Influence of Critical Storage Temperatures on Degradative Pathways of Pigments in Green Beans (*Phaseolus vulgaris* Cvs. Perona and Boby)

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In this work a study of critical storage temperatures on pigment degradation of green beans (*Phaseolus vulgaris*, cvs. Perona and Boby) was conducted. In this way, green beans kept better quality at 4 °C than either 8 or 12 °C, maintaining a bright green color and good texture. Nevertherless, temperatures of 4 °C induced chilling injury (CI) after eight days of storage, which became evident when the pods were transferred to 20 °C. Cold storage temperatures, 12, 8, and 4 °C, produced different changes on the green beans chlorophyll profile. Green beans of both cultivars, Perona and Boby, stored at 4 and 12 °C showed a continuous degradation of chlorophyll pigments during storage, while samples stored at 8 °C showed an increase of chlorophyll content at the first 15 days. Carotenoid pigments also suffered different changes during cold storage. Perona was the green beans cultivar which maintained the higher level of lutein, mainly when samples were stored at the most suitable temperature (8 °C).

Keywords: *Green beans; cold storage; chlorophylls; xanthophylls; shelf period; chilling; quality*

INTRODUCTION

Green beans (*Phaseolus vulgaris* L.) are one of the most popular vegetables because of their bright color, low calorie value, and pleasing taste. Also they are one of the horticultural produces with high levels of production in Europe and U.S.A. (Eskin, 1989). Green beans quality mainly depends on the cultivar and post-harvest practices (Stone and Young, 1985; Varseveld et al., 1985). Consumption of canned green beans has decreased in the last years, while that of frozen and fresh product increases continuously. In this sense, fresh produce is more appreciated by consumers due to its sensorial and nutritional characteristics.

There was as much as a 30% loss in weight in green beans during storage, while the moisture content of the pods remained the same (Guyer and Kramer, 1952). This loss is attributed to loss by respiration and transpiration. Green beans have a high respiration rate, averaging 212 mL of CO_2/kg/h (Sistrunk et al., 1989). One of the indicators of quality loss in green beans is chlorophyll.

Lewis et al. (1958) recommended a temperature of 7.2 °C as optimum to cold storage of green beans in air. However, other authors (Guyer and Kramer, 1950) found a significant loss in the green color of green beans stored 10 days in air at 10 °C, while the loss of color was not significant at 1 °C. Lieberman and Hardenburg (1954), working with broccoli at 24 °C found that the presence of some oxygen could cause yellowing.

Chlorophylls, the most widely distributed plant pigments, 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 to minimize these reactions in attemps to produce higher quality vegetable products (Schwartz and Lorenzo, 1991). However, recent studies (Ginsburg et al., 1994) have shown that chlorophyll degradation in plants continues beyond pheophorbide to colorless compounds. Heaton et al. (1996) 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), which can be utilized to help understand, and eventually help control, chlorophyll degradation in green tissues.

Fresh refrigerated prepared fruits and vegetables are becoming popular at the consumer and food service levels as convenience items, because the fresh products have been washed, peeled, cut, and packaged (sometimes under modified atmospheres). Such minimal processing of produce eliminates inedible portions as waste and brings about ready-to-use products (Huxsoll and Bolin, 1989). Green bean minimally processed products are not yet introduced in the commercial practices, but in Spain this vegetable is one of the most popular and is more consumed as a fresh product. In this sense, there is no previous work about the effects of critical temperatures of storage on chlorophyll and carotenoid pathways in fresh tissue of green beans using the individual pigment evaluation by HPLC. In the literature there is only one study about the HPLC determination of major pigments in the bean *Phaseolus*

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vulgaris (López-Hernández et al. (1993), but no identification and quantification of minor xanthophylls were made; only lutein and β-carotene together with chlrophylls were evaluated.

The main objective of the present work is to study the effects of critical temperatures on the chlorophyll and carotenoid pathways by using HPLC and the study of the probable development of chilling injury process during storage of two cultivar of green beans, cvs. Perona and Boby, to select the most suitable one for minimal processing in terms of color maintenance throught cold storage.

MATERIALS AND METHODS

Plant Material. Green beans (*Phaseolus vulgaris* L. cvs. Perona and Boby) were harvested in Granada (Southern Spain) and cool transported to Instituto del Frío (CSIC) (Madrid) within 12 h. Pods for cold storage studies were selected on the basis of their maturity and were disease free.

Storage Conditions. Green beans were stored at 4, 8, and 12 °C and 98% relative humidity. At each storage period a portion of each sample was transferred to another room cabinet at 20 °C, to establish their shelf life.

