Effect of Modified Atmosphere Packaging on the Flavonoids and Vitamin C Content of Minimally Processed Swiss Chard (*Beta vulgaris* Subspecies *cycla*)

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Five flavonoids from Swiss chard leaves (*Beta vulgaris* subsp. *cycla*) cultivar "green" were isolated and identified as vitexin 2″-xyloside and its 6″-malonated derivative, kaempferol 3-gentiobioside, isorhamnetin 3-gentiobioside, and isorhamnetin 3-vicianoside. 6″-Malonyl-2″-xylosyl vitexin is a new naturally occurring flavonoid. In fact, this is the first time that a flavonoid C-glycoside acylated with a dicarboxylic acid is reported in nature. The flavonoid content of fresh leaves (green cultivar) was in the range 2.4−3.0 mg/g fresh weight (f.w.). Cultivar "yellow" contained only flavone C-glycosides (2.1−2.3 mg/g f.w.), while the flavonols were not detected. Their vitamin C content was between 0.4 and 0.5 mg/g f.w., and after domestic processing an 80% loss was observed. Modified atmosphere packaging (MAP) (7% O_2 and 10% CO_2) had no effect on total flavonoid content after 8 days of storage, although it increased flavonoid extraction during cooking in boiling water. In contrast, vitamin C content decreased, especially in MAP-stored Swiss chard, to reach levels below 50% of the intial content after 8 days of cold storage.

Keywords: *Beta vulgaris subsp. cycla; Chenopodiaceae; Swiss chard; flavonoids; vitamin C; antioxidants; modified atmosphere packaging; cooking; minimally processed*

INTRODUCTION

Dietary flavonoids and vitamins C, E, and A are important in a well-balanced diet due to their antioxidant and free-radical scavenging activities, which play important roles in human nutrition (Stähelin et al., 1991a,b). Fruits and vegetables are rich in these natural antioxidants, and their consumption has been associated with lower incidence and lower mortality rates of cancer in several cohort and case-control studies for all common cancer sites (Ames et al., 1993). In addition, there is a highly significant negative association between intake of total fruit and vegetables and cardio- and cerebrovascular disease mortality (Verlangieri et al., 1985). The protection that fruits and vegetables provide against these diseases has been attributed to the various antioxidants including ascorbic acid and α-tocopherol (Leake, 1997). Recent studies have shown that the majority of the antioxidant activity of a fruit or vegetable may be from the flavonoids and other phenolic compounds (Cao et al., 1996; Wang et al., 1996).

Due to the nutritional importance of fruit and vegetable flavonoids and other phenolic compounds, in the past few years there has been an increasing interest in the evaluation of their changes with postharvest treatments. The effects of controlled atmosphere (Gil et al., 1997), minimal processing (Ferreres et al., 1996, 1997), and domestic processing (Price et al., 1997) on polyphenolic constituents have been recently studied. Modified atmospheres with elevated CO_2 and reduced O_2 have proved useful for the storage of minimally processed vegetables since they reduce respiratory activity (Ko et al., 1996) and prevent browning (Lopéz-Gálvez et al., 1996), microbial development (Babic and Watada, 1996), and product decay (Agar et al., 1990). However, the effect of controlled atmosphere on the ascorbate content of minimally processed fruits and vegetables has not been extensively studied (Barth and Zhuang, 1996; Wright and Kader, 1997). More research is required to identify those storage conditions that will minimize the degradation of antioxidant metabolites and vitamins.

The aim of the present work was the identification of the flavonoids in Swiss chard as well as the quantitation of these compounds and vitamin C (ascorbic plus dehydroascorbic acids) in the main cultivars produced in Spain. In addition, the effect of modified atmosphere packaging on the antioxidants content of minimally processed Swiss chard was also evaluated.

MATERIALS AND METHODS

Plant Material. Fresh Swiss chard leaves of green and yellow cultivars were purchased in a local market. Green tissue was separated from the white stems and cut in pieces of 2 × 2 cm immediately before extraction.

