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REGULAR ARTICLE

Behaviour of fresh cut broccoli under different modified atmosphere conditions

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ABSTRACT

Fresh cut broccoli is a product with an increasing demand due to its convenience and proved benefits on human health, but is very perishable due to its high respiration rate. In order to maintain quality and extend shelf-life, two different approaches were carried out. Firstly, samples were packaged in sealed trays under passive modified atmosphere, and half of them were heated with air (48°C for 3 hours). All trays were stored at 5 °C, and analyzed at 0, 7, 14 and 21 days. Basic physical-chemical parameters, chlorophyll a and b concentration, total phenolics, antioxidant activity, microbiological and sensory quality were assessed. Results showed that heat treated broccoli showed poor sensory quality due to off-odors and no increase in the shelf-life of the product was observed. For this reason, a second experiment was carried using passive and active modified atmospheres (10% O_2 and 5% CO_2) and compared to a control in air. Samples were also stored at 5 °C and analyzed at 0, 5, 10 and 21 days. Modified atmosphere, either active or passive, allowed maintaining broccoli florets shelf-life up to 21 days, with higher quality compared to the control, being most suitable the passive modified atmosphere due to its simplicity and lower cost.

Keywords: *Brassica oleracea* L. var. *Italica* cv. Parthenon; Heat treatment; Modified atmosphere packaging; Quality assessment; Shelf-life

INTRODUCTION

In recent years, conscious consumers have an increasing demand for healthy food. Broccoli (*Brassica oleracea* L. var *italica*) is an inflorescence highly valued due to its richness in glucosinolates, vitamins, antioxidants and other health-promoting phytochemicals (Yuan et al., 2010). In fact, Verhoeven et al., (1996) in their review indicated that epidemiological studies have shown an inverse association between the consumption of Brassica vegetables and the risk of cancer.Broccoli is a very perishable vegetable, and postharvest technologies should be applied, as modified atmosphere packaging, to extend its commercial life (Fernández-León et al., 2013a, Caleb et al., 2016).

Moreover, consumers demand not only healthy food, but easy-to-cook and easy-to-eat products. In that sense, the demand on broccoli has increased particularly, and broad types of broccoli products are now available in the market (Schreiner et al., 2007). Fresh-cut broccoli decays faster than intact heads. The quality loss is visually observed in symptoms such as loss of turgidity and yellowing (Izumi et al., 1996; Hansen et al., 2001; Eason et al., 2005, 2007; Fernández-León et al., 2013a).

Considering the above-mentioned factors, it is necessary to offer to the consumers a convenience product, and to evaluate different postharvest procedures aiming to preserve the quality of fresh-cut broccoli florets (Zhuang et al., 1995; Lemoine et al., 2008, 2009).

Heat treatment can be used as a postharvest technique to control pathongens, to modify tissue response to other types of stress and to maintain product quality during storage (Viña and Chaves, 2007), and it has shown evident advantages when compared with chemical treatments. Heat treatments have been successfully used in fresh cut vegetables as onion (Hong et al., 2000), celery (Viña and

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Chaves, 2007), leek (Tsouvaltzis et al., 2006), melon (Luna Guzmán et al., 1999) and garlic (Cantwell et al., 2003). For broccoli, hot air or water can delay senescence in intact heads (Funamoto et al., 2002, Perini et al., 2017) and in fresh-cut product (Lemoine et al., 2008, 2009; Costa et al., 2005).

Modified atmosphere packaging has been studied in order to assess whether this postharvest storage condition is an appropriate alternative to maintain physico-chemical, microbiological and sensory quality of fresh-cut broccoli. It is commonly accepted that broccoli can be benefitted by 1-2% O_2 with 5-10% CO_2 atmospheres at a temperature range of 0-5 °C, extending its shelf-life (Fernández-León et al., 2013b). However, it could be induced unpleasant sulfur-containing volatiles at these low O_2 levels (Izumi et al., 1996). It has been proved that it is not necessary to have these strict conditions to extend the postharvest life of broccoli, as Fernández-León et al., (2013b) established that a controlled atmosphere with 10 % O_2 and 5% CO_2 was very effective on maintaining the quality parameters of broccoli during 12 days. Moreover, Fernández-León et al., (2013a) found that modified atmosphere packaging with microperforated polypropylene at 5 °C allowed the retention of quality and functional values of fresh-cut broccoli cv. 'Parthenon'.

The aim of this work was to evaluate shelf-life of fresh-cut broccoli. In a first study, it was proved if the heat treatment helps to maintain the quality in broccoli florets and in a second study, diverse modified atmosphere packagings were tested (passive, active and a control) in order to establish the most suitable procedure to preserve physico-chemical, microbiological and sensory quality of fresh-cut broccoli.

MATERIALS AND METHODS

The work was divided into two subsequent experimental steps; first, modified atmosphere packaging and heat treatment was applied in order to extend the shelf-life of fresh-cut broccoli, then, in a second assay, three different atmospheres were used with the same aim.

Plant material

Broccoli (*Brassica oleracea* var. *italica*; cv. Parthenon) heads were obtained in February from two local producers (*CELAEX*, Puebla de la Calzada and *Cooperativa del Campo Juan XXIII*, Villafranco del Guadiana) in Badajoz, Spain. In both trials, approximately 15 kg of product were immediately transported to the INTAEX laboratory after harvesting, and stored in refrigeration at 0°C until the next day, when they were processed. Heads were prewashed with tap water, and then separated into florets with a sharp knife. Subsequently, florets were sanitized with chlorinated water (150 mg L^{-1} as sodium hypochlorite; pH=6.5) for 3 min at 4ºC.