Pigment Analysis. The study was carried out on green beans selected randomly for each storage condition and period; only the pods (without seeds) were used for pigment analysis. At each storage period samples were removed from storage, immediately frozen with liquid nitrogen, packed in aluminum foil bags, and stored at −80 °C until analysis (no more than 7 days).

Twenty grams of homogenized sample (made from chopped and sliced pods without thawing) was ground with 1−2 g of anhydrous sodium carbonate (PRS grade, Panreac, Spain), to adjust the pH to 8−9 to prevent chlorophyll conversion to pheophytin. Chilled acetone (80 mL) (HPLC grade, Lab-Scan, Ireland) was then added to the sample, and the mixture was first blended in an homogenizer (Omnimixer, Model ES-207, Omni International Inc., Gainsville, VA) and centrifuged at 4000*g* for 10 min (0−5 °C). The supernatant was transferred to a separatory funnel, and diethyl ether (HPLC grade, Lab-Scan, Ireland) and cold deionized water were added, as described by Cano (1991). After vigorous shaking and standing, the aqueous layer was discarded. The washing procedure was repeated 3−4 times to remove acetone. The diethyl ether layer was dehydrated with fluorometric analytical grade sodium sulfate anhydride (PRS grade, Panreac, Spain). This solution was filtered, transferred to a beaker, and evaporated under a stream of nitrogen. The residue was dissolved and diluted to 4 mL with chromatographic grade acetone (HPLC grade, Lab-Scan, Ireland). Duplicate 20 μL samples were injected for each for the HPLC analysis.

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

A combination of isocratic and gradient chromatography separated the oxygenated carotenoids (xanthophylls) from chlorophylls and the hydrocarbon carotenoids. A gradient mixture of methanol/water (75:25) (HPLC grade, Lab-Scan, Ireland), eluent A, and ethyl acetate (HPLC grade, Lab-Scan, Ireland), eluent B, was used, begining at time 0 until time 10 min, with a semifinal composition of eluent B (70%). The gradient eluent composition was followed at time 10 until time 20 min with a final composition of eluent B (100%). At the end of the run the column was reequilibrated by a new gradient condition begining at time 20 until time 30 min, with a final composition of eluent B (0%). The flow rate was 1 mL/min, and the runs were monitored at 430 nm.

HPLC Equipment. A Hewlett-Packard Model 1050 (quaternary solvent delivery) equipped with a Hewlett-Packard 1040A rapid-scanning UV/visible 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 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/visible spectrophotometer (Bodenseewerk, Germany).

Pigment Identification and Quantification. Chlorophyll and carotenoid pigments were identified according to their chromatographic behavior on HPLC and UV/visible absorption spectra, by comparing both their retention time and the absorption spectra to those of authentic chorophyll and carotenoid pigments previously isolated and separated from vegetable tissue (Cano, 1991) or commercial purchased: chlorophyll *a* (E_{662}^{M} = 8.2−8.6 × 10^4; E_{428}^{M} = 10.3−11.0 × 10^4); chlorophyll *b* (E_{644}^{M} = 5.3−5.6 × 10^4; E_{452}^{M} = 15.0−15.9 × 10^4); β-carotene (type IV, $E_{450}^{1\%}$ = 2450−2590; $E_{478}^{1\%}$ = 2160−2280); and lutein (approximately 70%) (Sigma-Aldrich, S.A., Spain). Spectral maximum of each peak was also compared with those found in the literature (Köst, 1988). Examination of the pigment functional groups was carried out using specific chemical tests (Liaanen-Jensen, 1971; Davies, 1976). Quantification of each pigment was carried out by external standard method using commercial pigment standards (Sigma-Aldrich, S.A., Spain), standard gifts from Hoffman La Roche (violaxanthin, flavoxanthin), or isolated and purified pigments from real green beans samples. Standard curves were prepared by plotting different concentrations of each pigment versus area measurement in HPLC chromatograms. The efficiency of the procedure was determined against a control sample fortified with 1.0, 0.5, and 0.2 mg/100 g fw of each pigment standards, mainly chlorophyll *a*, chlorophyll *b*, lutein, and β-carotene prior to start the extraction procedure. Recoveries ranged between 85 and 90% for chlorophyll *a* and chlorophyll *b*, and between 80 and 90% for xanthophyll, lutein, and β-carotene.

Data Analysis. Values are average of three independent determinations. These results were analyzed for variance (ANOVA) and statistical significance by T-test using a Statgraphics and for graphics using a Harward Graphics.