Flavonoid Extraction. Green cultivar was used for the isolation and identification of flavonoids. A sample of ca. 500 g fresh weight (f.w.) of green tissue was macerated overnight at room temperature with methanol−water (8:2 v/v). The extract was concentrated under reduced pressure (50 °C) until only the water remained, and the concentrate was centrifuged (6000 rpm, 5 min). The supernatant was mixed with the nonionic polymeric resin Amberlite XAD-2 (enough to fill a column of 3 × 50 cm) and placed in a magnetic stirrer for 4 h to allow flavonoid adsorption on the resin particles. The resin particles were then poured into the glass column and washed with distilled water (500 mL). Flavonoids were eluted with

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methanol (300 mL) and taken to dryness under reduced pressure (50 °C).

Flavonoid Isolation and Purification. The extract was submitted to reversed-phase low-pressure chromatography on a Lobar column (440 × 37 mm) LiChroprep RP18 (40−63 μm) (Merck, Darmstadt, Germany) using different methanol−water solutions (increasing the methanol percentage), and the separation of the different flavonoid fractions was readily followed on-line with a UV detector (340 nm). The composition of the different fractions was followed by analytical HPLC (see below). The different compounds were then purified by semipreparative HPLC on a Spherisorb ODS-2 column (25 × 1 cm, 5 μm particle size) (Tecnokroma, Barcelona, Spain), with different isocratic mixtures of methanol and water. Five different flavonoids (**1**−**5**) were isolated.

UV Spectrophotometry (λ_{max} (nm), methanol). (**1**) 271, 304i, 332; +NaOMe, 280, 329, 395; +$AlCl_3$, 274, 282i, 306, 358; +$AlCl_3$ + HCl: 276, 304, 343, 375i; +NaOAc, 280, 306i, 383; +NaOAc + H_3BO_3, 272, 311i, 336. (**2**) identical to **1**. (**3**) 267, 301i, 348; +NaOMe, 276, 327, 401; +$AlCl_3$, 275, 306, 359, 405i; +$AlCl_3$ + HCl, 275, 307, 347, 395i; +NaOAc, 275, 305, 378; +NaOAc + H_3BO_3, 268, 300i, 352. (**4**) 256, 268i, 294i, 355; +NaOMe, 272, 331, 415; +$AlCl_3$, 272, 309, 363, 404i; +$AlCl_3$ + HCl, 273, 305i, 362, 403i; +NaOAc, 275, 323, 394; +NaOAc + H_3BO_3, 257, 270i, 295i, 360. (**5**) 257, 269i, 348; +NaOMe, 272, 336, 406; +$AlCl_3$, 275, 304, 360, 401i; +$AlCl_3$ + HCl, 273, 302, 361, 399i; +NaOAc, 274, 324, 395; +NaOAc + H_3BO_3, 258, 229, 353.

Acid and Enzymatic Hydrolysis. Acid hydrolysis of the naturally occurring flavonoids was achieved by dissolving 1 mg of the isolated flavonoid in 1 mL of MeOH, 1 mL of 4 N HCl was added, and the solution was kept at 90 °C for 45 min. The aglycones were then extracted with diethyl ether, and the unhydrolyzed glycosides with ethyl acetate after removal of MeOH, while the sugars remained in the aqueous phase. The aglycones and sugars were identified by thin-layer chromatography against authentic markers and by their UV spectra in methanol and after the addition of the classical shift reagents (Mabry et al., 1970). Enzymatic hydrolysis was achieved by adding 0.5 mg of flavonoid and 3 mg of β-D-glucosidase (Sigma, St. Louis, MO), in 0.5 mL of 0.1 M citrate−phosphate buffer, pH 5 (37 °C, 24 h). The hydrolysis products were extracted with ethyl acetate, taken to dryness under reduced pressure (40 °C), and redissolved in methanol for HPLC analysis.

Paper Electrophoresis. This was achieved according to the method of Harborne and Boardley (1985) to detect the presence of acylation with organic dicarboxylic acids.

NMR Analyses. ^{1}H NMR spectra were recorded with a Varian 300 MHz instrument, in DMSO-d_6, with TMS as internal reference. ^{13}C NMR spectra were recorded with a Varian 60 MHz instrument, in DMSO-d_6, with TMS as internal reference.

