by preincubation of the sample with dithiothreitol (DTT). Data are expressed as milligrams of ascorbic acid on 100 g fresh weight.

**Antioxidant Capacity.** Ferric-reducing antioxidant power (FRAP) assay was based upon the methodology reported by Benzie and Strain (*20*). 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 consumed in each occasion. Ten grams of leaf tissue was homogenized with 20 mL of HPLC grade methanol, and the homogenate was filtered and centrifuged at 15000*g* 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 $\mu$M ferrous ion) produced by the addition of freshly prepared ammonium ferrous sulfate. FRAP was expressed as millimoles of ferrous ion per gram fresh weight.

**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 least significant difference (LSD) test. To correlate the antioxidant capacity and phenols, vitamin C content or flavonoids the regression analysis was carried out.

## RESULTS

No differences in water content were recorded in fresh-cut and intact leaves of spinach upon storage, indicating that no water loss occurred during storage.

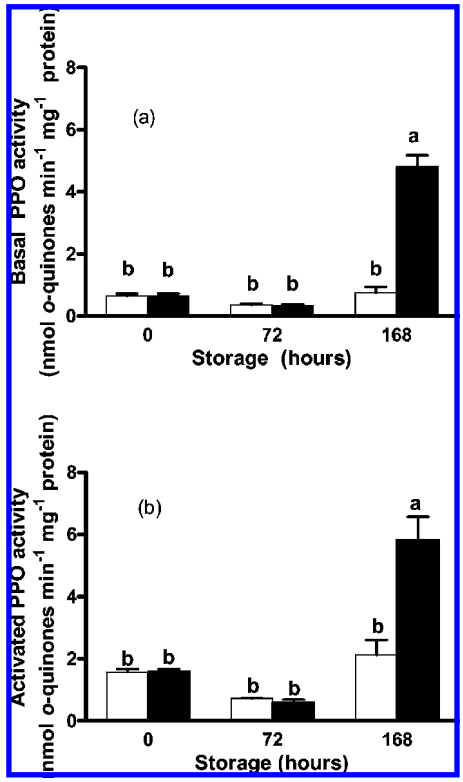


**Figure 1.** Basal (**a**) and SDS-activated (**b**) activity of PPO in intact (white) and fresh-cut (black) tissues of *Spinacia oleracea* during cold storage. The bars represent the mean of 3 replicates with standard deviation. Means of PPO activity followed by the same letter are not significantly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

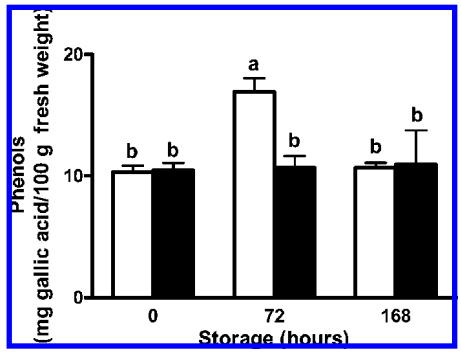


**Figure 2.** Phenol compounds in intact (white) and fresh-cut (black) tissues of *Spinacia oleracea* during cold storage. The bars represent the mean of 3 replicates with standard deviation. Means of phenol content followed by the same letter are not significantly different according to the LSD test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

The activity of basal and activated forms of PPO is shown in **Figure 1**. Both forms increased significantly in spinach leaves 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 basal form of PPO enzyme, which is activated by SDS, was already reported (*21*).

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

The main flavonoids identified in spinach by LC−MS/MS are 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 on the flavonoid concentration is summarized in **Figure 4**.

The most abundant flavonoid compound in spinach was by far the 5,3′,4′-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4′-glucuronide which accounted for about 25% of the total flavonoids in the fresh material. Both spinacetin-glucuronide and jaceidin 4′-glucuronide accounted for about 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 flavonoid content, which did not change in tissue of intact spinach upon storage while a significant increase was observed in fresh-cut leaves (**Figure 4**).

As shown in **Figure 5**, vitamin C (ASA + DHA) decreased significantly and rapidly upon storage in intact and fresh-cut leaf spinach. The lowest value of 4.83 mg of vitamin C per 100 g of fresh weight was recorded in intact leaves at the end of storage (see **Figure 5a**). No changes in DHA content were observed in intact spinach leaves upon storage while the behavior was different in fresh-cut products. In these samples vitamin C decreased significantly upon storage, but the loss in ASA was partially compensated by the increase in DHA values. All in all, at the end of the storage the concentration of vitamin C remained higher in fresh-cut products than in the intact leaves (see **Figure 5b**).

