

Health-Promoting Compounds in Broccoli as Influenced by Refrigerated Transport and Retail Sale Period

FERNANDO VALLEJO, FRANCISCO TOMÁS-BARBERÁN, AND
 CRISTINA GARCÍA-VIGUERA*

Laboratorio de Fitoquímica, Departamento de Ciencia y Tecnología de los Alimentos,
 CEBAS-CSIC, P.O. Box 4195, E-30080, Murcia, Spain

Total aliphatic and indole glucosinolates, phenolic compounds (flavonoids and hydroxycinnamoyl derivatives), and vitamin C contents were evaluated in freshly harvested broccoli (*Brassica oleracea* L., var. *italica*, cv. Marathon) inflorescences. These were film-wrapped and stored for 7 days at 1 °C to simulate a maximum period of commercial transport and distribution. After cold storage, inflorescences were kept for 3 days at 15 °C to simulate a retail sale period. For wrapping, low-density polyethylene (LDPE) of 11 μm thickness was used. Gas composition was about 17% O₂ and 2% CO₂ during cold storage and about 16% O₂ and 3–4% CO₂ during shelf life within packages. The predominant glucosinolates were 4-methylsulfinylbutyl-glucosinolate (glucoraphanin), 3-indolylmethyl-glucosinolate (glucobrassicin), and 1-methoxy-3-indolylmethyl-glucosinolate (neoglucobrassicin). The predominant hydroxycinnamoyl derivatives were identified as 1,2,2'-trisinapoylgentiobiose, 1,2-diferuloylgentiobiose, 1,2'-disinapoyl-2-feruloylgentiobiose, and 3-*O*-caffeoyl-quinic (neochlorogenic acid). Results showed major losses at the end of both periods, in comparison with broccoli at harvest. Thus, the respective losses, at the end of cold storage and retail periods, were 71–80% of total glucosinolates, 62–59% of total flavonoids, 51–44% of sinapic acid derivatives, and 73–74% caffeoyl-quinic acid derivatives. Slight differences in all compound concentrations between storage and retail sale periods were detected. Distribution and retail periods had minimal effects on vitamin C. Weight loss was monitored at the end of both periods.

KEYWORDS: Broccoli (*Brassica oleracea* L.); glucosinolates; vitamin C; phenolic compounds; MAP; postharvest; health-promoting; HPLC–MS

INTRODUCTION

Broccoli (*Brassica oleracea* L., var. *italica*, cv. Marathon) is a recognized health-promoting vegetable and one of the most important vegetables produced in the southeast of Spain. In the region of Murcia, about 70 000 tons are exported every year. The maximum time to reach a destination in some American, Asian, and European countries and to achieve its distribution from a logistic platform to retail sale is estimated to be about 6–7 days.

Epidemiological data show that a diet rich in cruciferous vegetables, such as broccoli, Brussels sprouts, and cauliflower, can reduce the risk from a number of cancers. At least some of the cancer chemoprotective activity of these vegetables is widely believed to be due to their content of minor dietary components such as glucosinolates (1–5). This is a large group of sulfur-containing compounds that occur in all the economically important Brassicaceae crops (6).

In addition, dietary flavonoids and vitamins C, E, and A are important in an optimal diet due to their antioxidant and free-

radical scavenging activities, which play important roles in human nutrition (7, 8). Due to the nutritional importance of vegetable flavonoids and other phenolic compounds, there is an increasing interest in evaluating their changes during postharvest treatments, such as effects of controlled atmosphere (9), minimal processing (10, 11), and domestic processing (12, 13).

A maximum of 4% weight loss is acceptable to avoid wilting, shrivelling, and senescence symptoms on the broccoli inflorescences (14). Benefits of high relative humidity (RH) within wrapped packages include weight loss alleviation, less cross-contamination, delaying senescence, and good retention of quality attributes, although risk of fungal development due to water condensations could increase (14).

Commonly, commercial handling conditions in Spain are rapid, humid, forced-air cooling to reach about 10 °C and hand-wrapping in 11–15 μm thickness low-density polyethylene (LDPE) under modified atmosphere packaging (MAP) conditions. To retain quality during storage and transport under controlled atmosphere (CA), O₂ levels between 1% and 3% and CO₂ levels between 5% and 10% at temperatures ranging from 0 to 5 °C have traditionally been recommended (15, 16).

* Corresponding author. E-mail cgviguera@cebas.csic.es.