RESULTS AND DISCUSSION

In green beans (cvs. Perona and Boby), three classes of pigments were identified: xanthophylls (mainly lutein), chlorophylls, and β-carotene. The most abundant pigments were identified as lutein, chlorophyll *b*, chlorophyll *a*, pheophytin *a*, and β-carotene, by comparison between their retention times and spectra with the authentic standard pigments. The minor pigments were identified as *trans*- and *cis*-neoxanthin (1 and 2), violaxanthin (3), luteoxanthin (4), 9-*cis*-neoxanthin (5), lutein epoxide (6), flavoxanthin (7), and the isomers cis of lutein, neolutein A and B (9 and 10) (Figure 1). Some differences in pigment profiles were observed among green beans cultivars. Both cultivars showed the presence of pheophytin *a*, being this chlorophyll derivative most abundant in cv. Perona than in Boby green beans extracts (Tables 1 and 2). López-Hernández et al. (1993) reported a study of HPLC determination of green bean, var. Helda, pigments showing the presence of chlorophylls and carotenoids; but they did not quantifie the minor xanthophylls, *cis*-neoxanthin, violaxanthin, luteoxanthin, lutein epoxide, flavoxanthin, and the isomers cis of lutein, which are evaluated in the present work as total minor xanthophylls content (Tables 1 and 2).

Tables 1 and 2 show the pigment content evolution (chlororophylls and carotenoids) during cold storage of green beans cvs. Perona and Boby, respectively. Both cultivars showed different changes in pigment composition when they were cold stored. Maximum storage