Assays description

In the first assay, after washing with chlorinated water, broccoli florets were centrifuged at 850 rpm for 30 s using an industrial centrifuge (K50 100 Kronen, Kehl am Rhein, Germany) to eliminate excess water, and then approximately 200 g of broccoli florets were placed in rigid polipropilene (PP) plastic trays (12 x 17 x 5 cm) which were thermally sealed on the top with a 35 mm thick PP film on an industrial packaging machine (Model Verpachkungs-Systeme, Western, Germany), passive modified atmosphere (PMA).

Half of the sealed trays (3 replicates x 4 sampling days) were randomly taken and stored at 5ºC and 90-95% RH (PMA), and the remaining sealed trays (3 replicates x 4 sampling days) were treated with hot air (H) at 48 ºC for 3 h in a Memmert oven (Schwabach, Germany) and they were left at room temperature and then stored at 5ºC and 90-95% RH (PMA+H).

Three replicates per treatment and sampling time were prepared, and trays were kept at 5 ºC and 90-95% RH. Quality analyses were conducted on the processing day and after 7, 15 and 21 days of cold storage.

Taking into account the results obtained on the first assay, the second experiment was focused on modified atmosphere without heat treatment. Active (AMA) and passive (PMA) modified atmosphere were assayed, and compared to a control (Control).

In the second assay, broccoli florets were conditioning as in the first assay and 16 sealed trays were prepared as described above (PMA), other 16 trays were injected with a gas mixture (10 % O_2 and 5% CO_2) and sealed (AMA), and the rest were sealed with the same plastic film and then macroperforations (2 mm diameter) were performed in order not to alter the atmospheric conditions (Control). All trays were stored at 5ºC and 95 % HR, and assayed on the processing day and after 5, 10 and 21 days of storage.

Physico-chemical analysis

Head space gas composition. O_2 and CO_2 contents of all trays were measured using an O_2 and CO_2 meter PAK 12P (Control y Suministros S.A, Barcelona, Spain). Each tray was used for a single determination.

Weight of each tray was recorded at the beginning and after storage, with a 0.01 g accuracy balance (Mettler, Madrid, Spain) and weight loss was expressed as percentage.

Firmness was measured using a Stable Micro Systems Texture Analyzer TA-XT2i (Stable Micro Systems, Aname, Spain) through a compression assay on ten broccoli florets per treatment. The force was applied to produce a 10% deformation with a 100mm aluminium plate. Force/deformation curves were recorded using the computer program of the texture analyzer. Firmness was expressed as the maximum force (N).

Color parameters (L*, a* and b*) were measured in color space CIELab and the hue angle (hº) and Cromaticity (C*) were then calculated. Measurements were performed with a Minolta CR-200 (Aquateknica, S.A., Madrid, Spain) using the illuminant D65, with diffuse illumination, a viewing angle of 0º and a measurement circular area of 8 mm diameter. Measurements were performed in 2 different locations of 10 broccoli florets.

Chlorophyll a and b contents were determined using multivariate calibration by means of partial least squares (PLS) (Fernández-León et al., 2010). The results were expressed as mg 100 g^{-1} of fresh weight (fw).

The determination of total phenolic content (TPC) was performed according to Fernández-León et al., (2013b) from 5 g of broccoli homogenate. The results were expressed as mg of chlorogenic acid equivalents 100 g^{-1} fw.

Total antioxidant activity (TAA) was evaluated according to the procedure proposed by Cano et al., (1998) slightly modified using ABTS. Results were expressed as mg of Trolox equivalents 100 g^{-1} fw.

Microbial analysis

Microbiological analyses were carried out on fresh broccoli florets before (F) and after disinfection with chlorinated water (F+D), and on each sampling time. To determine total coliform, *Escherichia coli*, aerobic mesophilic, psychrotrophic, and yeast and molds counts, standard enumeration methods ISO 4832:2006, NF V 08-053:1993, ISO 4833:2003, ISO 17410:2001 and ISO 7954:1988 were used (Nogales-Delgado et al., 2013), and the results were expressed as log CFU g^{-1}. The presence or absence of *Salmonella spp.* and *Listeria monocytogenes* was confirmed following EN ISO 6579: 2002 and EN ISO 11290-1:1996, respectively.

Sensory evaluation

The sensory analysis of fresh-cut broccoli was carried out by seven trained panelists in several fruit and vegetable products. Samples were evaluated at room temperature. Methodology by order of the UNE-ISO 8587:2010 was used. They were asked to order the samples by decreasing intensity of the following parameters: off-odors, color and appearance. For the first assay, they had also to group the samples by treatment.

Statistical analysis

For statistical studies SPSS 19.0 software was used (IBM SPSS statistic, USA). Data are expressed as means ± SD and were analyzed using a one-way analysis of variance (ANOVA). When ANOVA detected significant differences between mean values, means were compared using Tukey's HSD test. For sensory data analysis, Friedman test was used, as described above. Sensory evaluation data were analyzed with Friedman statistical test, considering 13.69 as critical F value for a significance level of 0.01, when working with 7 judges and 6 samples (Naes et al., 2010).

