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Extending shelf-life and nutritive value of green beans (*Phaseolus vulgaris* L.), by controlled atmosphere storage: micronutrients

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Abstract

Controlled atmosphere (CA) storage has become a useful technique for extending vegetables shelf-life. The aim of this work is to select the most suitable conditions for green beans storage under CA, for the maintenance of micronutrients (vitamins and mineral content). Samples were stored at 8 °C, under different conditions (normal atmospheric air, $5\%O_2+3\%CO_2$, $3\%O_2+3\%CO_2$ and $1\%O_2+3\%CO_2$), and analyzed periodically during 1 month for ash and individual mineral elements (atomic absorption spectroscopy), carotenoids (spectrophotometry), vitamins B_1 and B_2 (spectrofluorometry), vitamins B_6 (colorimetry) and vitamin C (HPLC–UV). Statistical analysis (ANOVA) and Duncan's tests were applied to the analytical data to evaluate the effects of the treatments applied. A weighted least squares polynomial plot was applied to vitamin C data to establish its degradation kinetics. The conditions 3% $O_2+3\%CO_2$ at 8 °C was selected as the best one to extend shelf-life and preserve the nutritive value of this vegetable, with a 75% total vitamin C retention at 8 days storage.
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Keywords: Green beans; *Phaseolus vulgaris* L.; Controlled atmospheres; Carotenoids; Vitamins; Mineral elements

1. Introduction

Micronutrients in foodstuffs include vitamins and mineral elements, as components which are found in very small amounts, but with an essential role in the metabolism of human body. Fruit and vegetables are particularly rich in those substances and, for this reason, the technological processes applied to these products should not reduce their contents.

Refrigeration (4–10 °C) and modified and/or controlled atmosphere (CA) have been proposed by several authors to extend shelf-life of fruits and vegetables, especially for those with high respiration rate, as is the case for green beans (Costa, Brecht, Sargent, & Huber, 1994; Heredia & Marañón, 1962; Watada & Morris, 1966; Wills, Mc Glasson, Graham, & Joyce, 1999).

CA storage applies lower O_2 and higher CO_2 levels in order to slow respiration rate and senescence processes in the vegetable. Several studies have been performed on the effects of CA on the physiological, microbiological and organoleptic properties of foods. However, not many studies focus on the nutritional changes induced by CA storage of vegetables products. Sánchez-Mata, Cámara, and Díez (2002) have reported higher and more stable amounts of moisture, total available carbohydrates, total soluble sugars, fructose and glucose, in green beans stored under 3% $O_2+3\%$ CO_2, than under atmospheric air storage or other different conditions of CA.

There are not many studies evaluating vitamin C and pigment variations in CA storage of vegetables. Some of them show that atmospheres with low levels of O_2 maintain higher levels of vitamin C in vegetables, since oxidation processes are reduced under these conditions (Barth, Kerbel, Perry, & Schmidt, 1993; Delaporte, 1971; Liao & Seib, 1987; Wright & Kader, 1997b); this phenomenon has also been observed in green beans (Cámara, Díez, Sánchez, & Torija, 1997).

Some authors have reported the effect of CA with low O_2 levels to retain carotenoid content in green beans; Zagory and Kader (1989) reported the degradation of carotenoids in carrots with 5% CO_2 levels, and it has

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been shown that vegetable storage under low levels of O_2 and high content of CO_2 influences both the reduction of the losses of carotenoids by oxidation, and the inhibition of its biosynthetic pathways (Kader, Zagory, & Kerbel, 1989; Wright & Kader, 1997a).

Apart from these studies on vitamin C and carotenoids, to the authors' knowledge, there are no previous data about the influence of CA on other micronutrients of green beans. For this reason, the aim of this work is to study the evolution of mineral elements, carotenoids, vitamins B (B_1, B_2, B_6) and vitamin C during CA storage of green beans, and to select the most suitable conditions for green bean storage under CA, from the point of view of the maintenance of their nutritive value.

