Effects of Oxygen Levels on Pigment Concentrations in Cold-Stored Green Beans (*Phaseolus vulgaris* L. Cv. Perona)

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Controlled-atmosphere (CA) effects on chlorophyll and carotenoid concentrations in green beans were studied. Green bean pods (cv. Perona) were stored at 8 °C and 98% relative humidity, with and without controlled atmospheres (samples: air, 5% O_2−3% CO_2; 3% O_2−3% CO_2; 1% O_2−3% CO_2), and then transferred to 20 °C to simulate ambient temperature. Green beans stored in air at 8 °C had good appearance for 18 days, whereas an atmosphere of 1% O_2−3% CO_2 extended the storage period to 22 days. Samples stored in other assayed atmospheres (5% O_2−3% CO_2 or 3% O_2−3% CO_2) had the same shelf life as the air-stored sample (18 days). Maximum chlorophyll accumulation in air-stored green beans took place at 6 days of storage, whereas the 1% O_2−3% CO_2 stored sample showed the maximum chlorophyll concentration at 13 days of storage, and no significant increase of this pigment was observed in the first 6 days. Degradation of chlorophylls *a* and *b* did not yield the corresponding pheophytin compounds, because the pheophytin content also showed a significant decrease during storage. Green bean carotenoids showed similar changes in chlorophyll concentrations during storage in air, 3% O_2−3% CO_2, and 5% O_2−3% CO_2.

Keywords: *Green beans; cold storage; controlled atmosphere; pigments; decay; shelf life*

INTRODUCTION

Discoloration of broken green bean pods and deterioration in quality attributes commonly occur during transporting and storage of the product. Controlled-atmosphere (CA) and modified-atmosphere (MA) transport and storage have been used to improve the shelf life and quality of several horticultural products (Calderon and Barkai-Golan, 1990). Green bean (*Phaseolus vulgaris* L.) is one of the most widely consumed vegetables in Spain, but fresh product cost is high at times during the year. Spanish consumers recognize and value the flavor and taste of fresh green beans, in contrast to those of canned or frozen beans.

Green bean quality mainly depends on the cultivar and postharvest practices utilized (Stone and Young, 1985; Varseveld et al., 1985). Consumption of canned green beans has decreased in recent years, while that of frozen and fresh product has increased. In this sense, fresh produce is more appreciated by consumers due to its sensorial and nutritional characteristics.

Green beans have a high respiration rate, averaging 212 mL of CO_2 kg^{-1} h^{-1} (Sistrunk et al., 1989). One of the indicators of decreased quality in green beans is loss of chlorophyll content (Lewis, 1958). This author recommended a temperature of 7.2 °C as optimum for the cold storage of green beans in ambient air. However, other authors (Guyer and Kramer, 1950) found a significant loss in the green color of green beans stored for 10 days in air at 10 °C, whereas the loss of color was not significant at 1 °C. Groeschel et al. (1966) reported that for green bean storage the oxygen concentration had to be reduced to 2% to decrease respiration to 60% of that in ambient air, whereas carbon dioxide had little or no effect on respiration. The greatest advantage of this CA storage for beans lies in improving the color of the product by inhibiting chlorophyll breakdown. Other authors such as Lieberman and Hardenburg (1954), working with broccoli at 24 °C, found that the presence of some oxygen could cause yellowing.

Chlorophylls are the most widely distributed plant pigments and are known to be easily degraded by conditions to which foods are exposed. Previous research has shown that most chlorophylls are converted to pheophytins and other derivatives during processing, causing dramatic color changes (from bright green to olive brown). A challenge to food processors has been to prevent or minimize these reactions in attempts to produce higher quality vegetable products (Schwartz and Lorenzo, 1991). In food products, chlorophyll degradation studies have shown that chlorophyll degrades to pheophorbide via either pheophytin or chlorophyllide (Minguez-Mosquera et al., 1989; Heaton et al., 1996a). However, Ginsburg et al. (1994) have shown that chlorophyll degradation in plant foods continues beyond pheophorbide to colorless compounds. Similarly, Heaton et al. (1996b) also reported that chlorophyll degradation in whole cold-stored cabbage heads did not lead to pheophorbide accumulation, leaving the degradation of pheophorbide to colorless byproducts as the only explanation. These mechanisms are summarized in a recent review on chlorophyll catabolism by Heaton and Marangoni (1996). Also, a kinetic model for chlorophyll degradation in green tissues through pheophorbide degradation to colorless compounds was reported by Marangoni (1996) and can be utilized to help understand, and eventually help control, chlorophyll degradation in green tissues.