As previously described for shipment (17), wrapped inflorescences are packed into telescopic corrugated fiberboard cartons of 60 × 40 × 15 cm for 8 kg, palletized, and then forced-air cooled to reach about 1–3 °C before transport. Temperatures between 1 and 5 °C for transport and distribution throughout logistic platform are generally used. Retail sale of wrapped broccoli takes about 2–3 days maximum at 15–20 °C (14).

Therefore, the purpose of the present work was to study the effect of MAP conditions created by wrapping on the postharvest behavior of health-promoting compounds present in the edible portions of fresh harvested broccoli inflorescences of Marathon cv. during simulated transport and distribution and retail sale periods. This report describes glucosinolate, phenolic compounds, and vitamin C levels among temperature and time periods in fresh-harvested broccoli inflorescences.

MATERIALS AND METHODS

Inflorescence Characteristics and Handling. Mature winter grown broccoli (*Brassica oleracea* L., var. *italica*, cv. Marathon) was hand harvested on February 24 from a field in the southeast of Murcia (Agrosol, S. C. L., Spain). Broccoli was grown according to integrated pest management cultural practices.

When the diameter of the inflorescence is larger than 1.0 dm and the compactness is at least 0.30 kg/dm (weight/diameter), broccoli is considered to be at its optimum harvesting condition for commercial purposes (14). Thus, inflorescences of broccoli were harvested when the diameter was about 1.1 dm and the weight was about 0.35–0.40 kg. Inflorescences were sorted and selected for uniform size, visual appearance, and freedom from defects.

Postharvest Treatments. Inflorescences were forced-air-cooled to reach 10 °C in 4 h and then wrapped with polymeric films at 10 °C (four inflorescences per replicate and three replicate per treatment were used). LDPE of 11 μm thickness (LDPE 11) (Filmwrap Plastic S. A. L.) was used. All the batches were placed inside 360 L gastight stainless steel chambers in a cold room at 1 ± 0.5 °C and 95% RH. The chambers were equipped with a renewal-humidification system with an air flow of 360 L/h (17). After 7 days of storage that simulated a maximum refrigerated transport and distribution period, batches of broccoli were transferred to 15 °C and 70–75% RH for 3 days to simulate a reasonable retail sale period.

Extraction and Desulfation of Glucosinolates. Desulfoglucosinolate contents were determined according to Kiddle et al. (18). Each sample (20 μL) was analyzed on a Merck-Hitachi HPLC system (Merck-Hitachi Ltd., Tokyo, Japan) consisting of a variable UV detector set at 227 nm and a Lichosphere RP-18 column (Merck, Darmstadt, Germany) (RP-18, 25 × 0.4 cm; 5 μm particle size). The mobile phase was a mixture of water (A) and acetonitrile (B). Desulfoglucosinolates were eluted off the column in 28 min with a linear gradient starting with 1% B and reaching 20% B at 28 min and 90% B at 30 min. The flow rate was 1.5 mL/min. Extraction and desulfation were done following procedures described in a previous work (13).

Extraction and Determination of Phenolic Compounds. The methodology for extraction of phenolic compounds has already been described (19). Samples (20 μL) were analyzed by HPLC (Merck-Hitachi pump L-6200) and a UV-Vis detector (L-7420) set at 320 and 360 nm. Separations were achieved on a LiChroCART column (Merck, Darmstadt, Germany, ODS-18, 25 × 0.4 cm; 5 μm particle size). The mobile phase was water–formic acid (95:5, v/v) (A) and methanol (B) eluting with a linear gradient, starting with 10% B to reach 15% B at 5 min, 30% B at 20 min., 50% B at 35 min, and 90% B at 40 min. The flow rate was 1 mL/min. Chromatograms were recorded at 320 and 360 nm. Caffeoyl-quinic acid derivatives were quantified as chlorogenic acid (5-caffeoylquinic acid, Sigma, St. Louis, MO), flavonoids as quercetin 3-rutinoside (Sigma St. Louis, MO), and sinapic acid derivatives as sinapic acid (Sigma St. Louis, MO). The results were expressed as mg/kg of broccoli fresh weight (fw).