2. Materials and methods

2.1. Samples

Fresh green beans (*Phaseolus vulgaris* L. cv. Perona), harvested in Almeria (Southeast of Spain), on April 1997 were selected for analysis. A previous temperature study had been done (Torija Isasa, Díez Marqués, Cámara Hurtado, Sánchez Mata, Fernandez Antoranz, & Peñuela Teruel, 1997), with 8 °C as the best temperature for the maintenance of vitamin C content of green beans. For this reason, samples were stored at 8 °C, 98% relative moisture and different CA conditions (normal atmospheric air, 5% O_2 + 3% CO_2, 3% O_2 + 3% CO_2 and 1% O_2 + 3% CO_2), during 22 days.

A representative sample was taken from each storage condition on days 0, 4, 11, 13, 18 and 22 (the last one only for 1% O_2 + 3% CO_2, due to the decay of green beans stored with higher levels of O_2 at the end of the storage). All the samples were cleaned and, after removing the tips and spoiled areas of the pods, they were homogenized with a domestic blender. A fraction of each sample was freeze-dried and reduced to fine particles for their preservation. Triplicate subsamples were taken for each analytical procedure.

2.2. Experimental procedures

Analytical determinations on fresh samples were:

moisture: desiccation at 100 ± 2 °C (FAO, 1989);
vitamin B_1: oxidation of thiamine to thiocrome using alkaline potasium ferrocianide, extraction of thiocrome with isobutanol and spectrofluorometry at 265/435 nm (AOAC, 1990);
vitamin B_2: oxidation of riboflavine using $KMnO_4$ and spectrofluorometric determination of the oxidised product, at 440/565 nm (AOAC, 1990);
vitamin B_6: diazotation of total vitamin B_6 using $NaNO_2$ + sulfanilic acid, and colorimetric measurement of total vitamin B_6 at 424 nm (Martínez Tamayo, Carballido, & Villanúa, 1964);
vitamin C: extraction in aqueous metaphosphoric acid and HPLC–UV determination as ascorbic acid (AA), before and afer reduction with dithiothreitol to dehydroascorbic acid (DHAA) (Sánchez-Mata, Cámara Hurtado, Díez-Marqués, & Torija-Isasa, 2000). Results for total vitamin C, AA and DHAA (by difference) were calculated.

Analytical determinations on freeze-dried samples were:
carotenoids: ether extraction after saponification with KOH/methanol, and spectrophotometric measurement at 424 nm (Guil Guerrero, 1994);
ashes and mineral elements: mineralization of samples at 450 °C and atomic absorption spectroscopy analysis for macroelements (Na, K, Ca, Mg) and microelements (Fe, Cu, Mn, Zn) determination (Torija, 1981).

2.3. Statistical analysis

The interpretation of analytical data was performed by the application of ANOVA, using the statistical F (Fischer) and a confidence level of 95%. Two factors of variation (time and composition of the storage atmosphere were considered). Duncan's test was applied to those parameters which showed a statistically significant variation in the samples due to any of these factors.

An unconstrained weighted least squares non-linear regression was applied to vitamin C data, in order to find the kinetics of vitamin C degradation in green beans for each storage condition, by using the SIMFIT computer package (ver. 1998, developed by W.G. Bardsley, University of Manchester).

3. Results and discussion

Total mineral content (ash) was higher for samples stored under 1% O_2 + 3% CO_2, and lower for 5% O_2 + 3% CO_2 stored green beans ($P < 0.05$). It showed an initial decrease in all the samples, followed by irregular variations (Table 1).

Cu and Zn were the most stable elements during all the storage periods considered, with no significant variations due to storage time for Cu and Zn, or to the atmosphere applied for Cu (Table 3). Ca, Mg and Mn were more stable during the first 11 days of storage, with more irregular behaviour at later stages of storage ($P < 0.05$). Ca, was more stable at low levels of O_2 and high levels of CO_2 ($P < 0.05$).

Na and K showed a significant decreasing tendency ($P < 0.05$) during storage (Fig. 1), especially in normal air and 5% O_2 + 3% CO_2 . Since minerals are not metabolized and therefore their contents should not change, variations of mineral content of green beans

during storage have been attributed to redistribution of mineral elements in the pods and possible microbial contamination at later stages of storage, in agreement with other authors (Bognår, Bohling, & Fort, 1990; Fernández Antoranz, 1998; Perring & Pearson, 1987; Zagory & Kader, 1989). Further research should be conducted on this subject in order to explain mineral elements behaviour.