Analytical HPLC. This was achieved on a reversed-phase column LiCrochart RP-18 (12.5 × 0.4 cm, 5 μm particle size) (Merck, Darmstadt, Germany), using as mobile phases water−formic acid (19:1, v:v) (A) and HPLC grade methanol (B). Elution was performed at a solvent flow rate of 1 mL min^{-1}, using a gradient starting with 10% B in A to reach 40% B at 30 min and 80% B at 40 min. Detection was achieved with a diode-array detector, and the UV spectra of the different compounds were recorded. Samples were passed through a 0.45 μm filter before analysis.

Cooking. Green tissue of Swiss chard was cut in small pieces 2 × 2 cm, washed with abundant water, and hand-centrifuged to remove surface moisture. Samples of 10 g fresh weight were submitted to boil with 50 mL of water for 10 min at 90 °C, and the water was filtered through filter paper Whatman No. 1 (see Table 3) and stored in an ice bath until HPLC analysis of the flavonoids the same day. The cooked plant material was then mixed with 50 mL of HPLC grade methanol, and homogenized (Ultraturrax T25 equipment, Janke & Kunkel, Staufen, Germany) at 20 000 rpm for 1 min. The homogenate was then filtered through filter paper and kept in ice until analysis the same day. Control samples (10 g f.w.) were directly homogenized with 50 mL of methanol under the conditions described above, filtered through filter paper, and kept in ice until analyzed. The different treatments were carried out in triplicate. All samples were filtered through 0.45 μm filters before analysis.

Vitamin C Determination. Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined by HPLC. Standard solutions, column conditioning, and derivatization procedures were as described by Zapata and Dufour (1992). Samples of 30 g f.w. (both fresh and processed tissues) were homogenized with 80 mL of MeOH/H_2O (5:95) plus citric acid (0.5 g/L) with EDTA (0.5 g/L) using an Ultraturrax homogenizer (24000 rpm). The homogenate was filtered through four layers of cheesecloth, and the extract was centrifuged at 5000 rpm for 5 min in a Centromix rotor (model 540, Selecta, Barcelona, Spain). After centrifugation, the pH was adjusted between 2.2 and 2.4 with HCl. The sample was passed through a C_{18} Sep-Pak cartridge (Waters, Milford, MA), previously activated with methanol followed by water and then the same volume of air. The first 5 mL was discarded and the next 3 mL were collected. The HPLC analysis of total vitamin C (AA plus DHAA) was achieved after derivatization of DHAA into the fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-*b*]quinoxaline-1-one (DFQ), with 1,2-phenylenediamine dihydrochloride (OPDA) (Zapata and Dufour, 1992). Freshly prepared OPDA solution was added to the water soluble fraction eluted from a C-18 solid-phase extraction cartridge Sep-Pak (OPDA extract; 1:3, v/v). The samples were left for 37 min at room temperature in the dark, and 20 μL was analyzed with a Merck-Hitachi (Tokyo, Japan) liquid chromatograph equipped with a L-4000 UV detector and a L-6000 pump. Separations of DFQ and AA were achieved on a Kromasil 100 C-18 column (25 × 0.4 cm; 5 μm particle size) (Tecnokroma, Barcelona, Spain). The mobile phase was methanol−water (5:95, v/v) containing 5 mM cetrimide and 50 mM potassium dihydrogen phosphate at a pH 4.5. The flow rate was 0.9 mL/min. The detector wavelength was initially set at 348 nm, and after elution of DFQ, it was manually shifted to 261 nm for AA detection.

Modified Atmosphere Packaging. Minimally processed ready-to-use Swiss chard (cultivar yellow) from a major vegetable processor (Vega Mayor S.A., Navarra, Spain) was evaluated. Products were delivered refrigerated to the laboratory after purchasing through a wholesale broker immediately after manufacture. Packages of 400 g were stored in their cartons protected from the light at 6 °C for 8 days. An air atmosphere was created in the bag by perforating the film with a needle. Gas samples were taken through a septum and changes of O_2 and CO_2 concentrations in the packages were monitored daily with a Perkin-Elmer (Norwalk, CT) Autosystem gas chromatograph equipped with a thermal conductivity detector (TCD). Samples were analyzed in triplicate for phenolics and vitamin C content at initial time and after 4 and 8 days of cold storage in both air and modified atmospheres.