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

No correlations among antioxidant capacity, phenols and total flavonoid content were 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 $r = 0.915$, $P < 0.001$, respectively in intact and cut spinach leaves). Therefore, the decrease of ASA observed in intact and fresh-cut spinach tissues is the major factor responsible for the decrease in antioxidant capacity of these tissues. When the correlation between specific flavonoids content and FRAP assay results was investigated, a significant correlation was found between patuletin-3-glucosyl-(1-6)[apiosyl(1-2)]-glucoside and antioxidant capacity ($r = 0.802$, $P < 0.05$ and $r = 0.899$, $P < 0.01$, respectively in intact and fresh-cut spinach tissues).

## DISCUSSION

At the end of one week (168 h) of cold storage fresh-cut spinach did not show any symptoms of color alteration that are typical of other leafy vegetables such as lettuce (*5, 22, 23*). Color alteration in fresh-cut fruits and vegetables is the direct consequence of PPO action on polyphenols (*4, 24*). 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 basal status (*25*) particularly in spinach leaves (*21*). When basal PPO was treated by SDS, its activity increased 2-fold at the various storage times both in intact and in fresh-cut tissues.

The fact that total PPO activity increased at the end of the storage in fresh-cut tissues indicated a de novo PPO synthesis

**Table 1.** LC−MS/MS Characteristics of the Main Flavonoids Present in the Extracts of Spinach

| compound[a] | peak ID no. | 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″-feruloylglucosyl)(1-6)[apiosyl(1-2)]-glucoside | 3 | 977 | 669 |
| | | | 345 |
| patuletin-diglucoside | 4 | 655 | 331 |
| spinacetin glucuronide | 5 | 521 | 345 |
| | | | 330 |
| jaceidin glucuronide | 6 | 535 | 359 |
| 5,3′,4′-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4′-glucuronide | 7 | 519 | 343 |
| | | | 328 |
| 5,4′-dihydroxy-3,3′-dimethoxy-6:7-methylenedioxiflavone-4′-glucuronide | 8 | 533 | 357 |
| | | | 342 |

[a] 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.

