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Food Chemistry 80 (2003) 309–315

Food Chemistry

www.elsevier.com/locate/foodchem

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

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Received 5 March 2002; received in revised form 11 June 2002; accepted 11 June 2002

Abstract

Controlled atmosphere (CA) storage has become a useful technique to extend vegetable shelf-life. The aim of this work is to select the most suitable conditions for green bean storage under CA, with the maintenance of macronutrients (proteins, total available carbohydrates, soluble sugars, neutral-detergent fibre and pectins). Samples were stored at 8°C, under different atmosphere 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 one month for dry matter (desiccation), proteins (Kjeldahl), total available carbohydrates (anthone), soluble sugars (HPLC), neutral-detergent fibre (gravimetry) and pectins (*m*-phenyphenol). Statistical analyses (ANOVA and Duncan tests) were applied to the analytical data to evaluate the effect of the treatments applied. The air composition 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.
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Keywords: Green beans; *Phaseolus vulgaris* L.; Controlled atmospheres; Proteins; Carbohydrates; Soluble sugars; Fibre; Pectins

1. Introduction

The quality and shelf-life of vegetables is related to all the biochemical processes which take place during post-harvest. Respiration induces heat emission, with a temperature increase, which affects metabolic processes and induces an acceleration of decay phenomena (Holdsworth, 1988). Green beans have intense respiration and heat emission, which limits shelf-life after harvest to 0–4 weeks maximum. This can be partially attributed to the intense metabolic activity of immature seeds inside the pods. During ripening processes, sugars condense to starch, with loss of sweetness, decrease in water content and increase of dietary fibre components (Bognår, Bohling, & Fort, 1990; Wills, Mc Glasson, Graham, & Joyce, 1999). On the other hand, at later stages of ripening (senescence), degradation processes increase, with the hydrolysis of starch and the consumption of soluble sugars on respiration (Muñoz Delgado, 1985).

In order to extend green bean shelf-life, several authors (Heredia & Marañón, 1962; Watada & Morris, 1966; Wills et al., 1999) have recommended the storage of this product at 4–10 °C. Modified and/or controlled atmosphere (CA) storage is also a useful technique for extending shelf-life of vegetables, especially for those which deteriorate quickly. In most vegetable products, when external O_2 pressure is low, an intense decrease of respiration activity is produced, which is attributed to reduction of oxidase activities, such as polyphenol-oxidase, ascorbate-oxidase and gycolic-oxidase (Kader, 1986; Solomos, 1982). Low O_2 levels can also induce the suppression of genes that codify for maturation associated enzymes, such as celulase, polygalacturonase, acid invertase, sucrose-phosphate-synthase and amine-cyclopropane-1-carboxylate-oxidase (Kanellis, Solomos, & Roubelakis-Angelakis, 1991) .

Kader (1997) reported that, in general, O_2 levels should be under 5% to obtain a decrease of respiration activity. In the case of green beans, Groeschel, Nelson, and Steinberg (1966) observed that 5% O_2 reduced their respiration rate by 20%, while levels of 2% O_2 reduced it by 40%. Storage conditions should be controlled to prevent anaerobic respiration of the product, which could induce alcohol and volatile compound production (with the appearance of strange flavours), as well as anaerobic microorganism proliferation. However Larson, Johnson, Barmore, and Hughes (1997) did not find any evidence of botulism toxin in different vegetables (including green

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PII: S0308-8146(02)00265-0

beans) packaged under modified atmosphere and inoculated with *Clostridium botulinum* (A, B and E type). Under 2% O_2 the metabolism of the vegetable turns to anaerobic, and this change is not due to cytochrome-oxidase, but to the reduction of ascorbate-oxidase, polyphenol-oxidase and gycolic-oxidase activities, which have 5 or 6 times less affinity for O_2 than cytochrome-oxidase (Zagory & Kader, 1989).

Reduction of O_2 levels and increasing CO_2 levels have synergic effects on vegetable respiration rate. Kader (1986) reported that CO_2 levels should be between 10 and 20% to obtain respiration suppression. However, these levels could produce physiological disorders in the vegetable (Henderson & Buescher, 1977; Costa, Bresht, Sargent, & Huber, 1994).