Extraction and Determination of Vitamin C. Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined according

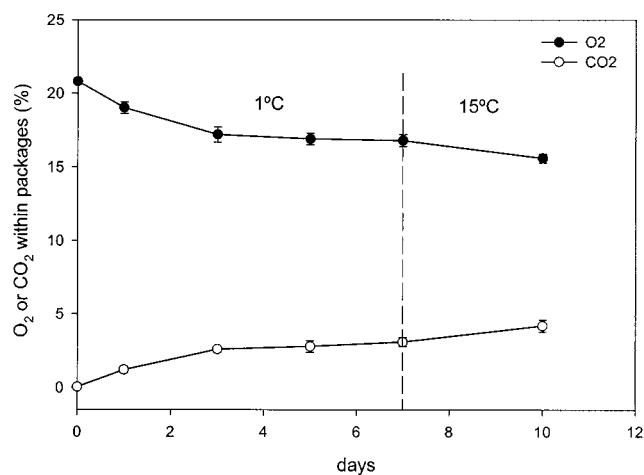


Figure 1. CO₂ and O₂ evolution within a package of broccoli during cold storage and shelf life periods.

to the method of Zapata and Dufour (20). HPLC analysis of vitamin C (AA + 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). Samples (20 μL) were 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 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. Standard solutions, column conditioning, and derivatization procedures have been previously described (19).

Gas Sampling. The changes in O₂ and CO₂ within the packages were monitored daily during both storage periods using a gas chromatograph (Shimadzu GC-14, Kyoto, Japan) equipped with a thermal conductivity detector (TCD).

HPLC–MS. These analyses were performed according to the methodology reported in a previous work (13), using an Agilent HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a binary pump (G1312A), autosampler (G1313 A), photodiode array detector (G1315) controlled by Agilent software (v. A.08.03), and degasser (G1322A), under the same chromatographic conditions above-described for HPLC analyses.

Statistical Analysis. A completely randomized experimental design was used, composed of groups of 12 inflorescences, with three replicates of four inflorescences each. A unifactorial analysis of variance (ANOVA) for each main factor was applied to the results. Mean values were compared using the least significant difference (LSD).

RESULTS AND DISCUSSION

Atmosphere Composition within Wrapped Inflorescences. During cold storage, O₂ within the packages declined from 20.8% to 17% after 3 days, followed by an equilibrium at 17%, while CO₂ had an opposite trend from 0.03% to 3% at the end of cold storage period. When exposed to shelf life conditions, O₂ dropped from 17% to 15% after 3 days while CO₂ increased from 3% to almost 5% (Figure 1). For cold storage and shelf life of broccoli, the atmosphere composition investigated was suitable according to that previously reported (17). No off-odors in any package were detected during the experiment. Weight loss (expressed as percentage of initial fresh weight) of broccoli at the end of cold storage and shelf life under MAP was always less than 0.1% at cold storage and retail sale periods.

Effects of Transport and Retail Sale Periods on Glucosinolates, Phenolic Compounds, and Vitamin C. These compounds were identified by their chromatographic behavior and

Table 1. HPLC–DAD–MS of Broccoli Inflorescence Phenolics and Glucosinolates

common name	structure	no. ^a	t _R (min)	HPLC–DAD (nm)	HPLC–MS (m/z)
Caffeoyl-quinic Derivatives					
neochlorogenic acid	3- <i>O</i> -caffeoyl-quinic	1	5.2	332, 295sh ^b	353, 179
chlorogenic acid	5- <i>O</i> -caffeoyl-quinic	2	8.4	332, 295sh	353, 179
Sinapic Acid Derivatives					
	1,2-disinapoylgentiobiose	3	29.2	328	753, 529, 223
	1-sinapoyl-2-feruloylgentiobiose	4	30.0	328, 295sh	723, 499, 223
	1,2,2'-trisinapoylgentiobiose ^c	6	30.7	328	959, 735
	1,2-disinapoyl-2-feruloylgentiobiose	7	31.4	328, 295sh	929, 705
	1-sinapoyl-2,2-diferuloylgentiobiose	8	32.3	320, 290sh	899, 705
	1,2,2'-trisinapoylgentiobiose ^c	9	33.7	328	959, 735
Feruloyl Acid Derivatives					
	1,2-diferuloylgentiobiose	5	30.4	328, 290sh	693, 499, 175
Aliphatic Glucosinolates					
glucoiberin	3-methylsulfinylpropyl-dsg ^d	1	5.1	227	366
progoitrin	2-hydroxy-3-butenyl-dsg	2	6.1	226	332
glucoraphanin	4-methylsulfinylbutyl-dsg	3	7.2	222	380
glucoalyssin	5-methylsulfinylpentyl-dsg	4	8.4	225	394
gluconapin	3-butenyl-dsg	5	9.0	225	316
glucobrassicinapin	4-pentenyl-dsg	6	16.6	225	
Aromatic/Indolyl Glucosinolates					
	4-hydroxy-3-indolylmethyl-dsg	7	12.4	222, 248, 280	407, 284, 223, 194, 146
glucobrassicin	3-indolylmethyl-dsg	8	19.1	222, 280	391, 284, 207, 130
gluconasturtiin	2-phenylethyl-dsg	9	21.7	221	
	4-methoxy-3-indolylmethyl-dsg	10	23.1	222, 246, 278	421, 284, 237, 194, 160
neoglucobrassicin	1-methoxy-3-indolylmethyl-dsg	11	29.1	222	421, 390, 237, 206, 130