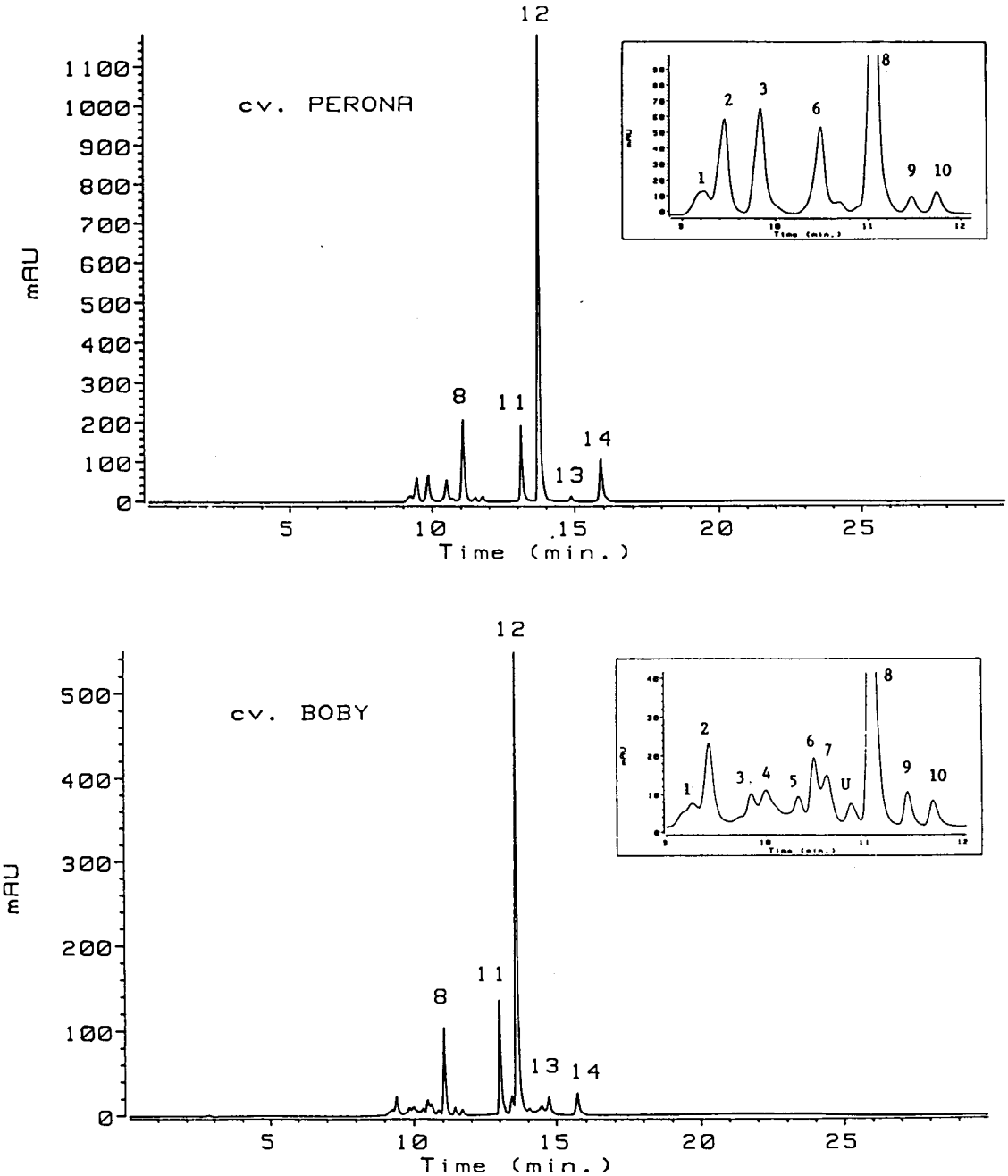

Figure 1. HPLC chromatograms of pigment extract from green beans, cvs. Perona and Boby. Peak identification: (1) neoxanthin; (2) *cis*-neoxanthin; (3) violaxanthin; (4) luteinxanthin; (5) 9-*cis*-neoxanthin; (6) lutein epoxide; (7) flavoxanthin; (8) lutein; (9) neolutein B; (10) neolutein A; (11) chlorophyll *b*; (12) chlorophyll *a*; (13) pheophytin *a*; and (14) *β*-carotene.

period for each green bean cultivar was established taking into account other quality factors together with tissue color, the other factors being tissue dehydration and withering or/and fungi developing. In this way, green beans cv. Perona can be successfully stored during 26 days at 8 °C; meanwhile in cv. Boby maximum the storage time was 17 days at this same temperature, in terms of overall quality of the product. Boby green beans showed a very great fungi development that severely limit their shelf life; also symptoms of tissue dehydration and withering were observed at this time.

Chlorophyll *a* showed a continuous decrease until 15 days in green beans cv. Perona stored at 12 °C, with 20 days being the maximum storage time for this temperature, showing a significant ($p < 0.05$) loss of chlorophyll *a*, 56% of the freshly harvested product. Chlorophyll *a* degradation was greatly affected when green beans cv. Perona were stored 15 days at 12 °C and transferred to 20 °C, producing a great loss of this pigment that severely limit the product shelf-period, Table 1. If green beans, cv. Perona, were stored at 8 °C, the maximum storage time was increased to 26 days (Table 1), since product appearance was acceptable; however, chlorophyll *a* showed a significant decrease from 15 days of storage (47%). Perona green beans stored at 4 °C showed a continuous decrease in chlorophyll *a*, which increases up to 33% of initial content at 26 days of storage. However, Boby green beans only were successfully stored for 17 days, when a temperature of 8 °C was employed, Table 2. This pigment, chlorophyll *a*, showed similar trends in Boby samples stored at the three assayed temperatures. A significative increase of chlorophyll *a* took place at seven days of storage, and from this date a continuous pigment

Table 1. Changes in Chlorophyll and Carotenoid Pigments in Green Beans Cv. Perona, Stored at Different Temperatures and Shelf Period at 20 °C