Buescher and Brown (1979) reported an indirect action of CO_2, increasing pH and slowing chlorophyll degradation, and Buescher and Adams (1983) reported a reduction on titratable acidity and an increase of soluble pectins in green beans stored under high levels of CO_2. From all the previous studies, temperatures near 8 °C, with 2–3% O_2 and 4–7% CO_2 concentrations are recommended to extend green bean shelf-life.

In general, fruits stored under modified or controlled atmospheres maintain high values of soluble sugars and organic acids, while vegetables show lower values for these compounds (Bognår et al. 1990; Romojaro et al., 1966). Soluble sugar variation is related to vegetable metabolism, as simple sugars are respiration substrates for vegetables. Arpaia, Labavitch, Greve, and Kader (1987) showed that CA maintained higher levels of uronic acids, galactose, arabinose, rhamnose, cellulose and starch in kiwis.

Many studies have been reported about the influence of CA on the physiological, microbiological and organoleptic aspects of foods; however, not many studies focus on the nutritional effects of these storage conditions on vegetables. Most of them study vitamin C and pigment variations (Cano, Monreal, De Ancos, & Alique, 1999; Kader, Zagory, & Kerbel, 1989; Weichmann, 1986; Wright & Kader, 1997a,b) but, to the authors knowledge, there are no previous data about the influence of CA on other nutrients in green beans.

For this reason, the aim of this work is to study the evolution of proteins, total available carbohydrates, soluble sugars, dietary fibre and pectins, during CA storage of green bean, and to select the most suitable conditions for the storage of this vegetable under CA, from the point of view of the maintenance of nutritive value.

2. Materials and methods

2.1. Samples

Fresh green beans (*Phaseolus vulgaris* L. cv. Perona), harvested in Almeria (South-East of Spain), in April 1997 were selected for analysis. A previous temperature study has been performed (Torija, Issasa et al. 1997), with the result of 8 °C as the best temperature for the maintenance of the nutritive value. For this reason, samples were stored at 8 °C, 98% relative humidity 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 preservation. Triplicate subsamples were taken for each analytical procedure.

2.2. Experimental procedures

Analyses were as follows:

moisture (before freeze-drying): desiccation at 100 ± 2 °C (FAO, 1989);
total proteins, by Kjeldahl procedure: acid digestion, distillation of NH_3 over N/10 H_2SO_4 and titration with N/10 NaOH (AOAC, 1990);
total available carbohydrates: starch hydrolysis with $HClO_4$, reaction with anthrone/H_2SO_4 and colorimetric measurement at 630 nm (Osborne & Voogt, 1986).Results are expressed as glucose content.
soluble sugars: ethanol extraction and HPLC-refractometric analysis (Sánchez Mata, Cámara Hurtado, & Díez Marqués, 2002).
neutral-detergent fiber: extraction with a neutral-detergent solution and gravimetric determination of insoluble residue (Oruña Concha, González Castro, LópezHernández, & Simal Lozano, 1996; Van Soest & Robertson, 1979).
pectins: hydrolysis of alcohol-insoluble material with H_2SO_4 and *m*-phenyphenol colorimetric determination at 530 nm (Blumenkrantz & Asboe-Hanse, 1973; Ahmed & Labavitch, 1977). Results are expressed as galacturonic acid content.

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.

3. Results and discussion

Samples stored under 3% O_2 + 3% CO_2, and especially 1% O_2 + 3% CO_2, maintained a lower and more stable water content ($P < 0.05$), which means a lower risk of microbial contamination (Table 1). Moisture slightly increased during storage, with oscillations lower than 1.6% of the initial content. This fact has been attributed to the high relative humidity used for green bean storage (98%) in order to avoid weight lost (Kader, 1988; Letang, 1997), and agrees with the observations of Fernández Antoranz (1998).

From the general composition parameters, the most stable one was total protein content. In most of the analyzed samples, a general slight decrease of protein content may be observed at final stages of storage (Table 1), probably due to a more intense protein and aminoacid degradation in this period. However, statistical analysis applied to the obtained data did not reveal significant variations of total protein content (Table 3) due to time or atmosphere used for storage of the samples ($P < 0.05$).