RESULTS AND DISCUSSION

Flavonoid Isolation and Identification. Five main flavonoids (**1**−**5**) were detected. The main flavonoids (**1** and **2**) had UV spectra of apigenin derivatives, and the other three (**3**−**5**) of 3-substituted flavonols (Mabry et al., 1970).

Compound **1** after acid hydrolysis yielded xylose and a mixture of 8-*C*-glucosylapigenin (vitexin) and 6-*C*-glucosylapigenin (isovitexin), identified by chromatographic comparisons with authentic markers. This is a typical behavior of flavone *C*-glycosides which under acidic treatment suffer the well-known Wessely−Moser rearrangement which opens the C-ring of the flavone and the original 8-*C*-glycoside is partly transformed to the 6-*C*-glycoside isomer (Gluchoff-Fiasson et al., 1989).

Table 1. ^{1}H NMR Data of Compounds 1−5 (300 MHz, DMSO-d_6)[a]

H	1	2	3	4	5
flavonoid					
H-3	6.78 s	6.81 s	–	–	–
H-6	6.22 s	6.24 s	6.18 d (2.0)	6.20 d (2.0)	6.18 d (2.0)
H-8	–	–	6.40 d (2.0)	6.43 d (2.0)	6.42 d (2.0)
H-2′	8.02 d (8.5)	7.95 d (8.5)	8.01 d (8.6)	7.94 d (2.0)	7.90 d (2.0)
H-3′	6.95 d (8.5)	6.94 d (8.5)	6.87 d (8.6)	–	–
H-5′	6.95 d (8.5)	6.94 d (8.5)	6.87 d (8.6)	6.91 d (8.4)	6.91 d (8.4)
H-6′	8.02 d (8.5)	7.95 d (8.5)	8.01 d (8.6)	7.50 dd (2.0, 8.4)	7.49 dd (2.0, 8.4)
OMe 3′	–	–	–	3.84 s	3.82 s
glucose					
H-1	4.78 d (10.0)	4.83 d (10.0)	5.36 d (7.0)	5.52 d (7.0)	5.45 d (7.5)
H-6_{A-B}		4.10 m			
terminal sugar					
H-1	3.87 d (7.0)	3.89 d (7.0)	4.02 d (7.5)	4.08 d (7.5)	3.96 d (7.5)
malonyl					
$-CH_2-$	–	3.30 s	–	–	–

[a] Coupling constants in Hz, d relative to solvent signal of DMSO-d_6.

Its UV spectrum in methanol, with a band II maximum of 271 nm, confirmed that this was an apigenin mono-C-glycoside. The UV study after the addition of the classical reagents showed that the hydroxyls at 5, 7, and 4′ were free (Mabry et al., 1970).

^{1}H NMR data (Table 1) confirmed the aglycone substitution pattern, and that the C-glycosylation was at the 8-position, since the response of the proton at 6-position was clearly observed, while that of the 8-position was not present. The anomeric proton of the glucose was in agreement with a *C*-β-D-glucopyranosyl appearing at a higher field (4.78 ppm) than the *O*-β-D-glucopyranosides (5.3−5.5 ppm), and with a coupling constant of protons 1 and 2 of 10 Hz, which is only 7 Hz in the *O*-glucosides (Markham and Geiger, 1993). The anomeric proton of the terminal sugar (xylose) indicated that this was a β-D-xylopyranoside.

The ^{13}C NMR analysis (Table 2) confirmed the substitution pattern of the flavone and the sugar sequence. In addition, it clearly indicated that the xylose residue was linked to the 2-position of the glucose, since the response of this carbon appeared downfield at 81.7 ppm. The ^{13}C NMR spectrum of compound **1** was in good agreement with that reported for 2″-xylosylvitexin (Table 2) isolated from *Setaria italica* (Poaceae) (Gluchoff-Fiasson et al., 1989).