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Table 1. Initial Physicochemical Characteristics of Green Beans (Cv. Perona)

characteristic[a]	
weight (g)	17.60 ± 4.36
firmness	
Kramer shear press (N/g)	93.71 ± 0.01
Warner−Bratzler (N)	27.68 ± 0.49
pH	5.62 ± 0.01
titratable acidity [g (100 g of malic acid)$^{-1}$]	0.12 ± 0.01
soluble solids (°Brix)	7.03 ± 0.01
moisture (g/100 g)	91.47 ± 0.01
color (Hunter *Lab*)	
*L**	56.75 ± 3.21
*a**	10.95 ± 0.28
*b**	28.38 ± 1.57
pigments [mg (100 g of fresh wt)$^{-1}$]	
chlorophyll *a*	3.67 ± 0.05
chlorophyll *b*	1.03 ± 0.02
pheophytin *a*	0.74 ± 0.04
lutein	0.15 ± 0.03
total minor xanthophylls	0.07 ± 0.01
β-carotene	0.02 ± 0.006

[a] Values are the mean of three independent determinations ± standard deviation.

In the present work, the main objective is the characterization of individual chlorophylls and carotenoids from green bean tissue and the effects of CA storage on their concentration. This study could be used as a very useful tool to tentatively establish the biochemical pathways of pigment changes in low-oxygen cold-stored green beans.

MATERIALS AND METHODS

Plant Material. Green beans (cv. Perona) were harvested in Granada (southern Spain) and cool-transported (7 °C) to Instituto del Frío (CSIC) (Madrid, Spain) within 12 h. Initial characteristics of green bean are given in Table 1. Pods for CA studies were selected on the basis of their maturity and were disease free.

CA Storage Conditions. Pods were stored for up to 18 or 22 days at 8 °C in glass containers through which the following humidified atmospheres flowed: air (21% O_2); 5% O_2−3% CO_2; 3% O_2−3% CO_2; 1% O_2−3% CO_2. A temperature of 20 °C was used also to transfer the CA cold-stored samples at different storage periods. Product quality, in terms of pigment content, was evaluated after 6, 13, 18, and 22 days of storage. Sample transference to 20 °C was made at 6, 13, and 18 days, depending on the sample.

Atmosphere Composition Analysis. The CO_2 and ethylene concentrations were determined from 1 mL gaseous samples taken from the inlet and outlet ports of the containers and injected into an infrared analyzer and a gas chromatograph equipped with a flame ionization detector, respectively. Standards of 0.5% CO_2 and C_2H_2 at 1 mL L^{-1} were used for calibration.

Pigment Analysis. The study was carried out on green beans randomly selected for each storage condition and period; only the pods (24 pods, without seeds) were used for pigment analysis. Samples from each storage period were immediately frozen with liquid nitrogen and stored at −80 °C until analyzed.

Three individual extractions from the same sample were carried out. Homogenized sample (20 g, made from chopped and sliced pods without thawing) was ground with 1−2 g of sodium carbonate, to adjust the pH to 8−9 to prevent chlorophyll conversion to pheophytin. Chilled acetone (80 mL) was then added to the sample, and the mixture was first blended in a homogenizer (Omnimixer, model ES-207, Omni International Inc., Gainsville, VA) and centrifuged at 4000*g* for 10 min (0−5 °C). Extractions with cold acetone were repeated (usually three times) until colorless. The supernatant was transferred to a separatory funnel where diethyl ether and cold deionized water were added, as described by Cano (1991). After vigorous shaking and then standing, the aqueous layer was discarded. This washing procedure was repeated three to four times to remove acetone. The diethyl ether layer was dehydrated with fluorometric analytical grade anhydrous sodium sulfate. This solution was filtered and transferred to a beaker and evaporated under a stream of nitrogen. The residue was dissolved and diluted to 4 mL with chromatographic grade acetone. Duplicate 20 μL samples of each were injected for the HPLC analyses.

Chromatographic Conditions. A combination of isocratic and gradient chromatography systems was used to separate the oxygenated carotenoids (xanthophylls) from chlorophylls and the hydrocarbon carotenoids. A gradient mixture of methanol/water (75:25, v/v, eluent A) and ethyl acetate (eluent B) was used, for 10 min, with a semifinal composition of eluent B of 70%. The gradient eluent composition was followed for a further 10 min with a final composition of eluent B of 100%. At the end of the run the column was re-equilibrated by a new gradient condition for a further 10 min, with a final composition of eluent B of 0%. The flow rate was 1 mL min^{-1}, and the analyses were monitored at 430 nm.