Statistical analysis of total available carbohydrates data showed significant differences due to time and atmosphere (Table 3). Green beans have a high respiration rate, because of the intense metabolic activity of the seeds (Wills et al., 1999). Groeschel et al. (1966), Arpaia et al. (1987) and Larson et al. (1997) reported that vegetable storage under atmospheres with low levels on O_2, maintains higher levels of starch and reduced respiration rates and carbohydrate consumption. Duncan's test (Fig. 1) showed that fresh samples had the highest carbohydrate contents, and distinguished the conditions 3% O_2 + 3% CO_2, and 1% O_2 + 3% CO_2, as those that maintained higher amounts of total available carbohydrates in green beans, presumably because of a lower respiration rate in these samples. This result agrees with Larson et al. (1997), who reported that the reduction of the respiration process in vegetables is efficient below 5% O_2.

Soluble sugars of the samples included fructose as the major sugar, glucose, sucrose and the polyalcohol myo-inositol (Table 2), reported previously in green beans by Souci, Fachmann, and Kraut (1994), Font Quer (2000) and Sánchez Mata et al. (2002). Myo-inositol acts as a galactose carrier in raffinose and stachyose synthesis, as well as a precursor for phytic and uronic acids. Two unknown sugars (U_1, U_2) have also been detected in some of the analyzed samples; although their identity could not be confirmed, they are presumed to be disaccharides (Sánchez Mata et al., 2002).

Total sugar content decreased significantly ($P < 0.05$), after an initial and slight increase until day 11 of storage, under most of the atmospheric conditions assayed (Fig. 1), with a total reduction of 44.9% from the initial value, after 22 days of storage on 1% O_2 + 3% CO_2. Samples stored under 3% O_2 + 3% CO_2 showed higher and more stable total soluble sugars, fructose and glucose contents ($P < 0.05$) than the other conditions assayed, which showed higher oscillations (Figs. 1 and 2). This result agree with Groeschel et al. (1966), who reported the slow respiratory activity of green beans stored under 3% O_2 + 3% CO_2.

Changes in soluble sugars of stored vegetables are due to the balance between anabolic and catabolic pro-

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

Atmosphere	Storage (days)	Moisture X±SD	Proteins X±SD	Carbohydrates X±SD	NDF X±SD	Pectins X±SD
	0	91.5±0.0	1.64±0.01	3.93±0.10	1.36±0.02	0.713±0.109
Normal air	4	91.9±0.0	1.52±0.02	2.76±0.05	1.28±0.02	0.627±0.059
	11	92.7±0.0	1.59±0.02	2.56±0.08	1.18±0.01	0.625±0.042
	13	91.9±0.0	1.56±0.11	3.29±0.04	1.21±0.06	0.782±0.044
	18	92.9±0.1	1.63±0.02	2.97±0.07	1.17±0.02	0.905±0.049
5% O_2 + 3% CO_2	4	92.2±0.0	1.71±0.02	2.99±0.26	1.26±0.04	0.552±0.076
	11	93.0±0.5	1.49±0.02	2.75±0.21	1.16±0.00	0.660±0.017
	13	92.4±0.5	1.67±0.01	3.18±0.04	1.24±0.03	1.044±0.055
	18	92.6±0.1	1.50±0.02	3.50±0.08	1.24±0.02	0.903±0.012
3% O_2 + 3% CO_2	4	91.7±0.1	1.59±0.09	3.63±0.19	1.33±0.05	0.557±0.037
	11	92.1±0.1	1.63±0.06	3.83±0.16	1.16±0.02	0.811±0.002
	13	92.3±0.1	1.56±0.02	3.29±0.15	1.31±0.03	0.635±0.004
	18	92.7±0.0	1.42±0.05	3.55±0.09	1.17±0.02	0.503±0.008
1% O_2 + 3% CO_2	4	92.0±0.0	1.50±0.05	3.73±0.35	1.19±0.02	0.452±0.014
	11	91.8±0.1	1.67±0.01	4.08±0.22	1.30±0.03	0.839±0.053
	13	92 2±0.4	1.65±0.04	3.30±0.17	1.22±0.03	0.571±0.100
	18	92.6±0.1	1.74±0.10	3.42±0.23	1.23±0.05	0.481±0.044
	22	92.0±0.0	1.54±0.01	3.37±0.03	1.11±0.04	0.545±0.015

X = mean value ($n = 3$); SD = standard deviation ($n-1$); NDF = neutral-detergent fibre.