RESULTS AND DISCUSSION

First experience

Fig. 1 shows the atmosphere composition inside the packages during storage. O_2 and CO_2 evolution was

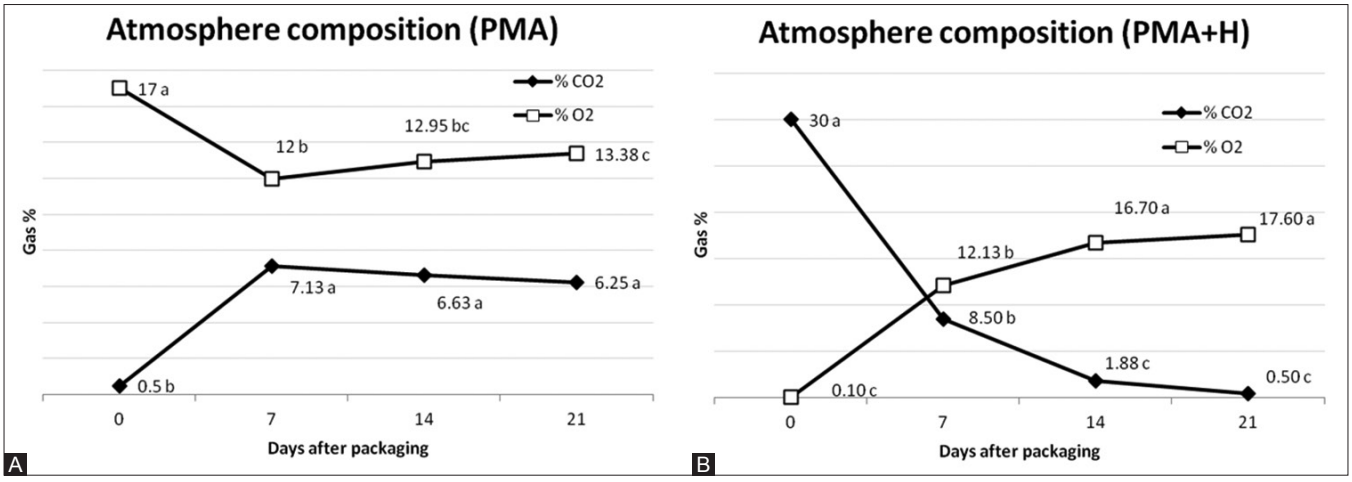

Fig 1. Gas composition within the trays of PMA (A) and PMA + H (B) during storage. Different letters for each gas and treatment indicate significant differences ($p<0.05$; n=3).

different in both treatments. In PMA (Fig. 1A), the percentage of O_2 decreased and CO_2 increased untill day 7, due to the high respiration rate of broccoli florets, when gas equilibrium was reached and remained almost constant during storage, according to other authors (Jia et al., 2009). On the other hand, in PMA+H (Fig. 1B) the initial gas composition was very different from PMA treatment, as it was measured just after heating (48ºC for 3 h) which caused a high stress on broccoli florets. Accordingly, the respiration rate dramatically increased during heat treatment and the gas composition inside the trays reached values of 30% CO_2 and 0.1% O_2. During storage, CO_2 concentration diminished and O_2 increased up to day 14, when gas equilibrium was reached and maintained until day 21. At the end of the storage, gas composition inside the PMA+H trays was very similar to that of atmospheric air, which can be attributed to a permeability modification of the plastic due to the heat treatment.

Results of weight loss, color, firmness, chlorophylls, TPC and TAA are shown in Table 1. There were significant differences between treatments in weight losses, being PMA+H the treatment with the highest losses, although they were always lower than 1 %. Condensation inside the trays was also observed in this treatment. These results disagree with those of Lemoine et al., (2009), who found lower water losses in heat treated florets.

Initial broccoli florets showed a C* value of 4.60, that increased after 7 days of storage in the two types of MA packages. After that, there was a decrease in MPA samples during storage, whereas MPA+H florets showed a slight increase. In all sampling days there were significant differences between both treatments (Table 1).

Among all color parameters, hº was the one that better describes the yellowish of the florets during storage, hº values close to 180 mean a greener color, as for fresh sample. There was a decrease since the first day of analysis, being the fall more drastic at day 21, but it must be pointed out that there were no significant differences between treatments.

There was a significant firmness loss in heat treated samples (PMA+H) after 14 days of storage (51%), that did not took place on PMA samples (21%), that could be attributed to water losses.

The highest concentration of chlorophyll pigments (a and b) were found in fresh broccoli florets (13.10 and 3.23 mg 100 g^{-1} fw, respectively) as they are very labile compounds. Chlorophylls decreased during storage with a decrement of 39 % for chlorophyll a and 44 % for chlorophyll b after 21 days at 5 ºC. Similarly, Lemoine et al., (2009) also found chlorophylls decreases in fresh-cut broccoli florets. At the end of the storage, PMA+H treatment had a higher concentration of chlorophylls pigments than PMA. In a similar way, Funamoto et al., (2002) found less yellowness in broccoli florets heated at 45 ºC for 14 minutes, and Büchert et al., (2011) also found higher chlorophyll contents in heat treated samples.