^a Compounds numbered according to HPLC elution order. ^b sh: spectrum shoulder. ^c Isomeric compounds. ^d dsg: desulfoglucosinolate

Table 2. Total Flavonoids and Total and Individual Caffeoyl-quinic Derivatives (mg/kg fw) and Analysis of Variance in Broccoli at Harvest, at the End of Cold Storage, and after Shelf Life under MAP, Showing the Percentage Losses of the Total Flavonoids and caffeoyl-quinic derivatives^a

temp	time	total flavonoids ^b	caffeoyl-quinic derivatives		
			1 ^c	2	total
	at harvest	532.7	67.0	19.8	86.8
1 °C	7 days	205.1	20.6	3.0	23.6
	% loss	61.5			72.8
15 °C	3 days more	217.5	19.1	3.1	22.2
	% loss	59.2			74.4
LSD Values and ANOVA ^d					
temp		0.2***	0.1***	0.1***	0.1***
time		0.2***	0.1***	0.1***	0.1***

^a Values represent the mean of three replicates ($n = 12$). ^b Flavonoids as rutin and caffeoyl-quinic derivatives as chlorogenic acid detected at 320 nm. ^c Peak identification in Table 1. ^d * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

UV spectra, HPLC-MS (Table 1), and chromatographic comparisons with authentic standards. The phenolic compounds pattern of the cultivar was similar to that described by other authors for this same cultivar (21). A large number of flavonoids (15–20 depending on the time period) were detected, although in small amounts which made their full identification difficult (thus, they are given as total flavonoids). The predominant hydroxycinnamoyl acids were identified as 1-sinapoyl-2-feruloylgentiobiose, 1,2-diferuloylgentiobiose, 1,2,2'-trisinapoylgentiobiose, and neochlorogenic acid (Tables 2 and 3).

In addition, 11 glucosinolates were found in broccoli inflorescences (Tables 4 and 5), in agreement with previous reports (22–25). These compounds were identified by their chromatographic behavior and UV spectra, HPLC-MS, and chromatographic comparisons with authentic markers (samples supplied by R. Bennett and B. Holst, IFR, Norwich). There were

Table 3. Total and Individual Sinapic and Feruloyl Derivative Levels (mg/kg fw) and Analysis of Variance in Broccoli at Harvest, at the End of Cold Storage, and after Shelf Life under MAP, Showing the Percentage Losses of the Total Sinapic and Feruloyl Derivatives^a

temp	time	sinapic and feruloyl derivatives ^b							total
		1 ^c	2	3	4	5	6	7	
	at harvest	23.4	50.8	30.6	35.6	6.7	1.2	3.2	151.5
1 °C	7 days	14.9	42.9	6.6	9.4	0.9	0.0	0.0	74.7
	% loss								50.7
15 °C	3 days more	15.0	45.0	7.6	12.7	4.8	0.0	0.0	85.0
	% loss								43.9
LSD Values and ANOVA ^d									
temp		0.1***	0.1***	0.1***	0.1***	0.1***	0.0***	0.0***	0.1***
time		0.1***	0.1***	0.1***	0.1***	0.1***	0.0***	0.0***	0.1***

^a Values represent the mean of three replicates ($n = 12$). ^b Sinapic and feruloyl derivatives as sinapic acid detected at 320 nm. ^c Peak identification in Table 1. ^d * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

significant differences in glucosinolate concentrations at both cold storage and retail sale when compared to samples at harvest (Tables 4 and 5). The predominant glucosinolates were glucoraphanin (aliphatic glucosinolate), followed by glucobrassicin and neoglucobrassicin (indolyl glucosinolates). The glucosinolate pattern found here was similar to that previously described (22–27). Compounds glucoraphanin, 4-hydroxy-glucobrassicin, glucobrassicin, 4-methoxy-glucobrassicin, and neoglucobrassicin (Tables 4 and 5) were common in every time period.