Table 2
Soluble sugar contents of green beans stored under controlled atmospheres (g/100 g on wet basis)

Atmosphere	Storage (days)	Fructose X±SD	Glucose X±SD	Sucrose X±SD	Myo-inositol X±SD	U_1 X±SD	U_2 X±SD	Total X±SD
	0	1.32±0.03	0.285±0.008	0.107±0.007	0.664±0.042	0.122±0.013	0.048±0.009	2.53±0.02
Normal air	4	1.27±0.03	0.282±0.005	0.273±0.023	0.644±0.023	0.113±0.012	0.046±0.009	2.59±0.08
	11	1.35±0.149	0.269±0.003	0.296±0.028	0.738±0.081	0.089±0.014	0.041±0.008	2.81±0.33
	13	0.99±0.06	0.231±0.006	0.487±0.040	0.536±0.005	0.092±0.011	ND	2.16±0.15
	18	0.89±0.08	0.245±0.007	0.235±0.025	0.489±0.044	0.123±0.002	ND	2.07±0.18
5% O_2 + 3% CO_2	4	1.38±0.05	0.449±0.010	0.212±0.016	0.659±0.037	0.165±0.016	0.045±0.009	2.89±0.10
	11	1.12±0.04	0.299±0.017	0.213±0.005	0.563±0.046	0.125±0.009	0.021±0.004	2.32±0.11
	13	0.99±0.08	0.261±0.008	0.211±0.013	0.507±0.071	0.109±0.011	ND	2.03±0.12
	18	0.98±0.04	0.241±0.003	0.204±0.012	0.434±0.037	0.120±0.012	ND	1.93±0.07
3% O_2 + 3% CO_2	4	1.25±0.10	0.415±0.046	0.249±0.027	0.570±0.052	0.127±0.008	ND	2.62±0.21
	11	1.26±0.06	0.349±0.030	0.363±0.012	0.638±0.028	0.116±0.025	ND	2.69±0.19
	13	1.11±0.04	0.334±0.009	0.257±0.039	0.571±0.075	0.102±0.015	ND	2.37±0.18
	18	1.12±0.07	0.330±0.020	0.274±0.046	0.488±0.064	0.060±0.001	ND	2.26±0.20
1% O_2 + 3% CO_2	4	1.07±0.09	0.233±0.028	0.228±0.028	0.487±0.041	0.135±0.013	ND	2.06±0.13
	11	1.28±0.01	0.385±0.008	0.311±0.018	0.589±0.026	0.164±0.001	ND	2.73±0.06
	13	0.937±0.07	0.293±0.011	0.379±0.048	0.433±0.012	0.092±0.011	ND	2.07±0.10
	18	0.997±0.07	0.149±0.014	0.338±0.020	0.618±0.016	ND	ND	2.07±0.13
	22	0.61±0.01	0.175±0.015	0.264±0.034	0.202±0.011	ND	ND	1.31±0.07

X = mean value ($n = 3$); SD = standard deviation ($n-1$), U_1, U_2 = unknown sugars; ND = non detected.

Table 3
Storage time and atmosphere influence on macronutrient composition of green beans (2 factors ANOVA analysis)

Nutrient	Time	Atmosphere
Moisture	+	+
Total proteins	−	−
Total available carbohydrates carbohydrates	+	+
Total soluble sugars	+	+
Fructose	+	+
Glucose	+	+
Sucrose	+	+
Myo-inositol	+	+
U_1	+	+
U_2	+	+
NDF	+	−
Pectic substances	+	+

+ = statistically significant variation; − = non statistically significant variation.

cesses. In general, during the first stages of ripening, some synthesis processes are going on, while degradation processes are starting (Muñoz Delgado, 1985; Wills et al., 1999). All these processes involve interconversions between different carbohydrates. The first increase of sugar content in green beans could be due to starch hydrolysis to release soluble sugars, and to the possible synthesis of some that may exist during the first stages of storage. On the other hand, the reduction in sugars content, after 11 days of storage, could be due to some sugar condensation to synthesize starch in the seeds (Bognår et al., 1990; Wills et al., 1999), but also to respiration processes which involve a high consumption of simple sugars, and other degradation processes which are intensified during the later stages of storage. This could explain the notable decrease of soluble sugars during the latter days of storage.