Phenolics are important compounds since they contribute to the maintenance of antioxidant status. Regarding to total phenols, PMA broccoli florets showed higher values than fresh sample (87.73 mg chlorogenic acid 100 g^{-1}) although they were not significantly different. On the other hand, PMA+H samples presented a 30 % decrease after 21 days of storage at 5 ºC, and always exhibited differences with PMA. Lemoine et al., (2009) found an important decrease in phenolic in heat treated samples after 7 days of storage, and then levels slightly increased. They explained that behavior as a consequence of a decrease in the activity of phenylalanine ammonia lyase enzyme (PAL) that has been related to phenolic compounds synthesis. Roura et al., (2008) found a reduction in PAL activity in fresh-cut lettuce, after a short heat treatment (water at 50 ºC during 2 min).

Fresh broccoli exhibits a wide range of compounds with antioxidant activity that has been reported to maintain

Table 1: Weight loss, color, firmness, chlorophylls and total phenols in broccoli florets stored under modified atmospheres (PMA and PMA+H)

Treatment	FRESH	PMA	PMA+H	PMA	PMA+H	PMA	PMA+H
Sampling date	0	7		14		21	
Weight loss	-	0.11±0.13c	0.54±0.12ab	0.22±0.00c	0.71±0.20[a]	0.27±0.11bc	0.66±0.18a
C*	4.60±2.33a	17.22±7.24c	15.42±2.08c	9.93±2.77b	16.24±2.14c	6.07±2.36a	17.98±5.09c
h°	177.18±56.52d	148.01±7.18c	146.05±2.36c	146.35±10.98c	144.75±3.11bc	125±18.4ab	116.45±6.93a
F max	12.13±2.12bc	11.98±1.78bc	11.42±3.61bc	9.63±1.28b	5.87±1.65a	12.88±2.24c	4.01±0.95a
Chlorophyll a[1]	13.10±0.35e	10.72±0.62d	9.59±0.3c	9.54±0.04bc	9.79±0.12c	7.82±0.1a	8.61±0.39ab
Chlorophyll b[1]	3.23±0.15d	2.51±0.08c	2.65±0.04c	2.46±0.06c	2.48±0.03c	1.73±0.05a	2.03±0.06b
TPC[2]	87.37±4.49bcd	101.74±11.89d	83.99±1.03bc	95.89±3.68cd	77.93±5.39ab	92.68±3.92bcd	67.08±6.98a
TAA[3]	72.48±2.47d	67.52±1.95d	64.06±4.96cd	53.4±3.68b	54.92±5.25bc	36.01±3.71a	37.61±3a

[1]Expressed as mg 100 g^{-1} fresh weight. [2]Expressed as mg chlorogenic acid 100g^{-1} fresh weight. [3]Expressed as mg Trolox 100ml^{-1} of broccoli extract. Values followed by different letters in the same row are significantly different ($p<0.05$)

human health (Zhang and Hamauzu, 2004), so antioxidant activity is an important characteristic for broccoli quality. The highest TAA value corresponded to fresh sample, and there was a decrease during storage registering values 50 % lower after 21 days. No significant differences between treatments were found though storage.

Microbial counts were under the limit of detection of the method (< 1 Log UFC g^{-1}) in all broccoli samples for total coliform and *E. coli*. Samples also showed absence of the pathogens *Salmonella spp.* and *L. monocytogenes*. Regarding aerobic mesophilic and psychrotrophic bacteria (Fig. 2 A, B), the behavior was similar; the sanitization process slightly reduced the counts that remained constant for PMA samples and during storage; while for heat treated broccoli florets (PMA+H) there was a significant reduction (55%). Heat treatment may result in the hydrolysis and/or thermal degradation of glucosinolates and various breakdown products are formed (isothiocyanates, nitriles, oxazolidinethiones, thiocyanate, and epithionitriles). Among the volatile products of glucosinolates degradation, isothiocyanates have been generally known as the most biologically active, being reported to possess broad-spectrum biological activity against bacterial (Lin et al., 2000). The values obtained in the present work were similar to those found by Brackett (1989) and higher than those reported by others (Olarte et al., 2009; Ansorena et al., 2011; Martínez-Hernández et al., 2013).

There were no differences between treatments during the storage in counts of yeasts and molds. A significant reduction (~3 Log UFC g^{-1}) was observed after sanitization and the first week of storage, increasing the counts afterwards. The heat treatment did not affect the viability of yeasts and molds.

Regarding to sensory evaluation, only the results for appearance are shown in Table 2. Off-odors and color results are not presented as they did not provide any additional information. There were significant differences between treatments, as the F test (13.72) was higher than the critical F value (13.69) of the Friedman's rank test ($p<0.01$).