At the first stages of storage, high variations of total carotenoid contents took place in all the samples

Table 1
Mineral contents of green beans stored under controlled atmospheres (mg/100 g on wet basis)

Atmosphere	Day	Ash (X±SD)	Na (X±SD)	K (X±SD)	Ca (X±SD)	Mg (X±SD)	Cu (X±SD)	Fe (X±SD)	Mn (X±SD)	Zn (X±SD)
Normal air	0	0.642±0.017	0.454±0.020	158±23	44.4±0.9	19.2±1.98	0.088±0.006	0.616±0.024	0.153±0.003	0.253±0.039
	4	0.577±0.021	0.404±0.006	141±33	45.9±0.8	18.1±1.32	0.084±0.005	0.600±0.006	0.141±0.001	0.224±0.016
	11	0.489±0.010	0.294±0.024	98±15	47.4±3.4	19.3±0.62	0.071±0.008	0.569±0.034	0.161±0.001	0.199±0.001
	13	0.601±0.009	0.305±0.027	92±10	42.6±4.2	17.4±0.53	0.071±0.012	0.486±0.024	0.146±0.012	0.285±0.025
	18	0.554±0.003	0.303±0.021	90±8	48.7±3.0	18.3±0.78	0.073±0.005	0.614±0.009	0.200±0.013	0.249±0.000
5% O_2 + 3% CO_2	4	0.496±0.007	0.423±0.033	142±15	42.5±1.3	18.9±0.30	0.085±0.005	0.554±0.045	0.158±0.001	0.264±0.055
	11	0.470±0.008	0.194±0.015	103±12	40.9±0.9	18.7±1.07	0.073±0.001	0.479±0.020	0.157±0.002	0.197±0.014
	13	0.576±0.019	0.184±0.027	106±15	46.9±1.2	18.9±1.03	0.088±0.006	0.579±0.021	0.179±0.003	0.263±0.004
	18	0.569±0.030	0.352±0.029	108±14	42.2±0.9	17.8±1.23	0.087±0.022	0.495±0.024	0.186±0.089	0.233±0.008
3% O_2 + 3% CO_2	4	0.600±0.103	0.526±0.021	167±19	42.7±1.3	17.3±0.58	0.089±0.005	0.516±0.033	0.132±0.002	0.253±0.024
	11	0.557±0.007	0.309±0.019	173±15	42.3±1.4	18.3±0.42	0.083±0.009	0.501±0.038	0.149±0.008	0.243±0.013
	13	0.573±0.017	0.288±0.029	118±22	39.6±2.0	11.6±1.04	0.082±0.009	0.527±0.030	0.123±0.007	0.211±0.035
	18	0.549±0.005	0.179±0.010	107±10	38.7±0.3	15.1±0.50	0.084±0.010	0.458±0.024	0.142±0.004	0.227±0.003
1% O_2 + 3% CO_2	4	0.550±0.001	0.361±0.042	117±20	42.2±2.8	17.3±1.39	0.075±0.001	0.575±0.035	0.139±0.009	0.270±0.057
	11	0.606±0.011	0.543±0.064	135±16	44.1±0.9	18.8±1.54	0.090±0.004	0.694±0.013	0.156±0.004	0.227±0.036
	13	0.574±0.006	0.349±0.028	125±13	39.9±4.1	15.6±1.95	0.080±0.009	0.579±0.032	0.129±0.012	0.239±0.015
	18	0.647±0.006	0.309±0.015	145±20	46.5±1.9	19.9±0.90	0.074±0.010	0.572±0.046	0.220±0.004	0.290±0.003
	22	0.584±0.014	0.260±0.015	95±13	41.5±2.7	18.4±2.27	0.068±0.005	0.517±0.041	0.154±0.010	0.281±0.037

X = mean value (n = 3); SD = standard deviation (n−1).