treatment			chlorophylls[a] (mg/100 g fw)				carotenoids[a] (mg/100 g fw)		
temperature	days[b]		chlorophyll *a*	chlorophyll *b*	pheophytin *a*	Chl *a*/Chl *b*	lutein	minor xanthophylls	β-carotene
sample 12 °C	0		17.85 ± 0.52	2.73 ± 0.07	0.31 ± 0.01	6.53	0.62 ± 0.01	0.41 ± 0.01	0.31 ± 0.01
	8		11.65 ± 1.04	1.85 ± 0.03	0.53 ± 0.1	6.29	0.47 ± 0.09	0.29 ± 0.01	0.16 ± 0.01
→ 20 °C		2	10.65 ± 0.37	2.51 ± 0.01	0.66 ± 0.03	4.24	0.55 ± 0.02	0.29 ± 0.02	0.17 ± 0.00
		4	9.6 ± 0.8	1.66 ± 0.19	0.55 ± 0.02	5.78	0.52 ± 0.06	0.28 ± 0.00	0.15 ± 0.02
		6	10.77 ± 0.95	2.65 ± 0.03	0.47 ± 0.06	4.06	0.47 ± 0.00	0.28 ± 0.01	0.13 ± 0.02
	15		7.89 ± 0.55	1.36 ± 0.03	0.66 ± 0.12	5.80	0.47 ± 0.02	0.28 ± 0.01	0.13 ± 0.00
→ 20 °C		2	4.83 ± 0.11	1.23 ± 0.02	0.45 ± 0.04	3.92	0.22 ± 0.00	0.27 ± 0.00	0.06 ± 0.00
		4	8.16 ± 0.6	1.36 ± 0.02	0.5 ± 0.01	6.00	0.24 ± 0.03	0.26 ± 0.01	0.08 ±0.00
	20		7.86 ± .16	1.97 ± 0.02	0.9 ± 0.03	3.98	0.74 ± 0.00	0.37 ± 0.02	0.16 ± 0.00
→ 20 °C		2							
	26								
sample 8 °C	0		17.85 ± 0.52	2.73 ± 0.08	0.31 ± 0.01	6.53	0.62 ± 0.01	0.35 ± 0.01	0.31 ± 0.01
	8		20.57 ± 0.07	0.57 ± 0.05	0.24 ± 0.08	36.08	0.63 ± 0.01	0.45 ± 0.01	0.35 ± 0.00
→ 20 °C		2	36.42 ± 1.83	4.05 ± 0.06	0.32 ± 0.01	8.99	1.31 ± 0.19	0.45 ± 0.02	0.57 ± 0.12
		4	33.55 ± 0.03	2.72 ± 0.03	0.24 ± 0.01	12.33	0.76 ± 0.01	0.43 ± 0.00	0.23 ± 0.00
		6	22.57 ± 0.93	3.52 ± 0.03	0.31 ± 0.02	6.41	0.98 ± 0.05	0.44 ± 0.01	0.28 ± 0.01
	15		20.98 ± 1.01	2.72 ± 0.42	0.27 ± 0.01	7.71	0.67 ± 0.04	0.44 ± 0.02	0.34 ± 0.02
→ 20 °C		2	20.31 ± 0.05	3.04 ± 0.04	0.59 ± 0.03	6.68	0.96 ± 0.05	0.42 ± 0.01	0.3 ± 0.00
		4	15.79 ± 0.49	2.41 ± 0.03	0.29 ± 0.00	6.55	0.71 ± 0.17	0.32 ± 0.00	0.24 ± 0.01
	20		9.28 ± 0.00	1.21 ± 0.01	0.13 ± 0.02	7.66	0.34 ± 0.02	0.26 ± 0.02	0.16 ± 0.01
→ 20 °C		2	7.89 ± 0.04	0.37 ± 0.00	0.11 ± 0.01	21.32	0.45 ± 0.01	0.21 ± 0.01	0.12 ± 0.00
	26		10.78 ± 0.30	1.6 ± 0.06	0.22 ± 0.00	6.73	0.47 ± 0.02	0.28 ± 0.01	0.17 ± 0.01
sample 4 °C	0		17.85 ± 0.52	2.73 ± 0.08	0.31 ± 0.01	6.53	0.62 ± 0.01	0.35 ± 0.01	0.31 ± 0.01
	8		16.4 ± 0.21	2.51 ± 0.05	0.19 ± 0.03	6.53	0.46 ± 0.01	0.28 ± 0.00	0.2 ± 0.00
→ 20 °C		2	23.11 ± 0.16	3.95 ± 0.04	0.46 ± 0.00	5.85	0.6 ± 0.01	0.27 ± 0.01	0.25 ± 0.00
		4	13.83 ± 0.02	2.14 ± 0.01	0.29 ± 0.02	6.46	0.36 ± 0.00	0.25 ± 0.00	0.15 ± 0.00
		6	20.72 ± 0.03	3.34 ± 0.02	0.35 ± 0.03	6.20	0.54 ± 0.00	0.26 ± 0.01	0.25 ± 0.01
	15		14.17 ± 0.05	2.02 ± 0.02	0.2 ± 0.07	7.01	0.39 ± 0.00	0.29 ± 0.01	0.19 ± 0.01
→ 20 °C		2	13.5 ± 0.53	2.27 ± 0.11	0.18 ± 0.02	5.94	0.45 ± 0.02	0.27 ± 0.01	0.18 ± 0.01
		4	12.86 ± 0.07	2.28 ± 0.02	0.51 ± 0.02	5.64	0.46 ± 0.01	0.28 ± 0.01	0.2 ± 0.00
	20		14.1 ± 0.42	2.1 ± 0.06	0.51 ± 0.01	6.71	0.39 ± 0.01	0.25 ± 0.02	0.17 ± 0.00
→ 20 °C		2	12.25 ± 0.07	1.05 ± 0.01	0.18 ± 0.2	11.66	0.35 ± 0.00	0.22 ± 0.01	0.13 ± 0.00
	26		11.99 ± 0.19	0.33 ± 0.01	0.11 ± 0.01	36.33	0.34 ± 0.00	0.22 ± 0.00	0.12 ± 0.00

[a] Values are the mean (±SD) of three independent determinations. [b] Days at the left column indicates the storage period at the assayed temperature and right day column indicates the shelf period by transfer to 20 °C fresh weight (fw).

degradation was observed. Boby green beans stored at the lower temperature, 4 °C, showed the greater chlorophyll *a* degradation (49% of initial value, 4.12 mg/100 g fw) at the end of storage time (17 days), Table 2.