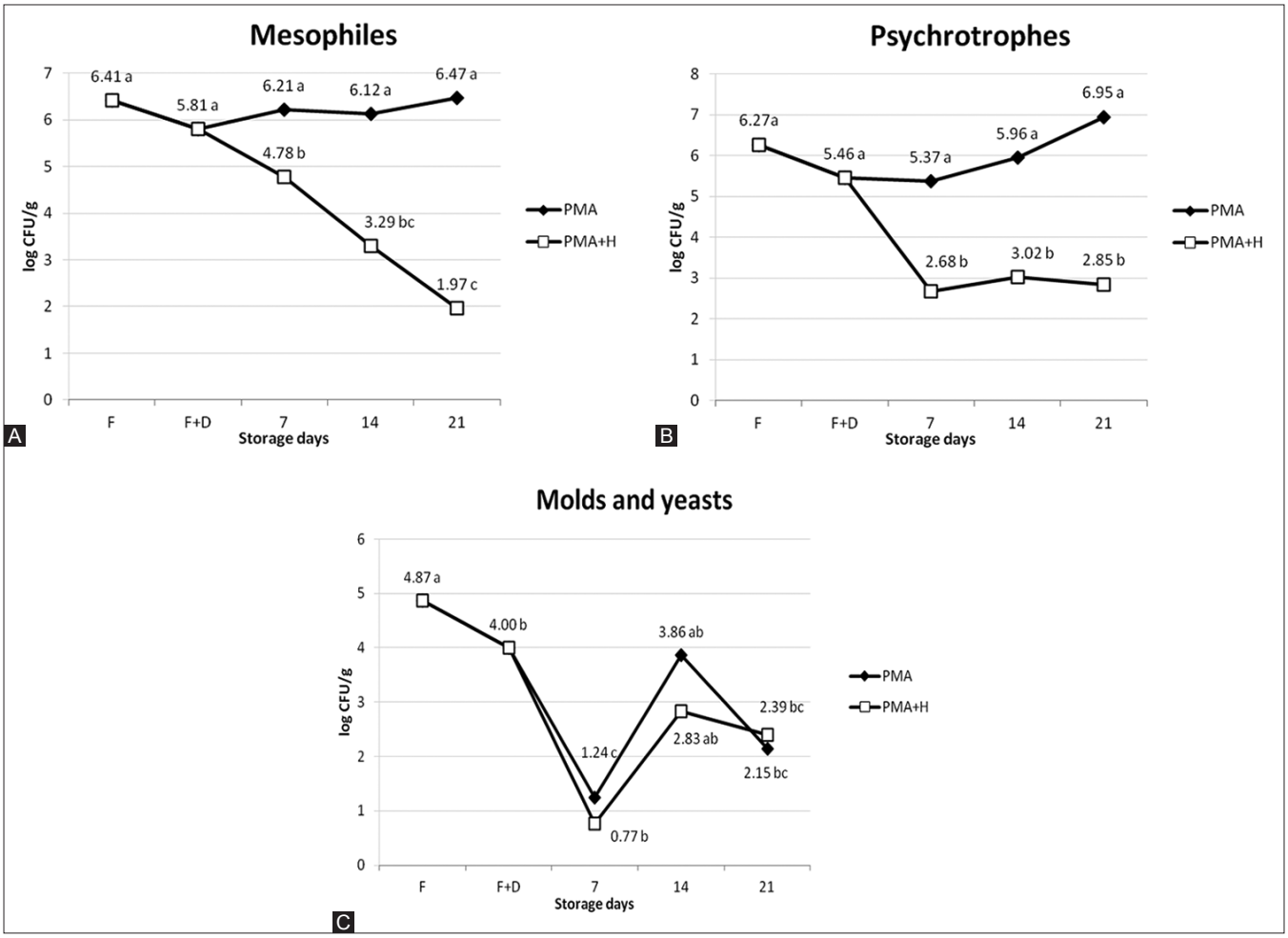

Fig 2. Microbial counts in PMA and PMA+H fresh-cut broccoli samples, before (F) and after disinfection (F+D), and during storage. Different letters for each treatment indicate significant differences ($p<0.05$; n=3).

The sum of the sample ranks (R) in the PMA treatment ranged between 12.5 and 15.5 whereas in the PMA+H between 34 and 35.5. As the difference of rank sums between samples, on the same treatment over time, was less than calculated Least Square Differences (LSD=13.72), there were no differences between them.

In the PMA+H for all sampling dates was observed water condensation, and the presence of off-odors, those were described as fungi, cheese, alcohol or moldy by the judges. Results indicate that panelists preferred PMA samples that exhibited better quality characteristics. So in the second experience, the study was focused on modified atmosphere, both active and passive.

Second experience

Fig. 3 shows the gas composition of the atmospheres around the florets. As expected, the gas composition of control samples did not change during storage and was similar to the atmospheric air due to the film macroperforations.

In the modified atmosphere packages, the equilibrium atmosphere was reached after 5 days and it was similar

Table 2: Results of the sensory test of PMA and PMA+H fresh-cut broccoli 'Parthenon' stored at 5°C

Treatment	PMA				PMA+H		PMA				PMA+H		PMA				PMA+H	
Sampling date	7						14						21					
Sample	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
Rank sums (R)	12.5	14	15.5	34	35.5	35.5	16.5	13	12.5	34.5	37	33.5	14	12.5	13.5	34.5	36	34.5
R mean per treatment		14			35			14			35			13.33			35	
Ftest per sampling date	48.24						48.65						45.88					
Ftest (day 7) = 48.25 > 13.69 LSD = 13.72																		