Fructose was the major sugar (43.0–51.9% of total sugar content) and its evolution was similar to total soluble sugars in all the atmospheres assayed, with a marked decrease after 11 days of storage (Table 2).

Glucose showed very irregular behaviour. In green beans stored under 5% O_2 + 3% CO_2 and 3% O_2 + 3% CO_2 glucose content increased up to 71.4 and 49.4% above the initial content during the first 4 days of storage. This phenomenon could be explained by a intense starch hydrolysis during this period. After this, green beans stored under 3% O_2 + 3% CO_2 maintained a more stable glucose content while it decreased in samples stored under 5% O_2 + 3% CO_2. This reduction could be related to a higher respiration rate in the samples but also to some sucrose and starch synthesis inside the seeds (Bognår et al., 1990; Wills et al., 1999).

The irregular oscillations in glucose content in stored green beans is due to the balance between glucose consumption on respiration and synthesis of other carbohydrates (mainly sucrose and starch) and its formation from starch hydrolysis and some residual synthesis that could still take place in the vegetable after its harvest (Bognår et al., 1990).

Sucrose showed a notable increase during the whole storage period (Table 2). At 4 days storage, this sugar reached more than double the initial content. After this, samples stored under 5% O_2 + 3% CO_2 maintained a more stable sucrose content ($P < 0.05$), while samples stored under the other conditions reached their max-

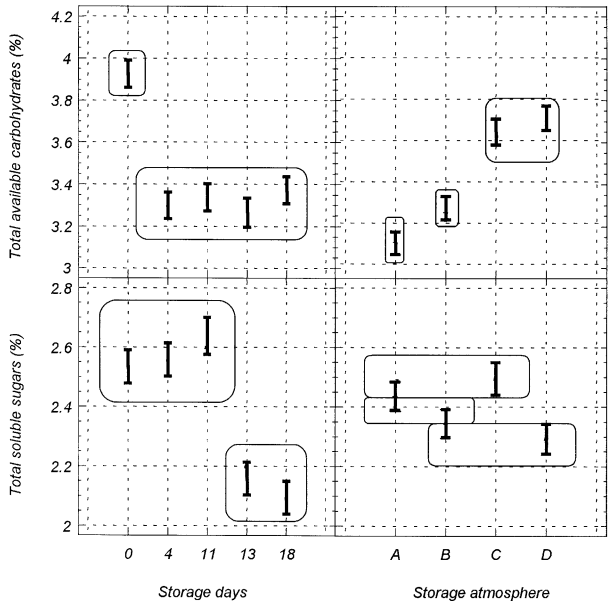

Fig. 1. Duncan's test (95% confidence level) to evaluate the influence of time and atmospheric conditions on total available carbohydrates and total soluble sugars of green beans stored under CA. A = Atmospheric air, B = 5% O_2 + 3% CO_2, C = 3% O_2 + 3% CO_2, D = 1% O_2 + 3% CO_2. Statistically different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