Similar amounts of vitamin C (considered as the sum of ascorbic and dehydroascorbic acid) were detected during the whole experiment.

An analysis of variance ANOVA (unifactorial analysis) for glucosinolates, hydroxycinnamoyl acid derivatives, and vitamin C/time period was done, and as a general trend, there were not significant differences in those compounds between transport and retail sale periods.

Table 4. Total and Individual Aliphatic Glucosinolate Levels ($\mu\text{mol/g}$ dw) and Analysis of Variance in Broccoli at Harvest, at the End of Cold Storage, and after Shelf Life under MAP, Showing the Percentage Losses of the Total Aliphatic Glucosinolates^a

temp	time	aliphatic glucosinolates						total
		1 ^b	2	3	4	5	6	
	at harvest	0.2	0.1	3.1	0.2	0.5	0.5	4.5
1 °C	7 days	0.1	0.1	1.6	0.2	0.2	0.5	2.7
% loss								40.0
15 °C	3 days more	0.1	0.1	1.1	0.3	0.1	0.3	2.0
% loss								66.6
LSD Values and ANOVA ^c								
temp		0.0***	0.0***	0.1***	0.0***	0.0***	0.1***	0.1***
time		0.0***	0.0***	0.1***	0.0***	0.0***	0.1***	0.2***

^a Values represent the mean of three replicates ($n = 12$). ^b Peak identification in Table 1. ^c * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Table 5. Total and Individual Aromatic/Indolyl Glucosinolate Levels ($\mu\text{mol/g}$ dw) and Analysis of Variance in Broccoli at Harvest, at the End of Cold Storage, and after Shelf Life under MAP, Showing the Percentage Losses of the Total Glucosinolates^a

temp	time	aromatic/indolyl glucosinolates					total 7–11	total 1–11 ^c
		7 ^b	8	9	10	11		
	at harvest	0.6	7.0	0.1	0.6	12.0	20.3	24.8
1 °C	7 days	0.6	2.6	0.1	0.6	0.5	4.4	7.1
% loss							78.3	71.4
15 °C	3 days more	0.6	1.4	0.0	0.4	0.5	2.9	4.9
% loss							85.7	80.2
LSD Values and ANOVA ^d								
temp		0.0***	0.1***	0.0***	0.1***	0.0***	0.1***	0.2***
time		0.0***	0.1***	0.0***	0.1***	0.1***	0.1***	0.2***

^a Values represent the mean of three replicates ($n = 12$). ^b Peak identification in Table 1. ^c See Table 4 for the values for glucosinolates 1–6. ^d * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Table 6. Vitamin C Levels (mg/100 g fw) and Analysis of Variance in Broccoli at Harvest, at the End of Cold Storage, and after Shelf Life under MAP, Showing the Percentage Losses of the Vitamin C^a

temp	time	ascorbic acid	dehydroascorbic acid	vitamin C
	at harvest	107.7	13.7	121.4
1 °C	7 days	81.6	37.1	118.8
% loss				2.4
15 °C	3 days more	66.5	40.7	107.2
% loss				13.2
LSD Values and ANOVA ^b				
temp		0.2***	0.1***	0.1***
time		0.2***	0.1***	0.1***

^a Values represent the mean of three replicates ($n = 12$). ^b * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Transport and Distribution Period (Cold Storage). Glucosinolates, total phenolics, and vitamin C levels were significantly affected by time and temperature (Tables 2–6). Total glucosinolates presented a high loss rate during cold storage in comparison with the amount at harvest (Table 5), mainly due to the fall of the major glucosinolates present in broccoli inflorescences, glucoraphanin, glucobrassicin, and neoglucobrassicin. Thus, the most relevant aliphatic glucosinolate, glucoraphanin (precursor of anticancer isothiocyanate sulforaphane), showed a big decrease (almost 50%) in contrast to that previously reported for broccoli under MAP at 4 °C (22). Our results are in disagreement with a previous report (23) that

showed an increase in total glucosinolates during 7 days of cold storage at 4 °C. A moderate loss rate also has been previously described (25), in disagreement to our value that reached 71% (Table 5). Total indolyl loss rate was higher than that of total aliphatic, mainly due to the large loss of neoglucobrassicin, which is the major affected individual glucosinolate.