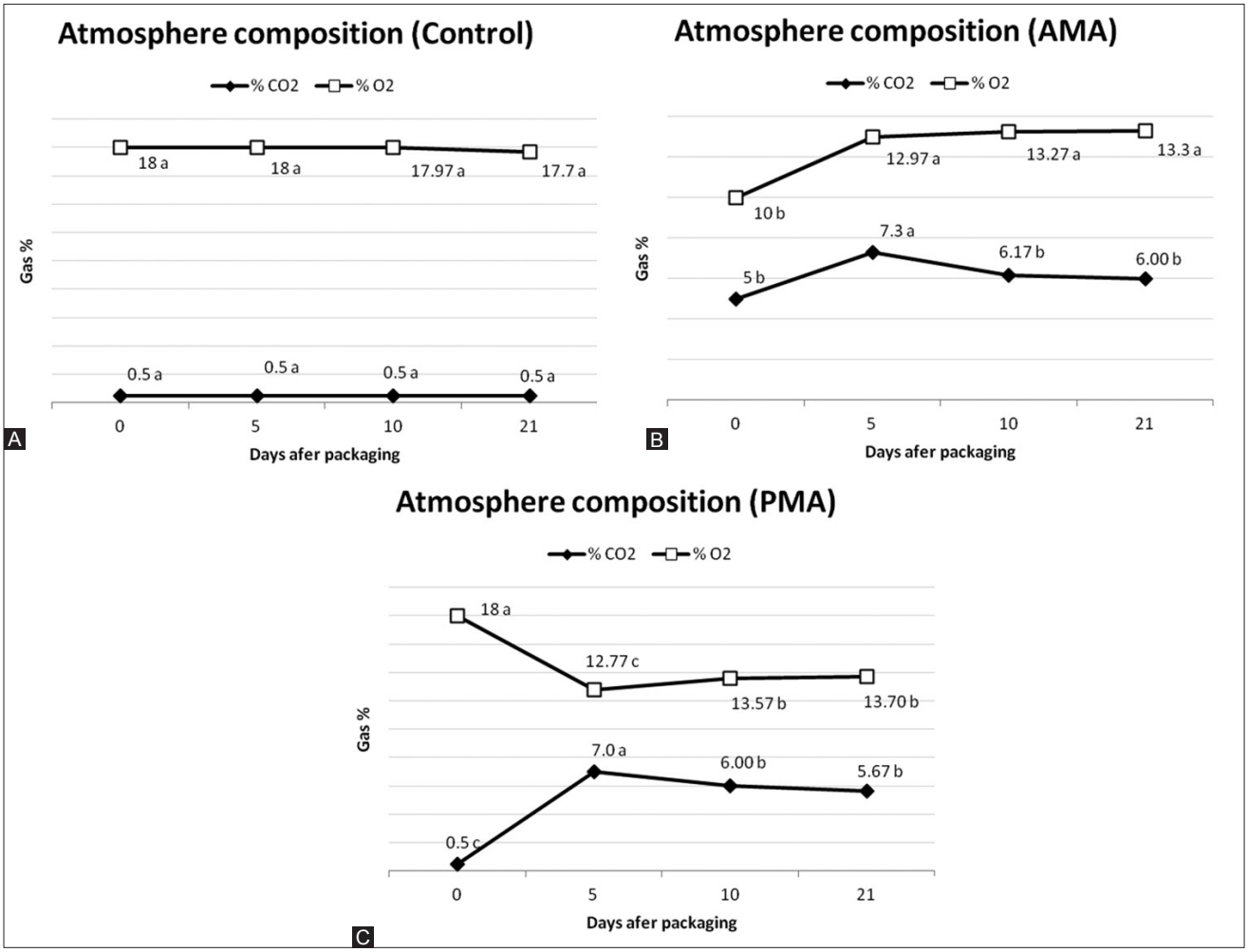

Fig 3. Gas composition within the trays of Control (A), PMA (B) and AMA (C) during storage. Different letters for each gas and treatment indicate significant differences ($p<0.05$; n=3).

in both treatments (13% O_2 and 6% CO_2), and remained almost constant up to the end of the storage. Similar results were obtained by Fernández-León et al., (2013a), using active modified atmosphere in broccoli with different plastic film.

As observed in Table 3, weight losses were always higher in Control samples, registering maximum levels of approximately 1% after 21 days of storage. Samples under modified atmospheres did not show differences between treatments. Weight losses of PMA and AMA samples were below 0.4 %, registering significant differences comparing to control samples only at the end of storage. Comparing with other works (Fernández-León et al., 2013a,b), the weight losses were relatively lower in our study. In control samples probably because of the use of a macroperforated film for sealing the trays, and in the modified atmosphere packages, the type of plastic film must be the responsible.

As it is well kwon, color is a very important quality attribute in broccoli as it is related to senescence processes that cause yellowing. Comparing to fresh sample, there was a diminishing in hº and in C* during storage in all treatments. Martínez-Hernández et al., (2013) had also previously reported a decrease in hº for broccoli florets. In general, no differences in hº and C* were observed among control and AMA and PMA broccoli samples throughout cold storage.

Firmness did not show significant differences between control and modified atmosphere treatments through storage. There was a slight increase at the end of storage, as previously reported by Ansorena et al., (2011) for broccoli florets during air storage.

Chlorophylls content in broccoli samples are shown in Table 3. There was a reduction in chlorophyll a content during storage for all treatments. In broccoli florets of AMA and PMA trays, chlorophyll a was always higher than in control samples, although these differences were significant only on day 21. On the other hand, the results showed that chlorophyll b remained without significant changes until day 5 for the control, while for the modified atmosphere samples (AMA and PMA) no significant differences were observed up to day 21 of cold storage. As it is known, chlorophyll a is responsible for blue-green colors while chlorophyll b is related to the yellow-green ones. The results obtained were slightly lower than other reported values (Fernández-León et al., 2013a,b). From the hereby presented data, it can be affirmed that the modified atmosphere treatments did not prevent chlorophylls degradation.