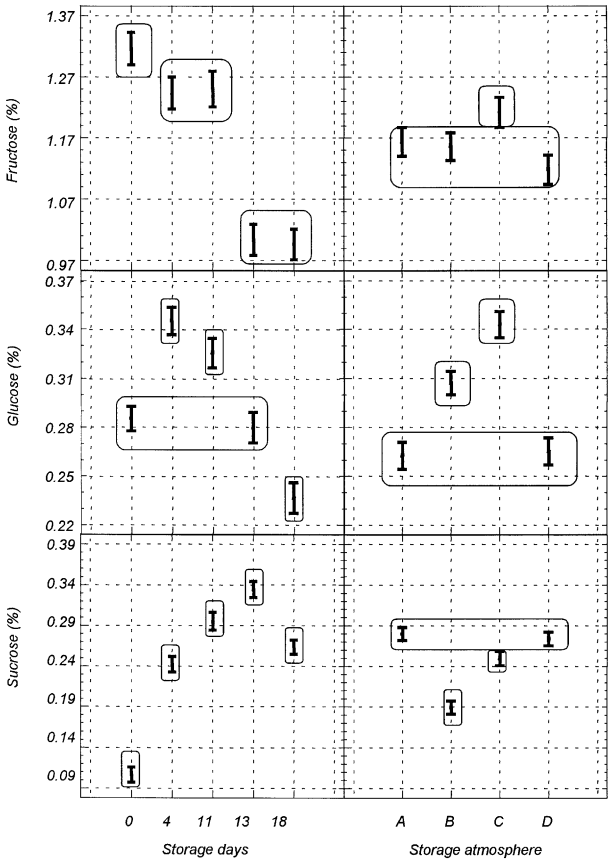

Fig. 2. Duncan's test (95% confidence level) to evaluate the influence of time and atmospheric conditions on fructose, glucose and sucrose of green beans stored under CA. A = Atmospheric air, B = 5% O_2 + 3% CO_2, C = 3% O_2 + 3% CO_2, D = 1% O_2 + 3% CO_2. Statistically different values ($P < 0.05$)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

imum value after 11–13 days, when sucrose content of green beans stored in normal air was more than 4 times the initial value. After this maximum, sucrose decreased (Fig. 2). These data confirm the synthesis of sucrose that takes place postharvest, reported previously by Parker and Stuart (1935). These authors reported that sucrose is the main sugar in green bean seeds (while the pods contain mainly reducing sugars) and that sucrose increases in the pods during green bean storage. The following decrease in sucrose content is due to its degradation, releasing fructose and glucose for plant respiration. The (fructose + glucose)/sucrose ratio was 14.9 on day 0, decreasing with storage time to 2.9–8.6, and always being higher in samples stored under 5% O_2 + 3% CO_2, due to a lower sucrose content than in the other storage conditions.

High contents of myo-inositol were found in analyzed samples (Table 2). Statistical analysis of the obtained data showed a lower content of this sugar in samples stored more than 11 days ($P < 0.05$) and more stable behaviour on green beans stored under 3% O_2 + 3% CO_2, with oscillations below 14.3% of initial content.

The unidentified sugar U_1 decreased in green beans stored with less O_2 (3% O_2 + 3% CO_2 and 1% O_2 + 3% CO_2) from day 11 until the end of the storage, and it was undetected in samples stored under 1% O_2 + 3% CO_2 at 18 and 22 days of storage. U_2 was only detected during the first 11 days of storage in green beans stored with higher proportions of O_2 (normal air and 5% O_2 + 3% CO_2), being undetected under the other conditions assayed. Both compounds seem to be susceptible to degradation at low O_2 atmospheres.

A 3% O_2 + 3% CO_2 atmosphere maintains total soluble sugars and individual soluble sugars (fructose and glucose) at more stable and higher values than the other conditions assayed for green bean storage. As these sugars are directly used as substrates for vegetable respiration, this result can be related to slower respiration processes in green beans stored under these conditions than under the other conditions applied.

Statistical analysis (Table 3) revealed that storage atmosphere does not significantly influence NDF content of green bean samples under the conditions applied ($P < 0.05$). This parameter suffered slight oscillations, below 8.1% of the initial value. Samples stored under 1% O_2 + 3% CO_2 showed the most intense decrease at the last stage of storage (Table 1), which can be attributed to hydrolysis of polysaccharides, in agreement with Femenía, Sánchez, Simal, and Roselló (1999).

Statistical analysis showed that samples under normal air and 5% O_2 + 3% CO_2 maintained a significantly higher content of pectins in the analyzed samples, than samples with lower levels of O_2 ($P < 0.05$). Pectic

substances (including soluble pectins and Ca-linked pectins) showed irregular variations (Table 1), due to the balance between synthesis of pectins from protopectins in the seeds, and degradation of pectins to galacturonic acid molecules, favoured in the pods when high levels of CO_2 are applied (Buescher & Adams, 1983; Parker & Stewart, 1935).

4. Conclusion

From the studied conditions, 3% O_2 + 3% CO_2 at 8 °C was selected as the best to preserve the nutritive value of this product (higher and more stable levels of moisture, total available carbohydrates, total soluble sugars, fructose and glucose), with lower risk of anaerobic processes, which could take place below 2% O_2.

Acknowledgements

This work has been funded by Comunidad Autónoma de Madrid (2-COR0023/94), and by a predoctoral grant for M.C. Sánchez-Mata, provided by the Spanish Government.