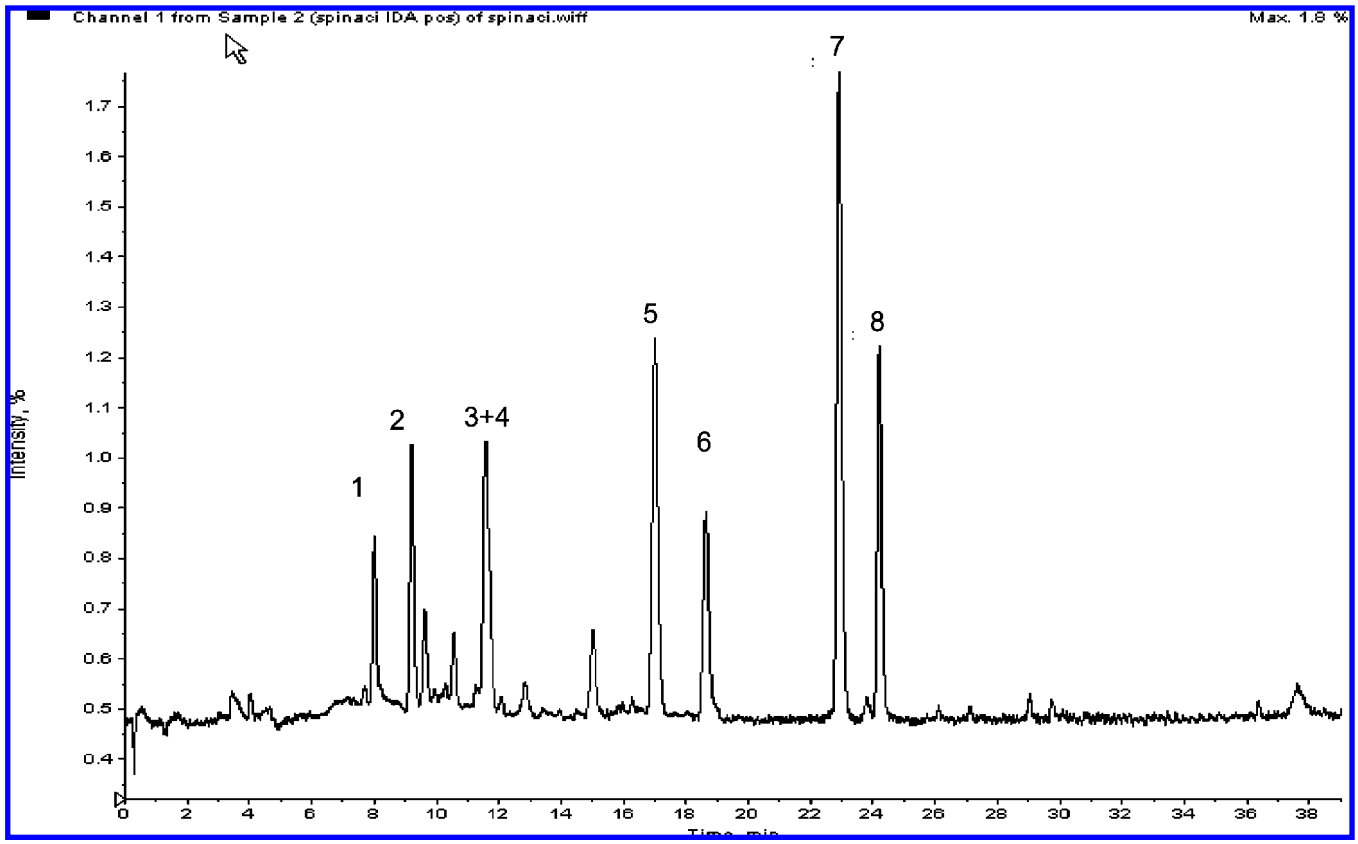


**Figure 3.** HPLC chromatogram of *Spinacia oleracea* flavonoids. Peak identification was based on MS/MS analysis reported in **Table 1**. 1: Patuletin-3-glucosyl-(1-6)[apiosyl(1-2)]-glucoside. 2: Spinacetin-3-glucosyl-(1-6)[apiosyl(1-2)]-glucoside. 3: Spinacetin-3-(2″-feroylglucosyl)(1-6)[apiosyl(1-2)]-glucoside. 5: Spinacetin glucoronide;. 6: Jaceidin glucuronide. 7: 5,3′,4′-Trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4′-glucuronide. 8: 5,4′-Dihydroxy-3,3′-dimethoxy-6:7-methylendioxyflavone-4′-glucuronide.

upon storage due to initial wounding. The decompartimentalization of cellular components, with subsequent release of proteases, caused a cascade of events leading to the activation of basal PPO (*26*). Although PPO activity increased significantly in fresh-cut tissues, they did not show any alteration in color attributable to enzymatic browning, and also the amount of phenol compounds, which are the substrates of PPO activity, did not change upon storage. This indicated that in spinach tissues wounding enhanced the activity of PPO, but its catalytic action did not cause an accumulation of quinones that polymerize forming brown pigments. This finding might be attributed to the lack of PPO substrates, but spinach contains plenty of flavonoids and phenolic acids that are described as good substrates for this enzyme (*27*, *28*). Therefore, the explanation for this behavior could be related to the high endogenous content of ascorbic acid of spinach. In fresh-cut tissues, as well as in intact leaves, ASA decreased upon storage. However, in fresh-cut tissues DHA significantly increased because of the conversion of ASA into DHA. As a consequence the ASA + DHA content in fresh tissues decreased less than in the intact leaves (−73 and −90% respectively in fresh-cut and intact tissues). It is well-known that ASA reduced oxidized substrates molecules back to their original state, therefore impairing their polymerization to brown pigment (*29*). 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