TPC evolution is summarized in Table 3. The initial TPC (87.58 mg chlorogenic acid $100g^{-1}$ fw) in fresh broccoli

Table 3: Weight loss, color, firmness, chlorophylls and total phenolic content in broccoli florets stored under modified atmospheres (AMA and PMA), and its comparison with control broccoli florets

Treatment	Fresh	CONTROL	AMA	PMA	CONTROL	AMA	PMA	CONTROL	AMA	PMA
Sampling date	0		5			10			21	
Weight loss	-	0.22±0.12bcd	0.00±0.00d	0.00±0.00d	0.44±0.00b	0.07±0.12cd	0.15±0.13bcd	1.04±0.13a	0.36±0.12bc	0.29±0.12bcd
C*	16.32±3.62ab	18.39±9.99a	15.99±6.03ab	11.95±2.81bc	6.12±3.62d	6.88±2.04d	9.73±3.33cd	6.56±4.94d	8.30±4.16cd	6.01±3.11d
hº	169.20±31.91a	144.78±5.77b	143.23±4.16b	143.94±31.7b	132.69±9.19bc	139.12±10.6bc	136.81±9.50bc	123.69±25.55c	129.67±10.64bc	127.01±16.77bc
F max	14.83±2.46ab	16.22±6.23abc	16.40±4.14abc	16.43±5.88abc	18.79±2.27abc	16.81±6.35abc	13.02±4.01a	21.43±4.69bc	21.92±6.65bc	22.93±7.52c
Chlorophyll a[1]	8.18±0.05 a	7.39±0.15b	7.14±0.54b	6.66±0.51bc	5.28±0.08def	6.07±0.22cd	5.61±0.28de	4.5±0.41f	5.47±0.13de	4.98±0.21de
Chlorophyll b[1]	1.40±1.30 a	1.30±1.30 a	1.40±0.03 a	1.44±0.01a	1.94±0.37b	1.47±0.01a	1.49±0.01a	2.92±0.15d	2.76±0.05cd	2.50±0.05c
TPC[2]	87.58±1.67 a	75.89±3.11bc	76.05±3.91bc	83.26±2.28ab	66.62±2.92de	72.77±3.07cd	77.14±2.69bc	27.54±2.08g	52.74±3.87f	58.77±2.96ef
TAA[3]	84.63±5.52 a	60.10±4.42cd	66.00±6.65bc	71.88±2.64b	53.56±2.61de	56.04±2.27cde	62.45±4.20bcd	35.00±1.16f	46.74±3.58e	48.08±3.73e

[1]Expressed as mg 100 g^{-1} fresh weight. [2]Expressed as mg chlorogenic acid 100g^{-1} fresh weight. [3]Expressed as mg Trolox 100ml^{-1} of broccoli extract. Values followed by different letters in the same row are significantly different ($p<0.05$)

was the higher value among all samples, but lower to those reported in literature (Fernández-León et al., 2013b). During storage there was a decrease in phenol contents that was more pronounced in control samples, compared to modified atmospheres on day 21 (27.54 vs. 52.74 and 58.77 mg chlorogenic acid $100g^{-1}$), with no significant TPC differences between modified atmosphere treatments throughout storage. These differences could be explained by the high respiration rate of broccoli stored under air conditions (Izumi et al., 1996) that might increase the degradation of TPC (Vallejo et al., 2003).

The evolution of TAA was similar to that of TPC. These compounds have a strong antioxidant activity as previously described by Fernández-León et al., (2013a,b). At the end of storage, the control samples had the lowest TAA value, significantly different to those of modified atmospheres samples. These results agree with those of Serrano et al., (2006), who pointed out that modified atmosphere packaging was an effective treatment for maintenance of TAA and TPC of broccoli during refrigerated storage.

As in the first assay, total coliform and *E. coli* counts were under the limit of detection of the method (< 1 Log UFC g^{-1}) and also showed absence of *Salmonella spp.* and *L. monocytogenes* in all broccoli samples. The low initial levels of microorganisms together with other production conditions are responsible for a high quality final fresh-cut product. Aerobic mesophilic and psychrotrophic bacteria showed a similar behavior (Fig. 4), with a reduction (1-2 Log UFC g^{-1}) after the sanitization process and an ulterior increase. In general there were no important differences among treatments with a result slightly lower for AMA at day 21. Our results were similar or lower than the reported by Lucera et al., (2011) and Martínez-Hernández et al., (2013). Yeats and molds counts also decreased (1.5 Log UFC g^{-1}) after sanitization, and the levels remain almost constant during storage (Fig. 4).