Compound **2** had identical UV spectral and hydrolytic characteristics as compound **1**, but eluted with a higher retention time in the HPLC analyses. This compound showed electrophoretic mobility at pH 4.4, but it did not migrate at pH 2.2 consistent with a flavonoid acylated with a dicarboxylic acid (Ferreres et al., 1989). This was confirmed by the behavior of compound **2** in acid (2.5 N HCl) at room temperature, where this was transformed into compound **1** after a few hours.

The ^{1}H NMR spectrum was similar to that of **1**, with the only differences of a new singlet with an integration of two protons at 3.3 ppm, consistent with the CH_2 of the malonyl residue (Markham and Geiger, 1993), and a multiplet integrating 2 protons appearing at 4.1 ppm, which could be related to the two protons at the 6-position of the glucose which were shifted downfield by the acylation with malonic acid (Withopf et al., 1997). These data are in agreement with those reported for 6-malonylglucosides which have been reported to be fairly common in nature.

The ^{13}C NMR analysis clearly showed the signals of the three carbons of the malonyl residue, and showed a downfield shift of the C-6″ carbon of the glucose from 61.0 to 64.9 ppm, as a consequence of the acylation, confirming that this was the position for the acylation, in agreement with other ^{13}C NMR data for 8-*C*-glucosylflavones acylated in 6-position of the glucose with 3-hydroxy-3-methylglutaric acid (Liu et al., 1994). Therefore, compound **2** was identified as 8-*C*-β-D-(2″-β-D-xylopyranosyl-6″-malonyl)glucopyranosyl apigenin (2″-xylosyl-6″-malonylvitexin), a new naturally occurring flavonoid (Figure 1). In addition, this is the first time that a flavone *C*-glycoside acylated with a dicarboxylic acid is reported in nature.

Table 2. ^{13}C NMR Data of Compounds 1 and 2 (60 MHz, DMSO-d_6)[a]

carbons	*b*	1	2
flavonoid			
2	163.6	163.7	163.7
3	102.2	102.4	102.7
4	181.9	181.9	182.1
5	161.1	161.1	161.3
6	98.1	98.2	98.2
7	163.1	162.9	162.8
8	103.4	103.7	103.4
9	156.5	156.6	156.7
10	103.6	103.7	104.0
1′	121.4	121.5	121.7
2′	128.7	128.8	128.6
3′	115.8	115.8	116.1
4′	160.4	160.5	160.8
5′	115.8	115.9	116.1
6′	128.7	128.8	128.6
C-glucosyl			
1″	71.4	71.5	71.8
2″	81.6	81.7	80.5
3″	78.2	78.2	78.3
4″	70.0	70.1	70.3
5″	80.7	80.8	78.0
6″	60.9	61.0	64.9
O-xylosyl			
1‴	105.6	105.7	105.8
2‴	73.5	73.6	73.7
3‴	75.7	75.8	75.9
4‴	69.2	69.2	69.4
5‴	65.3	65.4	65.6
malonyl			
CO			167.2
CH_2			41.4
COOH			168.0

[a] Coupling constants in Hz. [b] Gluchoff-Fiasson et al. (1989).

Compound **3** showed a UV spectrum as a 3-substituted kaempferol derivative (Mabry et al., 1970). Its UV analysis showed that the hydroxyls at 5, 7, and 4′

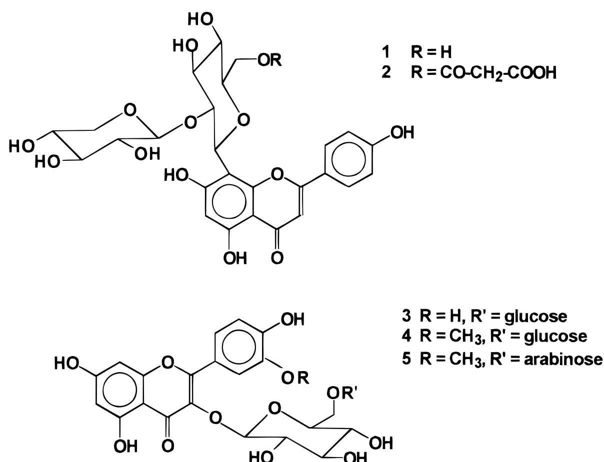

Figure 1. Flavonoids from Swiss chard (cultivar green).