Table 2
Vitamin contents of green beans stored under controlled atmospheres (mg/100 on wet basis)

Atmosphere	Storage days	Total carotenoids (X±SD)	Vitamin B_1 (X±SD)	Vitamin B_2 (X±SD)	Vitamin B_6 (X±SD)	Vitamin C (X±SD)	Ascorbic acid (X±SD)	Dehydroascorbic acid (X±SD)
Normal air	0	0.773±0.027	0.065±0.013	0.103±0.003	0.193±0.021	6.19±0.78	2.50±0.24	3.60±0.13
	4	0.675±0.116	0.042±0.004	0.089±0.004	0.163±0.014	3.57±0.13	2.48±0.41	1.43±0.06
	11	0.647±0.043	0.049±0.008	0.089±0.009	0.176±0.019	2.49±0.09	1.05±0.10	1.46±0.12
	13	0.633±0.094	0.046±0.009	0.088±0.008	ND	2.53±0.14	1.30±0.26	1.37±0.01
	18	0.725±0.076	0.037±0.006	0.107±0.006	ND	1.72±0.16	1.18±0.10	0.54±0.09
5% O_2 + 3% CO_2	4	0.980±0.020	0.040±0.008	0.091±0.009	0.163±0.014	3.86±0.24	1.89±0.06	1.96±0.10
	11	0.804±0.041	0.045±0.002	0.089±0.009	0.168±0.056	2.69±0.21	1.19±0.19	1.46±0.21
	13	0.769±0.048	0.042±0.001	0.111±0.015	ND	2.48±0.06	1.36±0.29	1.12±0.06
	18	0.671±0.037	0.040±0.003	0.124±0.001	ND	2.23±0.05	1.20±0.04	1.05±0.12
3% O_2 + 3% CO_2	4	0.529±0.086	0.043±0.004	0.102±0.008	0.146±0.012	6.02±0.14	2.47±0.31	3.55±0.38
	11	0.711±0.032	0.046±0.002	0.109±0.007	0.147±0.003	4.23±0.05	1.84±0.26	2.26±0.18
	13	0.711±0.080	0.041±0.003	0.095±0.007	ND	3.07±0.01	1.83±0.11	1.21±0.16
	18	0.740±0.048	0.032±0.008	0.101±0.008	ND	1.96±0.22	1.04±0.04	0.99±0.18
1% O_2 + 3% CO_2	4	0.545±0061	0.035±0.002	0.092±0.006	0.104±0.012	6.33±0.63	2.76±0.11	3.48±0.71
	11	0.621±0.061	0.051±0.004	0.115±0.005	0.123±0.057	5.06±0.47	1.91±0.32	3.23±0.15
	13	0.728±0.031	0.045±0.001	0.093±0.005	ND	3.89±0.27	1.60±0.05	2.32±0.29
	18	0.891±0.081	0.045±0.002	0.099±0.003	ND	3.70±0.09	1.99±0.09	1.70±0.00
	22	0.814±0.009	0.038±0.004	0.132±0.009	ND	2.12±0.14	1.32±0.01	0.81±0.10

X = mean value (n = 3); SD = standard deviation (n−1).

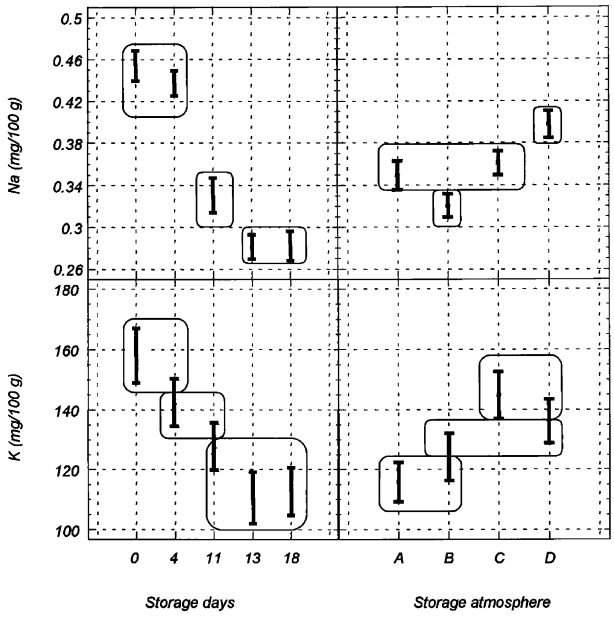

Fig. 1. Duncan's test (95% confidence level) to evaluate the influence of time and atmospheric conditions on Na and K of green beans stored under CA. A = Atmospheric air; B = 5% O_2 + 3% CO_2; C = 3% O_2 + 3% CO_2; D = 1% O_2 + 3% CO_2. Statistically different values ($P < 0.05$) have been grouped separately.