Storage studies conducted at 20 °C showed an accumulation of the most abundant pigments (chlorophyll *a*, chlorophyll *b*, and pheophytin *a*) up to the first four days; from this date the degradation pathways became more important, causing a significant decrease of their pigment content. Carotenoid content, lutein, and β-carotene also showed an increase during the first six days of storage at 20 °C, but again a significant decrease of these pigments was observed at the end of the assay (eight days, senescence).

Chlorophyll *b* continuously decreased up to 20 days of storage, at 12 and 4 °C (50% and 23%, respectively) for Perona green beans, being the maximum shelf life 20 days for samples stored at the higher assayed temperature (12 °C). However, samples stored at 8 °C suffered a maximum in this pigment at 15 days of storage, showing the greater chlorophyll *b* content in green beans stored at 8 °C for 15 days and transferred to 20 °C for 2 days (3.04 mg/100 g fw), Table 1. Boby green beans showed different behavior regarding chlorophyll *b* evolution, Table 2. Again, samples stored at 12 and 4 °C showed a similar trend. In these samples chlorophyll *b* content slightly increases up to eight days of storage. From this date all samples stored at chilling temperatures, 4, 8, and 12 °C, showed an evident decrease, being more important in Boby green beans stored at 4 °C (64% of initial chlorophyll *b* content).

Attending to the evolution of the most important chlorophyll derivative, **pheophytin *a***, which can be identified from the extracts of green beans (Figure 1), the effects of cooling temperatures were different depending on the green beans cultivar. In just harvested green beans, pheophytin *a* was present in pigment extracts in amounts of 0.31 and 0.55 mg/100 g fw, for Perona and Boby cultivars, respectively (Tables 1 and 2). In Perona samples, pheophytin *a* increased continuously up to 20 days of storage at 12 °C; meanwhile at 8 °C this derivative was almost unchanged up to 15 days. From this date pheophytin *a* disappeared up to 60% of the initial value. Storage at the lower assayed temperature, 4 °C, produced an evident degradation of pheophytin *a* (Table 1), which rends a total loss of 88% of the initial value. Taking into account that for this same sample, green beans cv. Perona stored at 4 °C, chlorophyll *a* also showed a continuous degradation. Other pigment degradative pathways differ in that only pheophynization must take place in green beans tissues as a consequence of chilling. However, green beans cv. Boby, stored at this same temperature (4 °C), showed an accumulation of pheophytin *a* (110%) at the first seven days of storage, which was degradated from this date up to the end of storage (17 days). Boby samples stored at 12 and 8 °C showed a continuous decrease of pheophytin *a*, which was more important for samples stored a 12 °C, possibly due to the high respiration rate of the green bean tissue at this temperature, Table 2.

Carotenoid pigment evolution in green beans was also affected by cooling temperatures, Tables 1 and 2. Total

Table 2. Changes in Chlorophyll and Carotenoid Pigments in Green Beans Cv. Boby, Stored at Different Temperatures and Shelf Period at 20 °C