HPLC Equipment. A Hewlett-Packard model 1050 (quaternary solvent delivery) equipped with a Hewlett-Packard 1040A rapid-scanning UV−vis photodiode array detector was employed. Data were stored and processed by means of a Hewlett-Packard model 9000/300 computing system and color Pro plotter. The HP-9000 computer with a built-in integration program was used to evaluate the peak area and peak height. Absorption spectra of isolated components in various solvents were recorded on a Perkin-Elmer Lambda 15 UV−vis spectrophotometer (Bodenseewerk, Germany).

Separation of pigments was performed on a C18 reverse phase Hypersil ODS column (25 cm × 4.6 mm i.d., 5 μm spherical particles) (Hewlett-Packard) with a guard column (2 cm length × 4.0 mm i.d.) packed with ODS-Hypersil C18 (5 μm particle size).

Pigment Identification. Chlorophyll and carotenoid pigments were identified according to their chromatographic behavior on HPLC and UV−vis absorption spectra, by comparing both their retention time and the absorption spectra to those of authentic chlorophyll and carotenoid pigments previously isolated and separated from vegetable tissue (Cano, 1991) or commercially (Sigma-Aldrich Co.) purchased; also, each spectral maximum was compared with those found in the literature (Köst, 1988). Examination of the pigment functional groups was carried out using specific chemical tests (Liianen-Jensen, 1971; Davies, 1976).

Data Analysis. Three samples were removed at each CA atmosphere and storage time. Values are the mean of three independent determinations for each sample. These results were analyzed for variance (ANOVA) and statistical significance by *t* test using a Statgraphics and for graphics using Harward Graphics software.

RESULTS AND DISCUSSION

In green beans (cv. Perona), three classes of pigments were identified: xanthophylls (mainly lutein), chlorophylls, and β-carotene. The most abundant pigments were identified as lutein, chlorophyll *b* (Chl b), chlorophyll *a* (Chl a), pheophytin *a* (Pheo a), and β-carotene, by comparison of their retention times and spectra with those of authentic standard pigments. The minor pigments were identified as neoxanthin, violaxanthin, luteoxanthin, 9-*cis*-neoxanthin, lutein 5,6-epoxide, and the *cis*-isomers of lutein and neoluteins A and B (Figure 1). Figure 1 also shows some of the changes induced by low-oxygen atmospheres during cold storage of green beans. The HPLC chromatograms of pigment extracts of green beans stored for 15 days at 8 °C and transferred to 20 °C for 2 days showed some differences from those of the raw green beans as an evident loss of Pheo *a* and

