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Designing a modified atmosphere packaging (MAP) for fresh-cut artichokes

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Abstract: 'Catanese' artichoke quarters were packaged in active modified atmosphere (5% O_2+10% CO_2) in four different materials or in air in macro-perforated bags used as control (CTRL), and stored at 4°C. Materials used for modified atmosphere packaging (MAP) were: polylactic acid (PLA), polylactid acid with one line of micro-perforation (PLA MF1), polypropilene with two lines of micro-perforations (PP MF2), and polypropylene + polyamide with two lines of micro-perforations (PP+PA MF2). Initially and after 2, 4, and 9 days, overall artichoke appearance, color and weight loss were monitored. O_2 and CO_2 concentrations within the packages were detected initially and after 1, 2, 17, 24.5, 41.5, 49.5, 120 and 216 h (end of the experiment). Also, at the last sampling date ethanol and acetaldehyde accumulations in artichoke tissue were measured. All the micro-perforated films maintained gas levels within the range of O_2>3% and CO_2<15%, defined as "safe", with a positive effect on quality: all samples remained above the limit of marketability until the end of the experiment, without significant differences among them, but showed a slight better overall appearance and, accordingly, a better retention of color parameters when compared with CTRL samples. Complete anaerobic condition (16% CO_2 and 0% O_2) developed in PLA bags where blackening of cut bracts and receptacle was observed, while black spots appeared on outer bracts, causing drastic quality reduction; samples fell below the limit of marketability after just 2 days. Also a significant accumulation of ethanol and acetaldehyde was found in these samples. Optimizing MAP made it possible to maintain the desired gas condition, with positive effects on quality of the produce in absence of any stabilizing treatment, for 9 days.

1. Introduction

Fresh-cut artichokes suffer several degradative reactions which limit their marketability. Enzymatic browning is caused by the oxidation of phenols, catalysed by polyphenol oxidase enzymes (PPO), with the subsequent formation of dark compounds (Lattanzio *et al.*, 1994; Tomás-Barberán and Espín, 2001). Non-enzymatic browning reactions are caused by iron-polyphenol complexes: chlorogenic acid, the most representative phenolic compound of artichoke heads, in presence of O_2 forms dark-coloured complexes with Fe^{3+}, while the same substrate in anoxic conditions forms colourless complexes with Fe^{2+}, but after exposure to air the Fe^{2+} complex is quickly oxidized to Fe^{3+} to give coloured compounds (Lattanzio, 2003). Also, mechanical wounding enhances a different array of enzymatic pathways, many of which are associated with volatile accumulation, such as ammonia, ethanol and acetaldehyde, which leads to the darkening of tissues and onset of off-flavors (Salunkhe and Do, 1976; Rolle and Chism, 1987).

The use of modified atmospheres can promote or, otherwise, inhibit these degradative reactions, and differences between beneficial and harmful effects of gas mixtures may be small. Increased levels of CO_2 are used in combination with low O_2 concentrations to maintain the visual quality of several types of fresh-cut produce (Beaudry, 1999). Carbon dioxide is considered a competitive inhibitor of PPO but increases in ammonia were observed in leafy tissues stored in high CO_2 (Cantwell *et al.*, 2010); similarly, if the O_2 level in the package decreases below the fermentation threshold, anaerobic respiration is triggered leading to the accumulation of anaerobic metabolites (i.e. ethanol and acetaldehyde) and stimulating the growth of some anaerobic pathogens (Oms-Oliu *et al.*, 2009). The presence of a very high CO_2 concentration (25%) in the storage atmosphere has been proved to be deleterious for fresh-cut artichokes (la Zazzera *et al.*, 2012), while only slight beneficial effects were observed at lower concentrations (5 and 15%). Other authors, combining a soy protein isolate enriched with cysteine (Cys) and different modified atmospheres (Active with 5 kPa O_2+15 kPa CO_2; active with 80 kPa O_2 and passive), found that the MA did not increase shelf-life compared to coated samples obtaining only 4 days of shelf-life, but the same authors refer an accumulation up to 30% of CO_2, which can be the cause of

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low shelf-life (Ghidelli *et al*., 2015). Therefore, the avoidance of extreme conditions in terms of CO_2 and O_2 concentrations within the package should be the main objective when designing a modified atmosphere packaging (MAP) system for fresh-cut artichokes.