References

Ahmed, A.E.R., & Labavitch, J. M. (1977). A simplified method for accurate determination of cell wall uronide content. *Journal of Food Biochemistry*, *1*, 361–367.

AOAC. (1990). *Official methods of analysis* (15th ed). *K. Herlich*. Arlington: Association of Official Analytical Chemist.

Arpaia, M. L., Labavitch, J. M., Greve, C., & Kader, A. A. (1987). Changes in the cell wall components of kiwifruit during storage in air or controlled atmosphere. *Journal of the American Society of Horticultural Science*, *112*(3), 474–481.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489.

Bognår, A., Bohling, H., & Fort, H. (1990). Nutrient retention in chilled foods. In T. R. Gormley (Ed.), *Chilled foods. The state of the art* (pp. 305–336). London: Elsevier Applied Science.

Buescher, R. W., & Adams, K. (1983). Basis for enhanced softening of snap beans (*Phaseolus vulgaris* L.) from postharvest exposure to CO_2. *Journal of the American Society of Horticultural Science*, *108*(1), 58–60.

Buescher, R. W., & Brown, H. (1979). Regulation of frozen snap bean quality by postharvest holding in CO_2 enriched atmospheres. *Journal of Food Science*, *44*, 1494–1497.

Cano, M. P., Monreal, M., De Ancos, B., & Alique, R. (1999). Effects of oxygen levels on pigment concentrations in cold-stored green beans (*Phaseolus vulgaris* L. cv. Perona). *Journal of Agricultural and Food Chemistry*, *46*, 4164–4170.

Costa, M. A. C., Brecht, J. K., Sargent, S. A., & Huber, D. J. (1994). Tolerance of snap beans to elevated CO_2 levels. *Proceedings of the Florida State Horticultural Science*, *107*, 271–273.

FAO. (1989). *Control de calidad en la elaboración de frutas y hortalizas*. Roma: Food and Agricultural Organization.

Femenía, A., Sánchez, E. S., Simal, S., & Roselló, C. (1999). Effect of temperature on the cell wall composition of raisins during storage under a modified atmosphere. *European Food Research and Technology*, *209*, 272–276.

Fernández Antoranz, C. (1998). *Influencia de la temperatura de almacenamiento en el valor nutritivo de distintas clases de judía verde* (Phaseolus vulgaris L.). Master degree thesis, Universidad Complutense de Madrid, Madrid.

Font Quer, P. (2000). *Plantas medicinales* (2nd ed). Madrid: Labor.

Groeschel, E. C., Nelson, A. I., & Steinberg, M. P. (1966). Changes in color and other characteristics of green bean stored in controlled refrigerated atmospheres. *Journal of Food Science*, *31*, 488–496.

Henderson, J. R., & Buescher, R. W. (1977). Effects of sulfur dioxide and CA on broken-end discoloration and processed quality attributes in snap beans. *Journal of the American Society of Horticultural Science*, *102*(6), 768–770.

Heredia, I., & Marañón, M. D. (1962). *Tecnología de conservación de alimentos, frutas y vegetales*. Madrid: Madrid.

Holdsworth, S. D. (1988). *Conservación de frutas y hortalizas*. Zaragoza: Acribia.

Kader, A. A. (1986). Biochemical and physiological basis for effects of controlled and modified atmospheres on fruits and vegetables. *Food Technology*, *40*(5), 99–104.

Kader, A.A. (1988). Influence of preharvest and postharvest environment on nutritional composition of fruits and vegetables. In B. Quebedeaux & F.A. Bliss. (Eds.) *Horticulture and human health. Contributions of fruits and vegetables. Proceedings of the 1st International Symposium on Horticulture and Human Health* (pp. 18–32). Arlington.

Kader, A. A. (1997). Biological bases of O_2 and CO_2 effects on postharvest-life of horticultural perishables. In M. E. Saltveit (Ed.), *Proc. of international controlled atmosphere research conference. CA'97 vol. 4* (pp. 160–163). Davis: University of California.

Kader, A. A., Zagory, D., & Kerbel, E. L. (1989). Modified atmosphere packaging of fruits and vegetables. *Critical Reviews of Food Science and Nutrition*, *28*(1), 1–30.