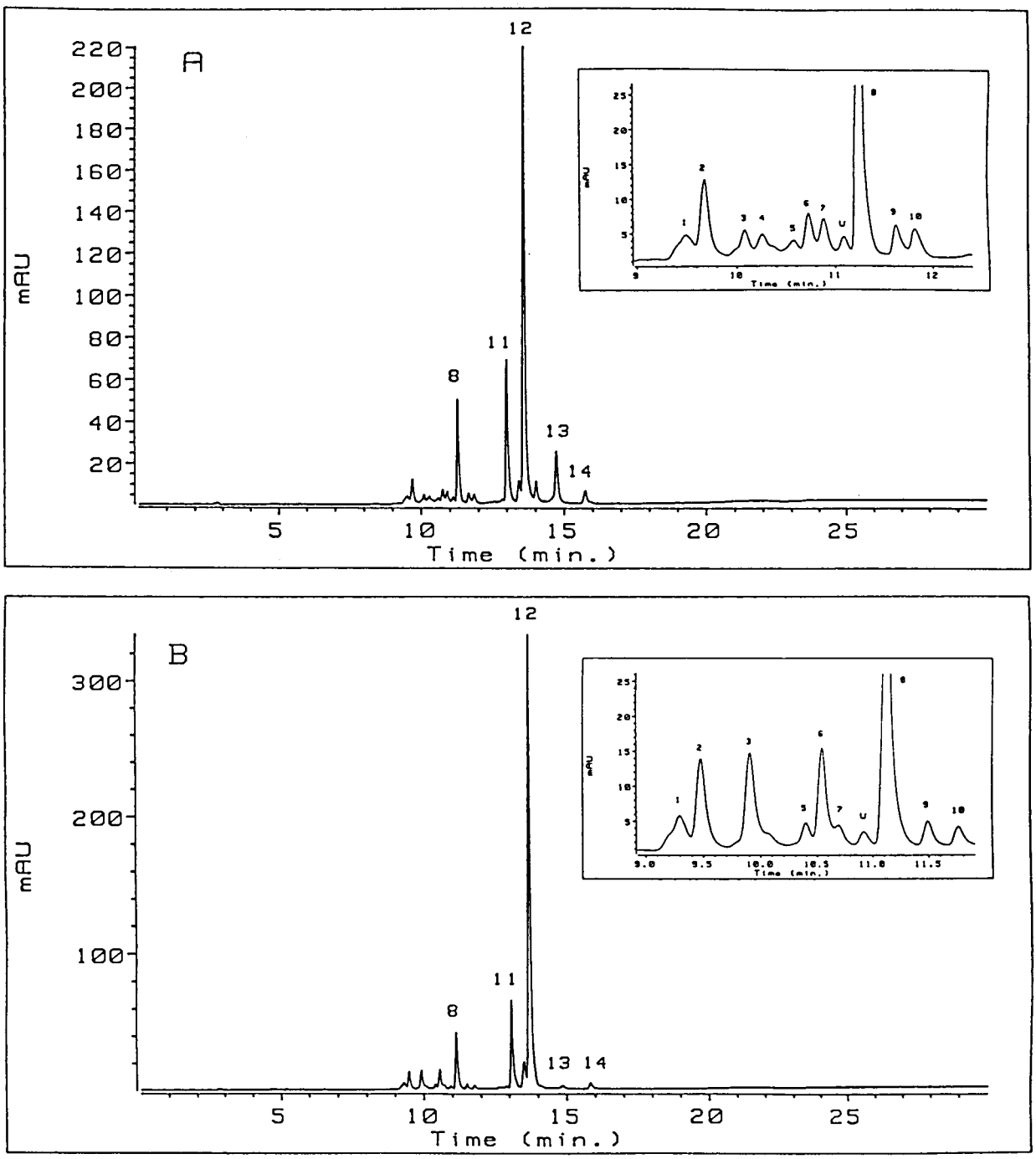

Figure 1. HPLC chromatograms of green beans (cv. Perona): (A) just-harvested green beans (0 days of air storage); (B) CA (5% O_2−3% CO_2) cold-stored green beans (15 days) at 8 °C and transferred to air at 20 °C for 2 days. Peak identification: (1) neoxanthin; (2) *cis*-neoxanthin; (3) violaxanthin; (4) luteoxanthin; (5) 9-*cis*-neoxanthin; (6) lutein 5,6-epoxide; (7) flavoxanthin; (8) lutein; (9) neolutein B; (10) neolutein A; (11) Chl *b*; (12) Chl *a*; (13) Pheo *a*; (14) β-carotene.

lutein and also an increase in the Chl *a*/Chl *b* ratio, which could indicate the acceleration of some degradation pathways due to the storage of green bean tissue at critical oxygen levels and by transfer to 20 °C.

The maximum storage period for each sample of CA- or air-stored green beans was established by taking into account quality factors such as tissue color, tissue dehydration and witherig, and/or fungi development. In this way some CA samples were successfully stored at 8 °C for 22 or 18 days, depending on the oxygen concentration (Tables 2 and 3).

Storage in Air. Figure 2 shows chlorophyll pigment changes for green beans stored at 8 °C in air and in CA. During the first 13 days of storage in air, the mean levels of Chl *a* and Chl *b* increased to maxima values of 6.74 and 1.09 mg (100 g of fresh wt)$^{-1}$, respectively. From this time, these chlorophylls decreased until the end of storage (18 days). Pheo *a* was the main degradation compound, which can easily be quantified in green bean tissue. Freshly harvested green beans contained 0.73 mg (100 g of fresh wt)$^{-1}$ of Pheo *a*. This concentration significantly decreased (by 90%), $p \leq 0.05$, in the first 6 days of storage and was only 0.035 mg (100 g of fresh wt)$^{-1}$ after 18 days of storage at 8 °C (Table 2). Transfer of air-stored green beans to 20 °C produced different effects depending on the cold storage time.