were free and that the sugars should be linked to the hydroxyl in the 3-position. After acid hydrolysis kaempferol and glucose were identified. Enzymatic hydrolysis with β-D-glucosidase released kaempferol 3-glucoside (as an intermediate) and kaempferol identified by chromatographic comparisons with authentic markers.

The ^{1}H NMR data confirmed the substitution pattern of the flavonol and showed that two β-D-glucopyranoses were present. The signal of the anomeric proton of the terminal glucose at 4.02 ppm indicated that the interglycosidic linkage was 1→6, since this signal for 1→2 diglucosides is in the range 4.6−4.7 ppm (Markham and Geiger, 1993). Therefore, compound **3** was identified as kaempferol 3-β-D-(6″-β-D-glucopyranosyl) glucopyranoside (kaempferol 3-gentiobioside).

Compound **4** had similar UV and hydrolytical behavior as compound **3**, but in this case isorhamnetin (3,5,7,4′-tetrahydroxy-3′-methoxyflavone) was the aglycone. This was confirmed by ^{1}H NMR, as well as the nature of the sugar moiety, which was identified as β-D-glucopyranosyl(1→6) β-D-glucopyranoside. Thus **4** was isorhamnetin 3-*O*-β-D-glucopyranosyl(1→6) β-D-glucopyranoside (isorhamnetin 3-gentiobioside) (Figure 1).

Compound **5** was also an isorhamnetin derivative but in this case glucose and arabinose were the sugars detected after acid hydrolysis. This compound was not hydrolyzed by β-D-glucosidase, showing that arabinose was the terminal sugar. A coupling constant of 7.5 Hz for H_1 and H_2 of the arabinose indicated that this was an α-L-arabinopyranoside, and the response for the anomeric proton at 3.96 ppm showed that arabinose was linked to the 6-position of the glucose directly linked to the flavonol, and that compound **5** was isorhamnetin 3-*O*-α-L-arabinopyranosyl(1→6) β-D-glucopyranoside (isorhamnetin 3-vicianoside). This compound had previously been reported from *Papaver orientale* (Papaveraceae) (Sakar et al., 1980).

Evaluation of Cultivar Composition. The flavonoid content of the photosynthetic tissue of both cultivars green and yellow was compared. The main difference between cultivars was that the yellow one lacked the flavonols **3**−**5** found in the green cultivar, and therefore the flavone C-glycosides were the only flavonoids detected (Table 3). The total flavonoid content was significantly higher in the green cultivar compared to the yellow cultivar (2.8 and 2.2 mg/g f.w., respectively). Concerning the relative amount of xylosylvitexin (**1**) and its malonated derivative (**2**), the deacylated compound was the main flavonoid in the green cultivar, while the acylated derivative was predominant in the yellow cultivar. In cultivar green, the flavonols were present in smaller concentrations than the C-glycosides, and in fact compound **3** could not be quantified due to the small amount present.

Concerning the vitamin C content (AA plus DHAA), both cultivars had similar amounts (around 0.45 mg/g f.w.). It was remarkable that only dehydroascorbic acid was detected in all the analyzed samples. Even when the sample was spiked with one mg of AA, this was completely and immediately transformed into dehydroascorbic acid, which was the only vitamin C form detected in the chromatograms. This could be associated with a high oxidase activity (ascorbate oxidase, polyphenol oxidase, cytochrome oxidase, and peroxidase) in the Swiss chard extracts. Wright and Kader (1997) mentioned other nonenzymatic degradation mechanisms of vitamin C in minimally processed products. These included light, oxygen, and washing with sodium hypochlorite.