Regarding to sensory evaluation, as in the first experience, color loss, off-odor development and aspect were evaluated by a panel during the storage period, and these parameters had similar behavior. No significant differences among the three treatments were detected by panelist until day 21, and in Table 4 are shown only the results for aspect at that date. There were significant differences between treatments, as F test (46.67) was higher than the critical F value (13.69). In order to detect the samples that were different, it was compared the rank sum of the treatment with the LSD value (13.72). Differences between the rank sums of control and modified atmosphere treatments (AMA and PMA) were 23 and 29, respectively, higher than LSD and consequently different to control. On the other hand there was no significant difference between rank sum of AMA and PMA. In general, control samples were the worst valued, as they showed larger color loss,

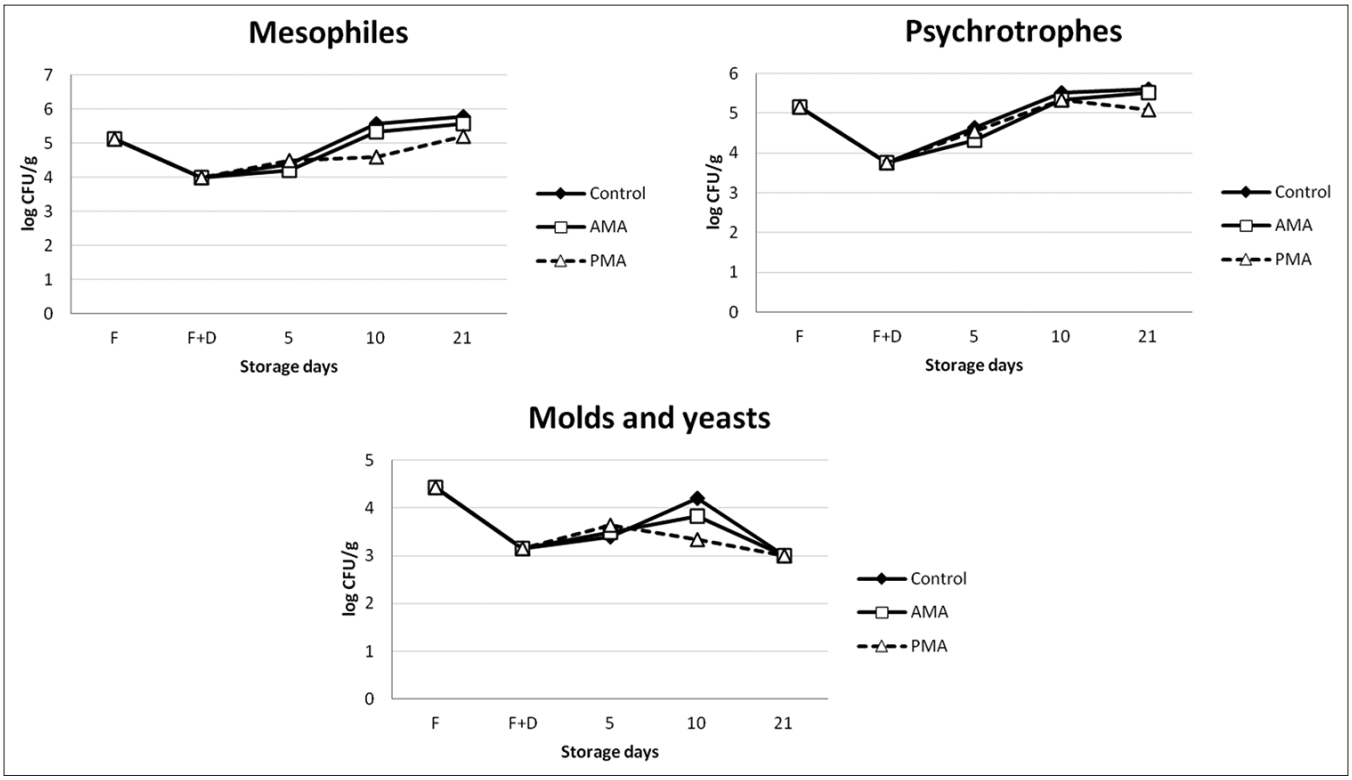

Fig 4. Microbial counts in Contrtol, AMA and PMA fresh-cut broccoli samples, before (F) and after disinfection (F+D), and during storage (n=3).

Table 4: Results of the sensoryl test of control, AMA and PMA fresh-cut broccoli 'parthenon' at day 21 of storage at 5°C

Sampling date	21					
Treatment	CONTROL		AMA		PMA	
Sample	A	B	C	D	E	F
Rank sums (R)	41	36	13.5	17.5	18	21
R mean per treatment	38.5		15.5		19.5	
Ftest	46.67					

presence of off-odors and a floret loose that gave them a poorest aspect.

CONCLUSIONS

Although heat treatment has been reported to be beneficial for chlorophyll retention, from our results it can be stated that it has a negative impact on sensory quality and shelf-life of fresh cut broccoli florets. High respiration rate of broccoli florets allowed a quick modification of the inner packages atmosphere, so there were no differences between active and passive modified atmosphere treatments. Compared to air storage, modified atmosphere samples maintained a higher quality in terms of weight loss, color, chlorophylls, phenolic compounds, total antioxidant activity and sensory characteristics up to 21 days, under the studied conditions. Taking in account those results, passive modified atmosphere is the most suitable storage technique for fresh cut broccoli due to its simplicity and lower cost.

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Authors' contributions

PFAH was in charge of acquisition of data and preparation of the manuscript. DGG and MCAY were responsible of the design of the experiment and were involved in data interpretation. DBG was involved in the physico-chemical and sensory analysis. JDA did the microbiological analysis. MJBG was the project manager and was involved data analysis and revised the manuscript.

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