Table 2. Changes in Chlorophyll Pigment Concentrations during CA Storage of Green Beans Cv. Perona at 8 °C

treatment/CA composition	days[b]	chlorophylls[a] [mg (100 g of fresh wt)$^{-1}$]			
		Chl *a*	Chl *b*	Pheo *a*	Chl *a*/Chl *b*
air	0	3.66 ± 0.04a	1.03 ± 0.02a	0.74 ± 0.02a	3.55
	6	6.37 ± 0.09bA	0.98 ± 0.01aA	0.08 ± 0.01bA	6.50
	13	6.74 ± 0.07cA	1.10 ± 0.03aA	0.05 ± 0.00cA	6.12
	18	6.15 ± 0.08dA	1.00 ± 0.05aA	0.03 ± 0.01cA	6.15
	22				
1% O_2−3% CO_2	0	3.66 ± 0.04a	1.03 ± 0.03a	0.74 ± 0.02a	3.55
	6	3.88 ± 0.09bB	0.90 ± 0.06aA	0.44 ± 0.04bB	4.31
	13	8.00 ± 0.10cB	1.52 ± 0.05bB	0.25 ± 0.02cB	5.26
	18	6.30 ± 0.07dA	1.17 ± 0.09cA	0.25 ± 0.03cB	5.38
	22	5.77 ± 0.03e	1.03 ± 0.06a	0.16 ± 0.03d	5.60
3% O_2−3% CO_2	0	3.66 ± 0.04a	1.03 ± 0.03a	0.74 ± 0.02a	3.55
	6	7.81 ± 0.07BC	1.54 ± 0.05bB	0.29 ± 0.08bC	5.07
	13	8.00 ± 0.06bB	1.58 ± 0.05bB	0.30 ± 0.07bB	5.06
	18	7.81 ± 0.06bB	1.35 ± 0.04cB	0.14 ± 0.05cC	5.78
	22				
5% O_2−3% CO_2	0	3.66 ± 0.04a	1.03 ± 0.03a	0.74 ± 0.02a	3.55
	6	10.27 ± 0.08bD	1.77 ± 0.07bC	0.13 ± 0.04bD	5.80
	13	10.84 ± 0.11bC	2.06 ± 0.08bC	0.16 ± 0.03bC	5.26
	18	7.62 ± 0.05cB	1.32 ± 0.05cB	0.12 ± 0.02bC	5.77
	22				

[a] Values are the mean (±SD) of three independent determinations. Different letters indicate significant differences ($p < 0.05$). Lower case letters indicate differences between storage times in the same treatment. Capital letters indicate differences between treatments at the same storage time. [b] Days indicate the storage period at 8 °C

Table 3. Changes in Carotenoid Pigment Concentrations during CA Storage of Green Beans Cv. Perona at 8 °C

treatment/CA composition	days[b]	carotenoids[a] [mg/100 g of fresh wt)$^{-1}$]		
		lutein	total minor xanthophylls	*β*-carotene
air	0	0.15 ± 0.02a	0.07 ± 0.01a	0.02 ± 0.01a
	6	0.15 ± 0.03aA	0.13 ± 0.03bAB	0.02 ± 0.00aA
	13	0.15 ± 0.02aA	0.14 ± 0.02bA	0.01 ± 0.00bA
	18	0.14 ± 0.02aA	0.13 ± 0.02bA	0.03 ± 0.01aAB
	22			
1% O_2−3% CO_2	0	0.15 ± 0.02a	0.07 ± 0.01a	0.02 ± 0.01a
	6	0.14 ± 0.01aA	0.08 ± 0.02aA	0.02 ± 0.01aA
	13	0.23 ± 0.02bB	0.14 ± 0.03bA	0.04 ± 0.01aB
	18	0.16(0.02aA	0.11 ± 0.01bA	0.02 ± 0.00aA
	22	0.16 ± 0.01a	0.10 ± 0.02b	0.02 ± 0.00a
3% O_2−3% CO_2	0	0.15 ± 0.02a	0.07 ± 0.01a	0.02 ± 0.01a
	6	0.22 ± 0.03bB	0.14 ± 0.02bB	0.01 ± 0.00aA
	13	0.21 ± 0.02bB	0.15 ± 0.03bA	0.05 ± 0.01bB
	18	0.20 ± 0.01bB	0.18 ± 0.02cB	0.01 ± 0.01aB
	22			
5% O_2−3% CO_2	0	0.15 ± 0.02a	0.07 ± 0.01a	0.02 ± 0.01a
	6	0.22 ± 0.03bB	0.19 ± 0.02bcC	0.02 ± 0.00aA
	13	0.22 ± 0.02bB	0.22 ± 0.03bB	0.02 ± 0.01aA
	18	0.18 ± 0.02bAB	0.17 ± 0.02cB	0.02 ± 0.00aA
	22			

[a] Values are the mean (±SD) of three independent determinations. Different letters indicate significant differences ($p < 0.05$). Lower case letters indicate differences between storage times in the same treatment. Capital letters indicate differences between treatments at the same storage time. [b] Days indicate the storage period at 8 °C.