Effect of Cooking. Processing Swiss chard by boiling in water to simulate domestic processing, extracted part of the flavonoids, and these were detected in the cooking waters. In cultivar green, around 50% of the flavonoids were extracted with water, while the rest remained in the cooked plant material. A similar behavior was observed for all the different flavonoids, and no selective or most favored extraction was observed for any specific flavonoid (Table 3). In cultivar yellow, the ratio of extracted flavonoids with water during cooking was smaller than the flavonoids remaining in the tissue, this being a difference with the green cultivar. The cooking process did not induce a transformation of the labile acylated derivative (**2**) into the deacylated derivative (**1**), and this was observed for both cultivars.

The effect of domestic processing on vitamin C content was also evaluated. A mean value ($n = 3$) of 0.09 mg/g f.w. was obtained, meaning that 80% of the vitamin C present in the initial tissue was lost during the cooking process.

Effect of Modified Atmosphere Packaging. Oxygen and carbon dioxide concentrations in the bags of minimally processed Swiss chard during storage at 6 °C for 8 days are shown in Figure 2. As a result of the respiration rate of the tissue and the gas diffusion characteristics of the film, the initial atmosphere was modified and varied during the storage period.

The total flavonoids increased significantly especially in MAP stored product, while this increase was not so significant in the air-stored Swiss chard (Table 4). No differences in the relative amounts of flavonoids were observed, suggesting that no metabolic alterations of the phenolic metabolism had occurred. When these samples were submitted to domestic processing (boiling) in this case no significant differences were found in the total flavonoids (Table 5), suggesting that the differences found between the applied treatments were due to a different facility of extraction. This indicates that in MAP-stored Swiss chard cellular structures are probably more fragile and the extraction either by homogenization with methanol (Table 3) or with water during domestic processing is easier than in those tissues which were stored in air. In conclusion, MAP-stored product loses more flavonoids during the cooking process than does product stored in air. This increased extraction

Table 3. Effect of Cooking on the Individual Flavonoid Content of Swiss Chard (Cultivars Green and Yellow)[a]

treatment	1	2	4	5	total
methanol before cooking					
green cv	1.93 (0.28)	0.34 (0.10)	0.39 (0.07)	0.10 (0.04)	2.76 a
yellow cv	0.75 (0.01)	1.44 (0.10)	–	–	2.19 b
water after cooking					
green cv	0.75 (0.30)	0.31 (0.12)	0.18 (0.11)	0.05 (0.03)	1.29
yellow cv	0.36 (0.05)	0.61 (0.01)	–	–	0.97 ns
methanol after cooking					
green cv	0.80 (0.16)	0.24 (0.09)	0.17 (0.05)	0.06 (0.02)	1.26
yellow cv	0.58 (0.05)	0.92 (0.10)	–	–	1.50 ns

[a] (**1**) Xylosylvitexin; (**2**) xylosylvitexin malonated; (**3**) kaempferol 3-gentiobioside, not quantified, present only on cultivar green as traces; (**4**) isorhamnetin 3-gentiobioside; (**5**) isorhamnetin 3-vicianoside. Values are mg g^{-1} fresh weight (standard deviation) ($n = 3$). Different letters indicate significant differences at $P = 0.05$; ns, not significant.

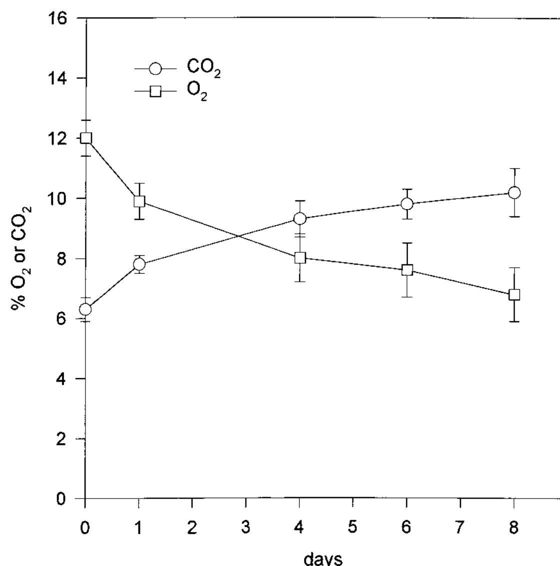

Figure 2. Oxygen and carbon dioxide concentrations in minimally processed Swiss chard packages.