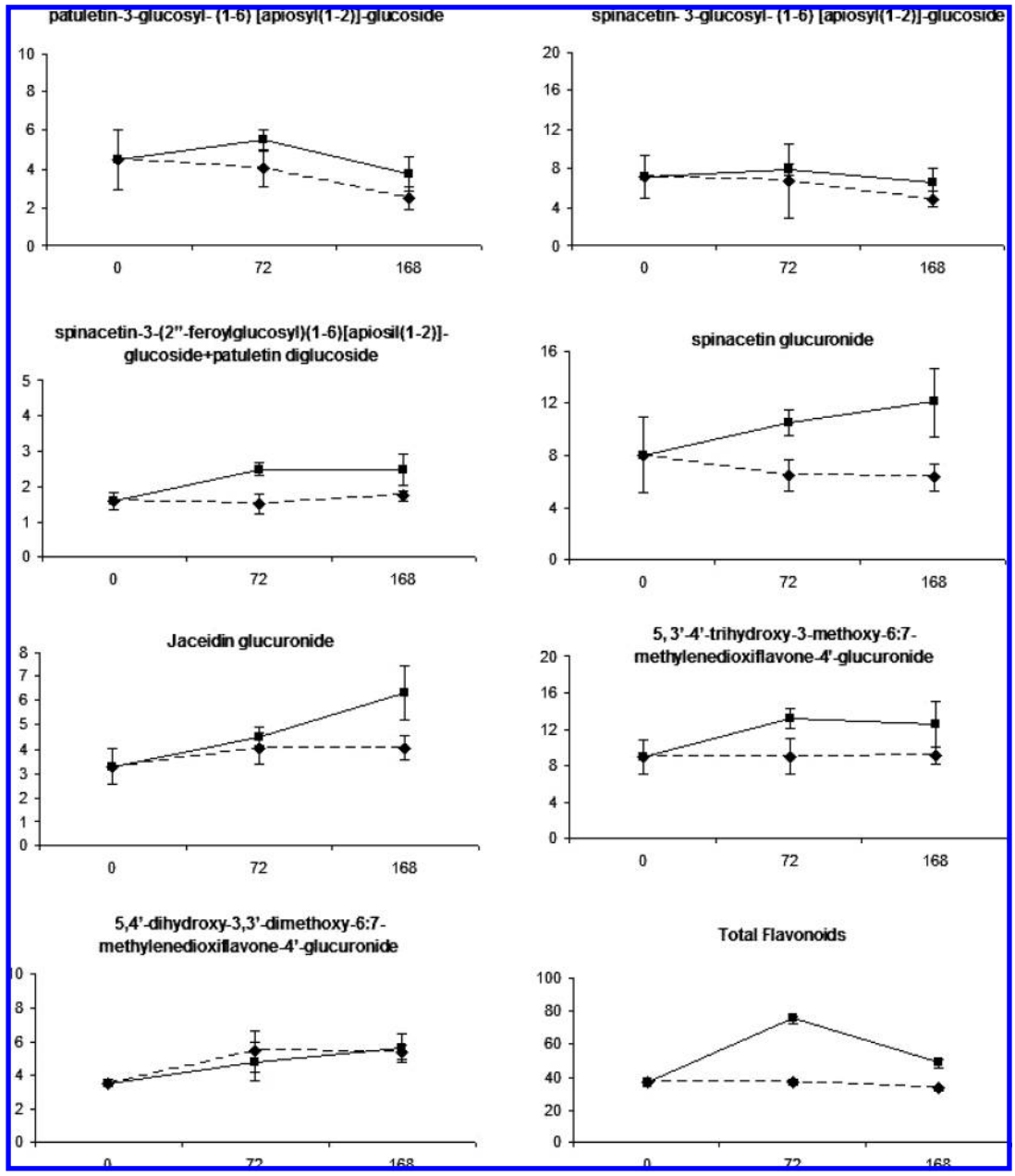


**Figure 4.** Flavonoid content expressed as milligrams of quercetin per 100 g of fresh weight in intact (closed circle) and fresh-cut (open circle) tissues of *Spinacia oleracea* during cold storage.

observed. This phenomenon was already described in other leafy vegetables characterized by a high ASA content such as rocket salad (*5*).

The role of ASA in preventing enzymatic browning is an important feature making spinach a suitable species for fresh-cut product. Despite the substantial decrease of vitamin C content fresh-cut spinach maintains most of its nutritional values due to the presence of other antioxidant compounds.

Total phenols increased significantly in intact spinach leaves only at 72 h of storage. This transient increase can be due to breakdown of glycosidic bonds leading to the formation of phenolic compounds which react better with the Folin−Ciocalteau (*30*). However this was not in line with the data obtained for the main phenolic compounds showed in **Figure 4**.

Few MS/MS data on spinach flavonoids are present in the literature. In a pivotal paper by Ferreres and co-workers (*15*) the main compounds were isolated and structures determined by NMR. After that, Bergquist et al. (*13*) investigated baby spinach by MS/MS, showing a flavonoid profile quite different from that here found for spinach which is similar to that reported by Ferreres and co-workers (*15*). Therefore the MS/MS data summarized in **Table 1** represented a useful tool for the metabolomic studies dealing with the flavonoid profile of different spinach varieties.