Green beans stored for 6 days at 8 °C and transferred to 20 °C for 2 days resulted in acceleration in the synthesis of Chl *a*, whereas samples maintained for 14 days lost Chl *a* (Figure 2). Green beans stored for 13 days and transferred to 20 °C also showed a slight decrease in Chl *a*. However, in this last sample an acceleration in Pheo *a* formation was observed.

Concentrations of carotenoid compounds in green beans also changed after cold storage at 8 °C followed by transfer to 20 °C (Figure 3). Lutein was the main carotenoid compound identified in green bean tissue, followed by *β*-carotene and other minor xanthophylls. Lutein showed a continual and slight decrease during cold storage at 8 °C in green beans. Only samples transferred to 20 °C had enhanced carotenoid synthesis, which gave a maximum lutein content in samples stored for 6 days at 8 °C and for 2 days at 20 °C. The sample stored for 13 days, which was transferred to 20 °C, showed an increase in lutein content with an increase in temperature (Figure 3). *β*-Carotene content increased during cold storage; green beans stored at 8 °C for 18 days showed an increase of 40% in the *β*-carotene content of freshly harvested green beans, and those transferred to 20 °C at different storage periods also produced a significant acceleration in the synthesis of pigment (Table 3).

CA Storage. Atmospheres containing 5% O_2 and 3% O_2 resulted in an acceleration of pigment synthesis and degradation, but the overall behavior was almost the same as that in the air-stored samples. Table 2 shows the evolution of chlorophyll pigment concentrations in air or CA storage. An atmosphere of 5% O_2−3% CO_2 produced greater accumulation of Chl *a* and Chl *b*, compared to other assay atmospheres. These CA-stored green beans showed Chl *a* and Chl *b* concentrations of 10.84 and 2.06 mg (100 g of fresh wt)$^{-1}$, respectively.