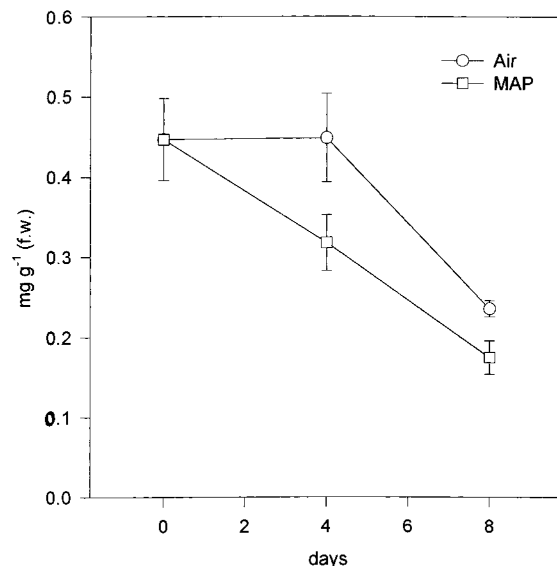

Figure 3. Effect of MAP on vitamin C content of minimally processed Swiss chard (cultivar yellow).

Table 4. Effect of MAP on Flavonoid Content of Minimally Processed Swiss Chard (Cultivar Yellow), Initially and after 8 Days of Cold Storage at 6 °C[a]

treatment	1	2	total
initial	0.75	1.44 a	2.19 a
air	0.81	1.57 ab	2.38 ab
MAP	0.92 ns	1.69 b	2.61 b

[a] (**1**) Xylosylvitexin; (**2**) xylosylvitexin malonated. Values are mean ($n = 3$) (mg g^{-1} fresh weight). Different letters indicate significant differences at $P = 0.05$; ns, not significant.

Table 5. Effect of Cooking and MAP on Flavonoid Content of Minimally Processed Swiss Chard (Cultivar Yellow), Initially and after 8 Days of Cold Storage in Air or MAP at 6 °C[a]

	before cooking	after cooking		
treatment	methanol	water	methanol	total
initial	2.19 a	0.97 a	1.50 a	2.47
air	2.38 ab	1.14 ab	1.35 ab	2.49
MAP	2.62 b	1.32 b	1.25 b	2.57 ns

[a] Values are mean ($n = 3$) (mg g^{-1} fresh weight). Different letters indicate significant differences at $P = 0.05$; ns, not significant.

during domestic processing of MAP-stored product can be considered as a negative factor when regarding antioxidant constituents, but it could also be regarded as a desirable feature, if toxic metabolites and salts (nitrates, oxalates, etc.) were considered (F. A. Tomás-Barberán, unpublished results).

Changes in vitamin C content of Swiss chard stored both in air and modified atmospheres are shown in Figure 3. It is interesting to note that vitamic C is better preserved in air than in MAP stored tissues. After 4 days of cold storage, some significant degradation was observed to reach less than 50% of the initial vitamin C content after 8 days. Storage in air for 4 days at 6 °C did not produce any vitamin C degradation. The effect of controlled atmosphere on AA retention has been described for some comodities, broccoli florets (Barth and Zhuang, 1996), strawberry and persimmon slices (Wright and Kader, 1997). However, Agar et al. (1997) observed a reduction in vitamin C content by high CO_2 levels (10−30%) in strawberries.

As the consumption of fresh-cut Swiss chard increases, it is important to take into account that processing (minimally processing or cooking) affects antioxidant phenolics and vitamin C content. It can be concluded that the antioxidant content of Swiss chard is lower after storage under modified atmosphere than in intact produce or minimally processed product stored in air.

ABBREVIATIONS USED

AA, ascorbic acid; DHAA, dehydroascorbic acid; OPDA 1,2-phenylenediamine dihydrochloride; DFQ, 3-(1,2-dihydroxyethyl)furo[3,4-b]quinoxaline-1-one; EDTA, ethylenediaminetetraacetic acid.

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