It has been shown that flavonoids are quite stable compounds during storage (28–30); however, in contrast with these authors and in agreement with others (31), we found that total flavonoid content decreased up to 61% of that measured at harvest (Table 2). An explanation for this could be the very high respiratory rate of broccoli (32) that could increase the metabolism and therefore the degradation of the phenolic compounds. Sinapic and caffeoyl-quinic derivatives showed the same trend: a high loss rate, up to 51% and 73%, respectively (Tables 2 and 3). However, the levels of the two identified caffeoyl-quinic derivative compounds were 4-fold lower than those found for sinapic and feruloyl acid derivatives. Among individual sinapic derivatives, 1-sinapoyl-2,2'-diferuloylgentiobiose and 1,2,2'-trisinapoylgentiobiose were not detected during this period.

The levels of vitamin C slightly decreased (2.4% loss) when compared with the initial values (Table 6). This behavior was clearly in contrast to that found for phenolic compounds and glucosinolates. This could be due to broccoli's potential for maintaining, during cold storage, the stability of vitamin C levels found in the fresh product. Thus, according to previous report (33), broccoli retain its vitamin C levels due to the protection of other oxygen scavengers. Those values (Table 6) were in agreement with a previous report for broccoli inflorescences under MAP during 6 days at 5 °C (34). However, our results were in contrast to that previously reported for Swiss chard under MAP, where the degradation reached around 60% after 8 days of cold storage (29).

Retail Sale Period (Shelf Life Storage). A similarity with transport period was found. Thus, the analysis of variance showed that glucosinolates, total phenolics, and vitamin C levels were significantly affected by time, temperature, and their interaction (Tables 2–6). Flavonoid content was slightly higher than in the cold storage period (Table 2), probably due to the weight loss that led to compound concentrations in the cells. However, across both transport and retail, total flavonoid content decreased almost 50% of that measured at harvest (Table 2). Sinapic and caffeic acid derivatives showed large loss rates of 44% and 74%, respectively (Tables 2 and 3). Individual sinapic derivatives 1-sinapoyl-2,2'-diferuloylgentiobiose and 1,2,2'-trisinapoylgentiobiose were not detected during this period. As happened in cold storage, caffeoyl-quinic derivatives were found in small amount (Table 2).

The highest decrease in total glucosinolate content (Table 5) was observed at this period, where the possible disruption of the old tissue (senescence after 10 days) and the temperature increase (15 °C) perhaps stimulated myrosinase activity and therefore reduced glucosinolate levels (35). Our results are in agreement with those previously reported (25) that showed loss rates of almost 80%.

Vitamin C levels slightly decreased (Table 6) in comparison with the cold storage period, with a high presence of the oxidized form (DHAA) of AA. However, a large amount of vitamin C was maintained during both periods.

In summary, slight changes in the amount of vitamin C were detected during transport and retail sale periods of broccoli. On the contrary, large losses in total flavonoids, hydroxycinnamoyl acid derivatives, and glucosinolates were detected for both periods in comparison with values of the broccoli at harvest. It

is remarkable that, during the cold transporting period, the main compound losses were detected, probably due to the long storage time (7 days). Thus, in general, the retail sale period maintained or increased slightly the loss rates in all compounds showed in cold storage, probably due to the short time period (3 days).

On the other hand, no significant effects of gas composition (Figure 1) within package on physiological disorders were found at the end of the 10 days. Thus, the good external broccoli appearance at the end of both periods did not correlate with the presence in broccoli inflorescences of health-promoting compounds. Therefore, a solution for the reduction of the losses of these compounds during cold storage period could be the limitation of exportation to maximum a of 2 or 3 days.

Finally, we can conclude that a long exportation and distribution would significantly reduce the levels of health-promoting compounds of broccoli under MAP. Thus, a faster logistic of transport and distribution system to retain health benefits might be necessary.

In general, our results were worse than we anticipated. The information available on these topics for Brassicas is very scarce and contradictory. Hence, more research on these topics is needed.

ACKNOWLEDGMENT

We thank Agrosol Co. S. C. L. for supplying the broccoli. We also thank Dr. M.I. Gil, Dr. J. C. Espín, and Dr. F. Ferreres, for their scientific support, and Dr. R. Bennett and B. Holst (IFR Norwich, UK), for providing glucosinolate standards and information on the analyzing method.