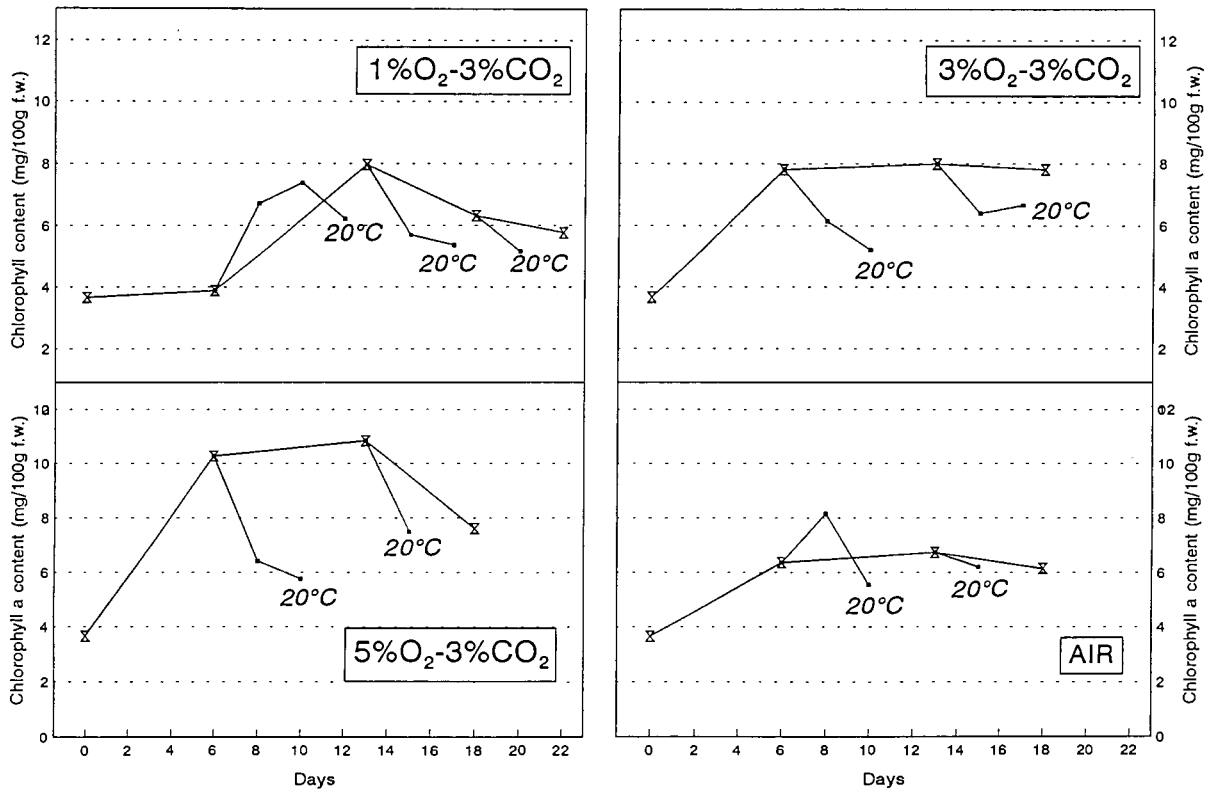

Figure 2. Changes in Chl *a* content in air- and CA-stored green beans (cv. Perona) at 8 °C and after transfer to 20 °C.

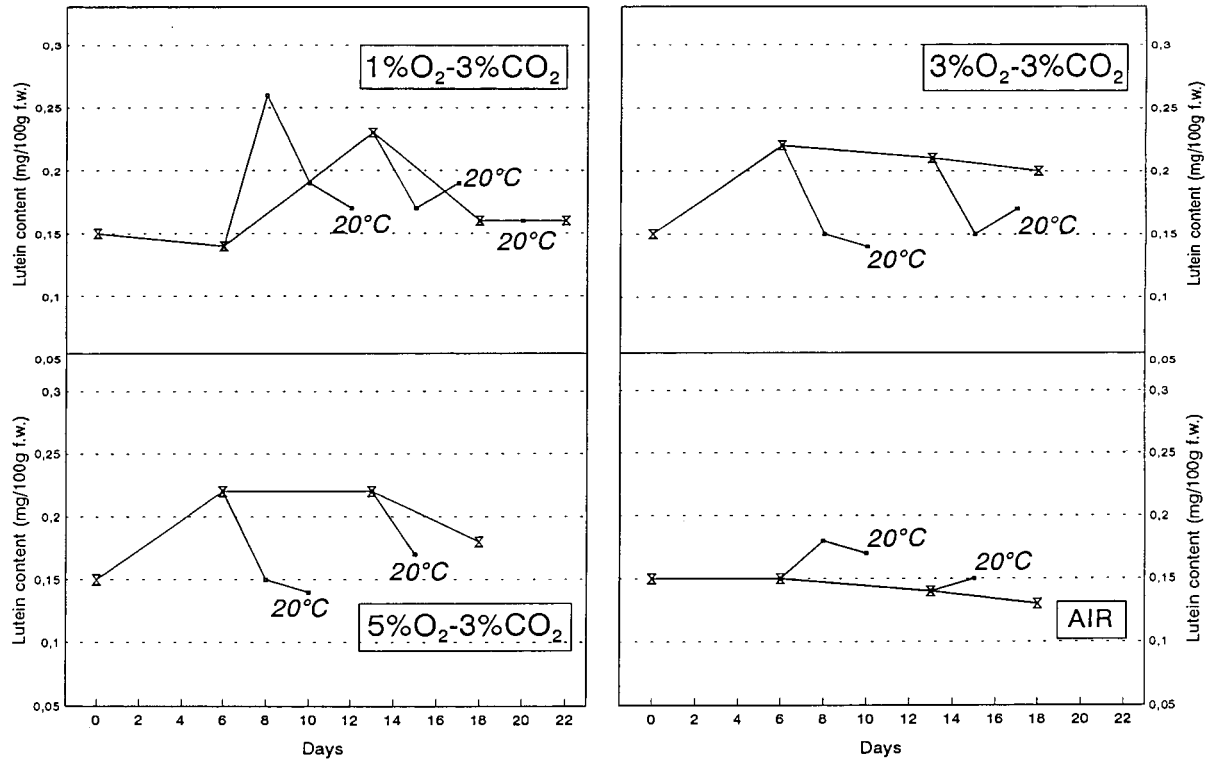

Figure 3. Changes in lutein content in air- and CA-stored green beans (cv. Perona) at 8 °C and after transfer to 20 °C.