treatment		chlorophylls[a] (mg/100 g fw)				carotenoids[a] (mg/100 g fw)		
temperature	days[b]	chlorophyll *a*	chlorophyll *b*	pheophytin *a*	Chl *a*/Chl *b*	lutein	minor xanthophylls	*β*-carotene
sample 12 °C	0	8.42 ± 0.86	1.91 ± 0.02	0.55 ± 0.06	4.40	0.25 ± 0.00	0.13 ± 0.01	0.07 ± 0.01
	7	12.22 ± 0.44	2.26 ± 0.44	0.39 ± 0.02	5.40	0.42 ± 0.06	0.2 ± 0.01	0.11 ± 0.02
→ 20 °C	2	8.55 ± 0.42	1.52 ± 0.00	0.17 ± 0.00	5.62	0.25 ± 0.02	0.18 ± 0.02	0.09 ± 0.01
	4							
	6							
	14	10.04 ± 0.03	1.84 ± 0.09	0.3 ± 0.00	5.45	0.28 ± 0.00	0.2 ± 0.01	0.11 ± 0.00
→ 20 °C	2							
	17							
sample 8 °C	0	8.43 ± 0.86	1.91± 0.02	0.55 ± 0.06	4.41	0.25 ± 0.00	0.13 ± 0.01	0.07 ± 0.01
	7	10.16 ± 0.52	1.73 ± 0.02	0.44 ± 0.01	5.87	0.22 ± 0.01	0.12 ± 0.01	0.07 ± 0.00
→ 20 °C	2	9.46 ± 0.72	1.8 ± 0.17	0.58 ± 0.08	5.25	0.22 ± 0.00	0.11 ± 0.00	0.08 ± 0.00
	4	5.97 ± 0.00	0.39 ± 0.01	0.30 ± 0.01	15.30	0.15 ± 0.01	0.09 ± 0.00	0.05 ± 0.00
	6	14.2 ± 0.65	2.55 ± 0.02	0.24 ± 0.01	5.56	0.45 ± 0.01	0.1 ± 0.02	0.14 ± 0.00
	14	7.96 ± 0.22	1.49 ± 0.01	0.39 ± 0.01	5.34	0.19 ± 0.00	0.09 ± 0.01	0.08 ± 0.00
→ 20 °C	2	8.55 ± 0.26	1.59 ± 0.01	0.3 ± 0.01	5.37	0.24 ± 0.00	0.07 ± 0.01	0.09 ± 0.00
	17	8.46 ± 0.07	1.58 ± 0.04	0.22 ± 0.01	5.35	0.24 ± 0.00	0.1 ± 0.02	0.09 ± 0.00
sample 4 °C	0	8.43 ± 0.86	1.91 ± 0.02	0.55 ± 0.06	4.41	0.25 ± 0.00	0.12 ± 0.01	0.07 ± 0.01
	7	11.14 ± 0.47	2.73 ± 0.34	1.17 ± 0.17	4.08	0.54 ± 0.00	0.23 ± 0.00	0.12 ± 0.00
→ 20 °C	2	17.65 ± 1.88	3.43 ± 0.03	0.83 ± 0.05	5.14	0.64 ± 0.01	0.27 ± 0.01	0.16 ± 0.01
	4	5.64 ± 0.33	1.08 ± 0.03	0.34 ± 0.05	5.22	0.22 ± 0.00	0.2 ± 0.00	0.11 ± 0.00
	6	6.1 ± 0.23	1.59 ± 0.00	0.42 ± 0.03	3.83	0.19 ± 0.01	0.09 ± 0.02	0.06 ± 0.00
	14	5.72 ± 0.6	0.98 ± 0.07	0.32 ± 0.00	5.83	0.08 ± 0.05	0.15 ± 0.00	0.02 ± 0.00
→ 20 °C	2	4.64 ± 0.6	0.94 ± 0.06	0.2 ± 0.00	4.93	0.03 ± 0.00	0.04 ± 0.00	0.02 ± 0.00
	17	4.12 ± 0.21	0.97 ± 0.01	0.26 ± 0.03	4.24	0.04 ± 0.00	0.02 ± 0.01	0.01 ± 0.00

[a] Values are the mean (±SD) of three independent determinations. [b] Days at left column indicates the storage period at the assayed temperature and right day column indicates the shelf period by transfer to 20 ° C fresh weight (fw).

xanthophylls, oxygenated carotenoids, showed similar evolution when samples were stored at 12 and 4 °C, meanwhile at 8 °C, xanthophylls showed a slight increase up to 15 days to continue with a significant degradation up to 26 days of storage. Storage at high temperature (12 °C) affected the respiration rate of the tissue, and this stress could rend an increase of degradative reactions. In contrast, chilling temperatures as 4 °C could produce a chloro- and chromoplast damage and consequently an increase in pigment degradation. However, in Boby green beans, the xanthophyll changes induced by critical storage temperatures were different than those observed for Perona cultivar, Table 1. HPLC analysis of Boby extracts showed that storage of the product at 8 °C produced the minor changes in xanthophyll content. However, samples stored at 12 and 4 °C showed a significant accumulation of xanthophylls up to 14 days of storage. Green beans stored at the lower assayed temperature (4 °C) showed the greater reduction in total xanthophyll content at the end of the storage (17 days), Table 2.

Lutein, the most abundant oxygenated carotenoid present in green beans extracts, showed a similar evolution of total xanthophylls during cold storage, Tables 1 and 2. In Perona green beans, lutein content was almost unchanged up to 15 days of storage when they were stored at 8 °C, meanwhile samples stored at 12 and 4 °C showed an slight decrease up to this date (Table 1). Boby green beans showed a slight modification of lutein content during storage at 12 °C, meanwhile the extreme temperatures produced an increase in lutein content in the first seven days of storage, but from this date the pigment degradation was up to 80%, Table 2.