LITERATURE CITED

- Wattenberg, L. W.; Hanley, A. B.; Garany, G.; Sparmins, V. L.; Lam, L. K. T.; Fenwick, G. R. In *Diet*; Hayashi, Y., et al., Eds.; Nutrition & Cancer Japan Sci. Soc. Press: Tokyo 1986; pp 193–203.
- Tawfiq, N.; Heaney, R. K.; Plumb, J. A.; Fenwick, G. R.; Musk, S. R.; Williamson, G. Dietary glucosinolates as blocking agents against carcinogenesis: glucosinolate breakdown products assessed by induction of quinone reductase activity in murine hepallc7 cells. *Carcinogenesis* **1995**, *16*, 1191–1194.
- Fahey, J. W.; Zhang, Y.; Talalay, P. Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10367–10372.
- Rosa, E.; Heaney, R. K.; Fenwick, G. R.; Portas, C. A. M. Glucosinolates in crop plants. *Hortic. Rev.* **1997**, *19*, 99–215.
- Zhang, F.; Evans, E. J.; Bilsborrow, P. E.; Schnug, E.; Syers, J. K. Correction for protein content in the determination of the glucosinolate content of rapeseed by the X-RF method. *J. Sci. Food Agric.* **1992**, *58*, 431–433.
- Howard, L. A.; Jeffery, E. H.; Wallig, M. A.; Klein, B. P. Glucosinolates and their breakdown products in food and food products. *J. Food Sci.* **1997**, *62*, 1098–1100.
- Stähelin, H. B.; Gey, K. F.; Eichholzer, M.; Lüdin, E.; Bernasconi, F.; Thurneysen, J.; Brubacher, G. Plasma antioxidant vitamins and subsequent cancer mortality in the 12-year follow-up of the prospective Basel study. *Am. J. Epidemiol.* **1991**, *133*, 766–775.
- Stähelin, H. B.; Gey, K. F.; Eichholzer, M.; Lüdin, E. β -Carotene and cancer prevention: The Basel study. *Am. J. Clin. Nutr.* **1991**, *53*, 265S–269S.
- Gil, M. I.; Holcroft, D. M.; Kader, A. A. Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *J. Agric. Food Chem.* **1997**, *45*, 1662–1667.
- Ferreres, F.; Gil, M. I.; Tomás-Barberán, F. A. Anthocyanins and flavonoids from shredded red onion and changes during storage in perforated films. *Food Res. Int.* **1996**, *29*, 389–395.
- Ferreres, F.; Gil, M. I.; Castañer, M.; Tomás-Barberán, F. A. Phenolic metabolites in red pigmented lettuce (*Lactuca sativa*). Changes with minimal processing and cold storage. *J. Agric. Food Chem.* **1997**, *45*, 4249–4254.
- Price, K. R.; Bacon, J. R.; Rhodes, M. J. C. Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion (*Allium cepa*). *J. Agric. Food Chem.* **1997**, *45*, 938–942.
- Vallejo, F.; Tomás-Barberán, F. A.; García-Viguera, C. Glucosinolates and vitamin C content in edible parts of broccoli inflorescences after domestic cooking. *Eur. Food Res. Technol.* **2002**, *215*, 310–316.
- Namesny, A. Bróculi. In *Post-recolección de hortalizas*; Ediciones de Horticultura: Reus, Spain, 1996; Vol. 1., pp 281–298.
- Kader, A. A.; Zagory, D.; Kerbel, E. L. Modified atmosphere packaging of fruits and vegetables. *Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 1–30.
- Makhlof, J.; Castaigne, F.; Arul, J.; Willemot, C.; Gosselin, A. Long-term storage of broccoli under controlled atmosphere. *HortScience* **1989**, *24*, 637–639.
- Artés, F.; Vallejo, F.; Martínez, J. A. Quality of broccoli as influenced by film wrapping during shipment. *Eur. Food Res. Technol.* **2001**, *213*, 480–483.
- Kiddle, G. A.; Bennett, R. N.; Botting, N. P.; Davidson, N. E.; Robertson, A. A. B.; Wallsgrove, R. M. High performance liquid-chromatography separation of natural and synthetic desulfoglucosinolates and their chemical validation by spectroscopic, NMR, and CI-MS methods. *Phytochem. Anal.* **2001**, *12*, 226–242.
- Vallejo, F.; Tomás-Barberán, F. A.; García-Viguera, C. Potential bioactive compounds in health-promoting from broccoli inflorescences cultivars growing in Spain. *J. Sci. Food Agric.* **2002**, *82*, 1293–1297.
- Zapata, S.; Dufour, J. P. Ascorbic, dehydroascorbic and isoascorbic acid simultaneous determinations by reverse phase ion interaction HPLC. *J. Food Sci.* **1992**, *57*, 506–511.
- Price, K. R.; Casuscelli, F.; Colpouhoun, I. J.; Rhodes, M. J. C. Composition and content of flavonol glycosides in broccoli inflorescences (*Brassica oleracea* L.) and their fate during cooking. *J. Sci. Food Agric.* **1998**, *77*, 468–472.
- Rangkadilok, N.; Tomkins, B.; Nicolas, M. E.; Premier, R. R.; Bennett, R. N.; Eagling, D. R.; Taylor, P. W. The effect of post-harvest and packaging treatments on glucoraphanin concentration in broccoli (*Brassica oleracea* var. *italica*). *J. Agric. Food Chem.* **2002**, *50*, 7386–7391.
- Hansen, M.; Moller, P.; Sorensen, H.; Cantwell de Trejo, M. Glucosinolates in broccoli stored under controlled atmosphere. *J. Am. Soc. Hortic. Sci.* **1995**, *120*, 1069–1074.
- Kushad, M. K.; Brown, A. F.; Kurilich, A. C.; Juvik, J. A.; Klein, B. P.; Wallig, M. A.; Jeffery, E. H. Variation of glucosinolates in vegetable crops of *Brassica oleracea* L. *J. Agric. Food Chem.* **1999**, *47*, 1541–1548.
- Rodrigues, A. S.; Rosa, E. A. S. Effect of postharvest treatments on the level of glucosinolates in broccoli. *J. Sci. Food Agric.* **1999**, *79*, 1028–1032.
- Rodrigues, A. S.; Rosa, E. A. S. Total and individual glucosinolate content in 11 broccoli cultivars grown in early and late seasons. *HortScience* **2001**, *36*, 56–59.
- Lewis, J.; Fenwick, G. R.; Gray, A. R. Glucosinolates in Brassica vegetables: green-curded cauliflower (*Brassica oleracea* L. *botrytis* group) and purple-headed broccoli (*B. oleracea* L. *italica* group). *Lebensm.-Wissu.-Technol.* **1991**, *24*, 361–363.
- Gil, M. I.; Castañer, M.; Ferreres, F.; Artés, F.; Tomás-Barberán, F. A. Modified-atmosphere packaging of minimally processed 'Lollo Rosso' (*Lactuca sativa*). Phenolic metabolites and quality changes. *Z. Lebensm. Unters. Forsch. A* **1998**, *206*, 350–354.
- Gil, M. I.; Ferreres, F.; Tomás-Barberán, F. A. Effect of modified-atmosphere packaging on the flavonoids and vitamin C of minimally processed 'Swiss chard' (*Beta vulgaris* subspecies *cycla*). *J. Agric. Food Chem.* **1998**, *46*, 2007–2012.