Kanellis, A. K., Solomos, T., & Roubelakis-Angelakis, A. K. (1991). Suppression of cellulase and polygaracturonase and induction of alcohol dehydrogenase isoenzyme in avocado fruit mesocarp subjected to low oxygen stress. *Plant Physiology*, *96*, 269–274.

Larson, A. E., Johnson, E. A., Barmore, C. R., & Hughes, M. D. (1997). Evaluation of the botulism hazard from vegetables in modified atmosphere packaging. *Journal of Food Protection*, *60*(10), 1208–1214.

Letang, G. (1997). Frutas y hortalizas: refrigeración y pérdida de agua. *Alimentación, Equipos y Tecnología., abr*., 29–36.

Muñoz Delgado, J. A. (1985). *Refrigeración y congelación de alimentos vegetales*. Madrid: Fundación Española de la Nutrición.

Oruña Concha, M. J., González Castro, M. J., López Hernández, J., & Simal Lozano, J. (1996). Evolution of sugar, starch, pectin and insoluble fibre contents in frozen green beans and Padrón peppers. *Deutsche Lebensmittel Rundschau*, *92*(9), 278–281.

Osborne, D. R., & Voogt, P. (1986). *Análisis de los nutrientes de los alimentos*. Zaragoza: Acribia.

Parker, M. W., & Stuart, N. W. (1935). Changes in the chemical composition of green snap beans after harvest. *Bulletin of The University of Maryland Agricultural Experiment Station*, *383*, 291–313.

Romojaro, F., Riquelme, F., Pretel, M. T., Martínez, G., Serrano, M., Martínez, C., Lozano, P., Segura, P., & Luna, P. A. (1966). *Nuevas tecnologías de conservación de frutas y hortalizas: atmósferas modificadas*. Madrid: Mundi Prensa.

Sánchez Mata, M. C., Cámara Hurtado, M., & Díez Marqués, C. (2002). Optimization of soluble sugar extraction on green beans and their identification and quantification by HPLC. *European Food Research and Technology*, *214*(3), 254–258.

Solomos, T. (1982). Effect of low oxygen concentration on fruit respiration: nature of respiratory diminution. In D. G. Richardson, & M. Meheriuk (Eds.), *Controlled atmospheres for storage and transport of perishable agricultural commodities*. Beaverton: Timber Press.

Souci, S. W., Fachmann, W., & Kraut, H. (1994). *Food composition and nutritional tables* (5th ed). Stuttgart: Medpharm Scientific.

Torija Isasa, M.E., Díez Marqués, C., Cámara Hurtado, M., Sánchez Mata, M.C., Fernández Antoranz, C. & Peñuela Teruel, M.J. (1997). Influence of storage temperature on the micronutrient content of green bean (*Phaseolus vulgaris* L.). In *Proceedings of 1st International Convention Food Ingredients: New Technologies. Fruits and Vegetables*. (pp. 72–79). Cuneo: UNIDO.

Van Soest, P. J., & Robertson, J. B. (1979). *Systems of analysis evaluating fibrous feeds*. Ithaca: Cornell University.

Watada, A. E., & Morris, L. L. (1966). Post-harvest behaviour of snap bean cultivars. *American Society of Horticultural Science*, *89*, 375–380.

Weichmann, J. (1986). The effect of controlled-atmosphere storage on the sensory and nutritional quality of fruits and vegetables. *Horticultural Reviews*, *8*, 101–127.

Wills, R., Mc Glasson, B., Graham, D., & Joyce, D. (1999). *Introducción a la fisiología y manipulación postcosecha de frutas, hortalizas y plantas ornamentales*. Zaragoza: Acribia.

Wright, K. P., & Kader, A. A. (1997a). Effect of controlled atmosphere storage on the quality and carotenoid content of sliced persimmons and peaches. *Postharvest Biology and Technology*, *10*, 89–97.

Wright, W. P., & Kader, A. A. (1997b). Effect of slicing and controlled-atmosphere storage on the ascorbate content and quality of strawberries and persimmons. *Postharvest Biology and Technology*, *10*, 39–48.

Zagory, D., & Kader, A. A. (1989). Quality maintenance in fresh fruits and vegetables by controlled atmospheres. In J. J. Jen (Ed.), *Quality factors of fruits and vegetables. Chemistry and technology*. Los Angeles: American Chemical Society.