Table 3
Storage time and atmosphere influences on micronutrient composition of green beans (two factor ANOVA analysis)

Nutrient	Time	Atmosphere
Total carotenoids	+	+
Vitamin B_1	+	–
Vitamin B_2	+	+
Vitamin B_6	+	–
Total vitamin C	+	+
Ascorbic acid	+	+
Dehydroascorbic acid	+	+
Ash	+	+
Na	+	+
K	+	+
Ca	–	+
Mg	+	+
Cu	–	–
Fe	+	+
Mn	+	+
Zn	–	+

+ = statistically significant variation.
– = non statistically significant variation

(Table 2). Samples 5% O_2 + 3% CO_2 maintained a higher total carotenoid content ($P < 0.05$), with a increase up to 34.7% of their initial level, followed by a decrease. Several authors have reported some carotenoid synthesis in stored green beans (Cano, Monreal, De Ancos, & Alique, 1999; Gross, 1991). Monreal, De Ancos, and Cano (1999) reported an acceleration of those processes at 8 °C while, at other temperatures degradation processes are more important. According to Kader et al. (1989) and Wright and Kader (1997a), low levels of O_2 and high CO_2 influences both the reduction of the losses of carotenoids by oxidation, and the inhibition of biosynthetic pathways.

Vitamins B showed different behaviour in green beans during storage. As can be observed from Table 3, the evolution of vitamin B_1 was independent of the storage atmosphere applied ($P < 0.05$), which agrees with Watada, Kim, Kim, and Harris (1987), and it decreased significantly during storage ($P < 0.05$) (Table 2). Although some thiamine synthesis may occur in green beans postharvest (Lee & Chichester, 1974; Watada et al., 1987), the decrease is due to degradation of this vitamin by thiaminases. Different compounds, such as polyphenols, flavonoids and vitamin B_2, act as inducters of thiaminases, while fructose and inositol inhibit this reaction, protecting vitamin B_1 from degradation (Dwivedy & Arnold, 1973; Ottaway, 1993). Myo-inositol has also been determined in these samples (Sánchez et al., 2002), and a positive correlation (95% confidence level) between vitamin B_1 and myo-inositol content was found.

Vitamin B_2 showed an irregular evolution during storage (Table 2), with higher levels in CA-stored green beans ($P < 0.05$). At later stages of storage an increase was observed, which may be related to microbial contamination in the samples. Similar irregular variations have been reported by Augustin et al. (1978) and Watada et al. (1987) during vegetable storage.

Vitamin B_6 showed an abrupt reduction on day 13 (Table 2) to non-detectable levels (detection limit of the analytical technique 0.026 mg/100 g), which could be attributed to hydroxylation of pyridoxine in the presence of ascorbic acid (Tadera, Arima, Yoshino, Yagi, & Kobayashi, 1986). Lower O_2 atmospheres (1% O_2 + 3% CO_2) maintained only lower levels of this vitamin in the analyzed samples ($P < 0.05$).

Vitamin C was found in green beans as AA (40.3%) and also as DHAA (59.7%), which was the predominant form (Table 2), as the pH of green beans was 5.6. Statistical analysis (Fig. 2) showed that time and atmosphere of storage significantly influenced vitamin C content of green beans, in both AA and DHAA forms ($P > 0.05$). Those samples stored with lower levels of O_2, were always higher as oxidation processes are reduced under these conditions.

Vitamin C degradation is due to autoxidation and also enzymatic degradation (ascorbate–oxydase, polyphenol–oxidase, cytochrome–oxidase, peroxidase) (Barth et al., 1993; Erdman & Klein, 1982). Ascorbate–oxidase has a maximum activity at pH 4.5–5.5 (Maccarone, D'Andrea, Salucci, Avigliano, & Finazzi Agro, 1993), so high levels of DHAA in vegetables at pH higher than 4 have been reported by some authors (Wills et al., 1999; Wright &