The Chl *a*/Chl *b* ratio also had a maximum value at 6 days of storage (5.80), indicating that Chl *a* synthesis was more important than Chl *b* synthesis in these tissues. Transfer to 20 °C after different periods of storage at 8 °C showed an evident loss of Chl *a*, except for samples CA (1% O_2−3% CO_2) stored for 6 days and transferred to air at 20 °C (Figure 2).

An atmosphere containing 1% O_2−3%−CO_2 increased the shelf life of cold-stored green beans to 22 days (Table 2); pods showed a very good appearance and texture, and no chilling symptoms were observed. For chlorophyll concentration, this atmosphere (1% O_2) caused a latent period, during which chlorophyll synthesis did not take place for the first 6 days. After 6 days of storage, these CA-stored samples showed a typical accumulation of Chl *a* and Chl *b* until 13 days [8 and

1.52 mg (100 g of fresh wt)$^{-1}$, respectively] (Table 2). Green beans stored for 6 days at 8 °C and transferred to 20 °C for 2 days showed an increase in the synthesis of chlorophyll and carotenoid compounds; in samples stored for more than 6 days at 8 °C and then transferred to 20 °C, significant degradation took place (Tables 2 and 3). Other authors (Bastrash et al., 1993) reported similar results in their studies for CA storage of broccoli florets. CA storage (6% O_2−2% CO_2) resulted in extended storage of broccoli florets from 5 to 7 weeks. This CA-stored broccoli showed delayed yellowing, prolonged chlorophyll retention, and reduced development of mold and offensive odors.

Green beans stored at all controlled atmospheres showed a continuous decrease in Pheo *a* content (Table 2). CA treatment also produced an accumulation of Chl *b* (53%) after 18 days of storage at 8 °C (Table 2). Chl *a* content in CA-stored (3% O_2−3% CO_2) product showed an increase similar to that of samples CA-stored at 1% O_2−3% CO_2. However, higher concentrations of oxygen accelerated the accumulation of Chl *a* up to 3-fold, the initial content of just-harvested green beans (Table 1). Yamauchi and Watada (1993) reported that chlorophylls *a* and *b* decreased less in parsley leaves held in 10% O_2−10% CO_2 CA, but neither the pathway of chlorophyll degradation nor the xanthophyll products were altered by the presence of ethylene or CA. In the present work, there were no peaks identified in HPLC chromatograms that corresponded to chlorophyllides, which is in contrast to the above-mentioned study (Figure 1). Only Pheo *a* can be identified as the main chlorophyll degradative compound during green bean storage (Table 2).

Lutein, as the major carotenoid in green bean tissue, showed an accumulation after 13 days of storage in samples stored at 1% O_2−3% CO_2. This atmosphere produced no significant loss of lutein at the maximum storage time for this sample (22 days; Table 3). If these green beans CA-stored for 6 days were transferred to 20 °C, the lutein content showed an acceleration in synthesis up to 0.26 mg (100 g of fresh wt)$^{-1}$ (Table 3). However, if the transfer was made after 13 days of CA storage, the lutein content diminished, indicating that the green bean tissue was affected by the levels of oxygen (Figure 3). β-Carotene concentration was almost unaltered by storage when samples were CA-stored at 5% O_2−3% CO_2 and 3% O_2−3% CO_2 (Table 3). However, lower levels of oxygen (1% O_2−3% CO_2) produced a significant 2-fold increase in this carotenoid after 13 days of storage (Table 3). Total minor xanthophylls [neoxanthin, *cis*-neoxanthin, violaxanthin, lutein 5,6-epoxide, *cis*-luteins (neoluteins A and neolutein B)] also showed greater accumulation in green bean tissue CA-stored in 5% O_2−3% CO_2 for 13 days. This fact could be related to the availability of oxygen necessary for the synthesis of these oxygenated carotenoids.

Color loss of green beans was reduced with low O_2 levels in combination with moderate CO_2 concentrations. This response to these atmospheres is typical of many green tissues (Salveit, 1989). In the present work, CA storage (1% O_2−3% CO_2) of green beans (cv. Perona) prolonged the product shelf life to 22 days due to retention of pigments and pod appearance. A temperature of 8 °C together with a 1% oxygen CA was beneficial to retain the postharvest quality of green beans. These beneficial effects were especially noticeable when green beans were transferred to air at 20 °C.

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