Data demonstrated that cold storage and cutting had some effects on flavonoid concentration and their profile. During storage the total flavonoid content remained quite constant in intact leaves and showed a slight increase in fresh-cut spinach. Interestingly, the increase observed during storage is limited to some specific compounds 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 (*31*). Other compounds such as patuletin-3-glucosyl-(1-6)[apiosyl(1-2)]-glucoside and spinacetin-glucuronide decreased significantly also

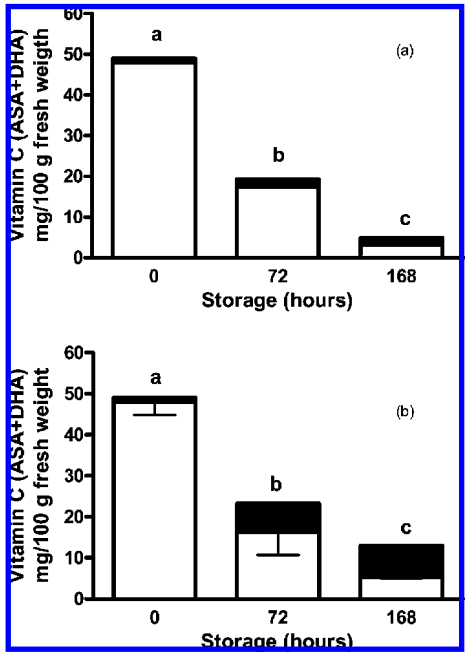


**Figure 5.** Vitamin C [ascorbic acid (white), dehydroascorbic (black)] content in intact (**a**) and fresh-cut (**b**) tissues of *Spinacia oleracea* during cold storage. The bars represent the mean of 3 replicates. Means of vitamin C content followed by the same letter are not significantly different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

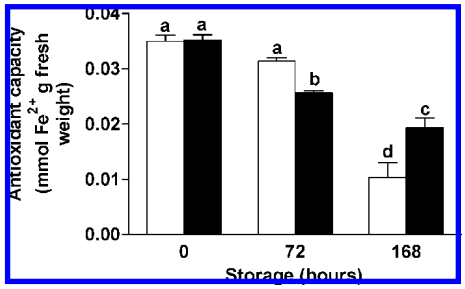


**Figure 6.** Antioxidant capacity determined by FRAP assay in intact (white) and fresh-cut (black) tissues of *Spinacia oleracea* during cold storage. The bars represent the mean of 3 replicates. Means of antioxidant capacity followed by the same letter are not significantly different according to the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

in intact spinach leaves whereas the other flavonoids remained rather stable or showed no response to storage as already reported in literature (*10*). In particular Bergquist et al. (*13*) 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 capacity 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 (*32*). In accordance to the structure−activity relationship reported by Rice-Evans and Miller (*32*) 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 capacity.

Antioxidant capacity was linearly correlated also with ASA content both in intact and fresh-cut spinach tissues. The decrease in ascorbic content during cold storage was already reported in the literature for spinach (*33*), but the difference between intact leaves and fresh-cut product is shown here for the first time.

Actually the literature reports contrasting evidence about the relationship between phenols and antioxidant capacity; some authors have observed a positive relationship (*34*), while others reported a weak one (*35*). The lack of correlation between phenol concentration and antioxidant capacity in this work is odd taking into account that these two methods are based on similar redox reactions. This behavior might be explained by the high concentration of vitamin C in spinach and by its drop during storage. In fact, FRAP assay is very sensitive to vitamin C (*36*), that is way there is a high correlation between antioxidant activity value determined by FRAP assay and vitamin C concentration during storage. Therefore the total antioxidant capacity can not be correlated in the same samples with the phenol compounds which remained roughly constant.

In conclusion these data suggested that spinach represents a very suitable leafy vegetable for the as fresh-cut products. It does not show the phenomenon of enzymatic browning likely because of the high content of ASA. Vitamin C changed at a lower extent in fresh-cut spinach as compared to intact spinach even if the antioxidant capacity decreased significantly both in fresh-cut and in intact leaf. 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, thus keeping in this minimally processed product most of the nutritional value present in fresh spinach.

## ACKNOWLEDGMENT

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