***β*-Carotene** did not significantly change in Perona green beans stored during 15 days at 8 °C, Table 1. However, when storage was carried out at 12 and 4 °C *β*-carotene showed a significant decrease up to the end of storage. There were no differences between storage temperatures in *β*-carotene content at 20 days, but green beans stored at 4 °C showed other quality problems at 26 days of storage and a temperature of 12 °C only can be successfully employed for a period of 20 days. Different results were obtained for Boby green beans. In these samples, *β*-carotene showed a significant increase at temperatures of 12 and 4 °C. From seven days of storage at 4 °C, *β*-carotene content suffered a great decrease (80% of the fresh harvested product content), while at 8 °C a continuous increase was shown through the entire storage period.

Results obtained from the experiments of cold stored product shelf-period at 20 °C at different temperature regimes and times are also shown in Tables 1 and 2. Perona green beans stored at the three assayed temperatures, 12, 8, and 4 °C, at different periods up to 15 days, and tranferred to 20 °C showed an increase in the chlorophyll *a* synthesis probably due to the tissue respiration increase being more evident in samples stored at 8 and 4 °C, Table 1. However, samples stored since 15 days suffered a significative chlorophyll *a* degradation when transfer to 20 °C was carried out. This fact severely limits the shelf-period of Perona green beans stored at chilling temperatures, producing tissue yellowing. Green beans, cv. Boby, only showed an increase in chlorophyll *a* due to the sample transfer, when they were stored at 4 °C for seven days, Table 2. Boby green beans stored at 8 °C showed a significant ($p < 0.05$) decrease in chlorophyll *a* content when the transfer was carried out at nine days of storage.

Some authors (Groeschel et al., 1966; James, 1953) stated that yellowing in green beans is caused by breakdown of chlorophyll. They suggested that breakdown of the protein which is attached to the chlorophyll molecule within the chloroplasts removes the natural protection it affords the chlorophyll. The chlorophyll is then labile. Also these authors reported that the rate

of tissue respiration was related to chlorophyll breakdown and that the green beans stored in air are subject to low-temperature damage "chilling injury".

Lutein content changes due to cold stored samples transferred to 20 °C are shown in Tables 1 and 2. Perona green beans stored at 8 °C showed an evident acceleration on lutein synthesis when cold stored 7, 15, and 20 days at 8 °C; samples were transferred to 20 °C, Table 1. However, Perona samples stored at 12 °C only showed this increase in lutein content when the transfer was carried out at seven days of storage. Green beans stored at 12 °C for 15 days showed a significant degradation of lutein due to the transfer, indicating that this tissue was stressed as a consequence of the high storage temperature. Similar results were obtained regarding to Boby green beans, Table 2. Cold storage at 12 °C severely limits the shelf-period in terms of tissue color. Green beans stored at low temperature, 4 °C, showed developing of latent chilling injury symptoms when transfer was carried out at prolonged storage times. Perona samples stored 20 days at this temperature showed a significant acceleration of lutein degradation, and Boby samples showed this behavior when transfer was made at 14 days of cold storage, Tables 1 and 2.

In the present work, chilling injury symptoms observed in 4 °C green beans were more severe than the observed ones in samples stored at the higher assayed temperatures. This fact could indicate that the chilling injury could induce severe damage in the chloroplast membranes and accelerate the degradative pathways of pigments. The changes in chlorophyll pigments in green beans during cold storage are related to synthesis and degradation processes, depending on the maturity stage of the product and the storage conditions. Both green beans cultivars showed the presence of chlorophyll *a*, chlorophyll *b*, and pheophytin *a*, but no other chlorophyllic derivatives as chlorophyllides or pheophorbides were identified in HPLC extracts, Figure 1. The rapid disappearance of the chlorophyllic pigments as storage time advance coincided with the transformation of the chloroplasts. Also, chloropigments are suceptible to many degradation reactions, either chemical or enzymatic. Major chemical degradation routes are associated with pheophytinization, epimerization, and pyrolisis and also with hydroxylation, oxidation, or photooxidation (Mangos and Berger, 1997). In this sense, in green beans tissue the pheophytinization was not the only degradation route which takes place in fresh tissue. The fact that the chlorophyll *a*/chlorophyll *b* ratio tended to increase in both cultivars stored at all three assayed temperatures, except for cv. Perona stored at 12 °C, and that chlorophyll *b* disappeared more rapidly than chlorophyll *a* would seem to indicate that chlorophyllase enzyme has a higher affinity for the substrate from *b* series, Table 1. In green beans, cv. Perona stored at 12 °C, chlorophyll *a* disappearing more rapidly than chlorophyll *b* could indicate that at this temperature the normal degradation pathways were affected due to the high respiration rate of the tissue, and this fact significantly reduced the storage shelf-period of this vegetable product.

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