- (30) Van der Sluis, A. A.; Dekker, M.; De Jager, A.; Jongen, W. M. Activity and concentration of polyphenolic antioxidants in apple: Effect of cultivar, harvest year, and storage conditions. *J. Agric. Food Chem.* **2001**, *49*, 3606–3613.
- (31) DuPont, M. S.; Mondin, Z.; Williamson, G.; Price, K. R. Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. *J. Agric. Food Chem.* **2000**, *48*, 3957–3964.
- (32) Izumi, H.; Watada, A. E.; Douglas, W. Optimum O₂ or CO₂ atmosphere for storing broccoli inflorescences at various temperatures. *J. Am. Soc. Hortic. Sci.* **1996**, *121*, 127–131.
- (33) Davey, M. W.; Van Montagu, M.; Inzé, D.; Sanmartín, M.; Kanellis, A.; Smirnoff, N.; Benzie, I. J. J.; Strain, J. J.; Favell, D.; Fletcher, J. Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *J. Sci. Food Agric.* **2000**, *80*, 825–860.
- (34) Barth, M. M.; Zhuang, H. Packaging design affects antioxidant vitamin retention and quality of broccoli inflorescences during postharvest storage. *Postharvest Biol. Technol.* **1996**, *9*, 141–150.
- (35) Cole, R. A. Volatile components produced during ontogeny of some cultivated crucifers. *J. Sci. Food Agric.* **1980**, *31*, 549–557.

Received for review October 22, 2002. Revised manuscript received February 3, 2003. Accepted February 3, 2003. The authors are grateful to the Fundación Seneca and Consejería de Agricultura, Agua y Medio Ambiente (AGR/6/FS/99), and the Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT/AGL2001-1125) for their financial support of this work, and F.V. is indebted for a grant.

JF021065J