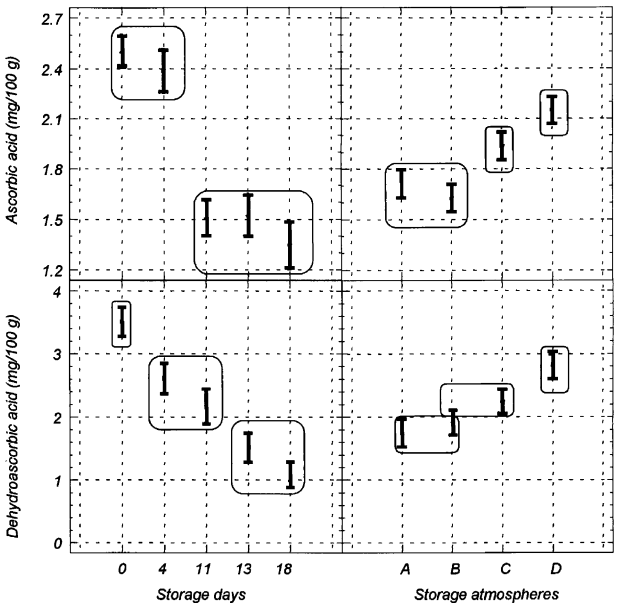

Fig. 2. Duncan's test (95% confidence level) to evaluate the influence of time and atmospheric conditions on ascorbic acid and dehydroascorbic acid of green beans stored under CA. A = Atmospheric air; B = 5% O_2 + 3% CO_2; C = 3% O_2 + 3% CO_2; D = 1% O_2 + 3% CO_2. Statistically different values ($P < 0.05$) have been grouped separately.

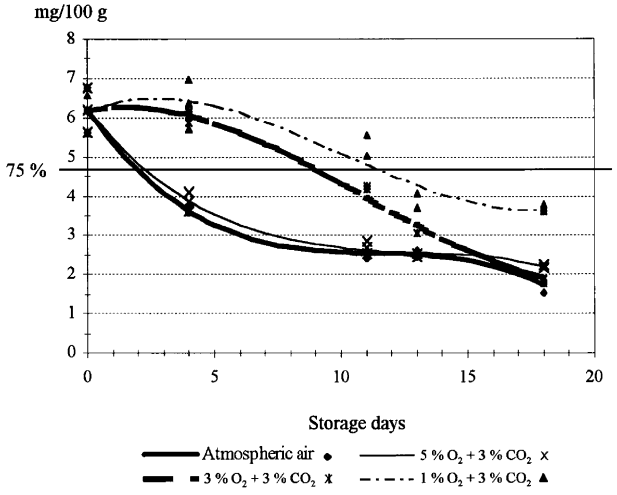

Fig. 3. Weighted least squares polynomial plot of vitamin C degradation in green beans stored under controlled atmospheres.

Kader, 1997b), which agree with the high proportion of DHAA found for the analyzed green beans.

In most of the analyzed samples, the decrease was more intense for DHAA than for AA, due to its higher lability and the irreversibility of its degradation, which have been previously described by Klein (1987) and Nobile and Woodhill (1981). These results agree with those reported by Wright and Kader (1997b) on strawberries and persimmons stored under CA.

Vitamin C degradation in green beans can be adjusted to a polynomic kinetic model (Fig. 3). The time necessary to preserve 75% of the initial content of vitamin C ($t_{75\%}$) has been estimated. This value was only 2 days for green beans stored in normal atmospheric air; the modification of O_2 and CO_2 levels in the surrounding atmosphere gave values of 2.3, 8.1 and 11.2 days, respectively for 5% O_2 + 3% CO_2, 3% O_2 + 3% CO_2 and 1% O_2 + 3% CO_2. From these results, 5% O_2 + 3% CO_2 is not efficient to preserve vitamin C in green beans compared with normal air, while the other conditions assayed maintained vitamin C levels in the product for a longer time.

4. Conclusions

Reduction of atmospheric O_2 to 5% with 3% CO_2 is not efficient to extend the shelf-life or the nutritive value of green beans. From the studied conditions, 3% O_2 + 3% CO_2 at 8 °C was selected as the best to preserve vitamin B_2 and vitamin C in this product. Under these conditions, green beans maintain a very good nutritive quality (retaining more than 75% of initial vitamin C content) during 8 days, with 13 days as the maximum storage time for obtaining a vegetable of optimal nutritive quality.

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