In this work an active MAP was designed to maintain inside the package, at a steady state, a gas concentration of 5% O_2+10% CO_2 (target atmosphere) or at least within a "safety range" ($O_2 \geq 3\% + CO_2 \leq 15\%$) as defined in previous experiments. The use of micro-perforated films, allowing a greater permeability to O_2 and CO_2 than non micro-perforated films, should be recommended for fresh-cut produce with a very high respiration rate, like artichoke (Kader, 2002), since an accumulation of CO_2 can still be reached within the package but avoids an extreme concentration and total O_2 depletion. A simple recyclable film (polypropylene), a two-layer non recyclable film (polypropylene and polyamide), and a bio-based compostable film (polylactic acid), with different levels of micro-perforations, were used in this work as MAP materials.

2. Materials and Methods

Freshly harvested artichokes (*Cynara scolymus* L. 'Catanese') were collected in the area of Brindisi (Italy). In previous studies (Cabezas-Serrano *et al*., 2009), 'Catanese' showed the best attitude for processing as a fresh-cut produce compared to other cultivars, as it is less susceptible to post-cutting degradation phenomena. Raw heads were immersed for 2 min in a 100 mg L^{-1} sodium hypochloride solution in order to reduce the microbiological contamination and eliminate residual soil particles. Artichokes were then hand-trimmed using sharp stainless steel knives in order to remove external bracts, leaves and stalks; heads were washed in a NaOCl solution (100 mg L^{-1} of free chlorine). After washing, head trimming was completed by further removal of external greener and tougher bracts (inedible fraction) so as to keep just the innermost tender bracts, and by cutting about 2 cm from the top. Finally, artichokes were cut into quarters and closed in active modified atmosphere packaging (MAP) with the initial gas composition of 5% O_2+10% CO_2 using a Tecnovac packaging machine (Mod. T520, Grassobbio, BG; Italy). Four different packaging materials were used (Table 1): polylactic acid (PLA), polylactid acid with one line of micro-perforation (PLA MF1), polypropilene with two lines of micro-perforations (PP MF2), and polypropylene + polyamide with two lines of micro-perforations (PP+PA MF2). Control samples were passively packaged in macro-perforated PP MF2 bags in which additional macro holes (four per side) were manually made with a needle, insuring the complete gas exchange through the film and no atmosphere modification inside the packaging, while protecting the product from excessive weight loss (CTRL).

Evolution of gas composition inside the package, once it has been sealed, is due to the gas permeability of the plastic material used, to the respiration rate of the packaged commodity and, consequently, to the ratio between the package dimension and product weight. In order to reach and maintain the target atmosphere within the bags, packaging dimensions for a produce weight of 150 g were optimized knowing the film properties (Table 1), and the desired Gas Transmission Rate for O_2 (OTR) and CO_2 (CO_2TR) being respectively 221 ml m^{-2} day^{-1} for O_2 and 354 ml m^{-2} day^{-1} for $CO_{2,}$ using the following formula:

$$A = \frac{W * RR}{GTR * (\%G_{atm} - \%G_{pkg})}$$

where A = packaging surface (m^2); W = product weight (kg); RR = respiration rate (ml kg^{-1} day^{-1}); GTR= gas transmission rate (ml m^{-2} day^{-1}) % G_{atm} = percentage of the gas in the atmosphere (Bar) ; % G_{pkg} = percentage of the gas in the packaging (Bar).

Only for PLA MF1 was a produce weight of 120 g used, and the optimal dimension could not be optimized since, based on its low GTR, too-large and not feasible bag dimensions would have been needed.

All samples were stored at 4°C. Initially and after 2, 4, and 9 days, artichoke colour, overall appearance and weight loss, were monitored. O_2 and CO_2 concentrations

Table 1 - Film material and packaging characteristics

Film material	Packaging dimension (cm)	Thickness (µm)	Diameter of holes (µm)	Number of holes/m2	OTR (ml m^{-2} day^{-1} bar^{-1})	ß (CO_2TR/OTR)	WVTR (gm^{-2} day^{-1})
PLA	20x25	30	-	-	570	4.2	317
PLA MF1	10.6X17	30	60	138	570	4.2	317
PP MF2	24.6X13	30	60	160	1100*	2.4*	5*
PP+PA MF2	22.5X25	67	60	222	29*	2.75*	2.01*

* refers to the film without micro perforation.

PLA= polylactic acid without micro-perforation; PLA MF1= polylactid acid with one line of micro-perforation; PP MF2= polypropilene with two lines of micro-perforations; PP+PA MF2= polypropylene + polyamide with two lines of micro-perforations; OTR= Oxygen Transmission Rate; β = CO_2TR/OTR; WVTR = Water Vapor Transmission Rate.

within the packages were detected initially and after 1, 2, 17, 24.5, 41.5, 49.5, 120 and 216 h (end of experiment). On the last sampling date, also ethanol and acetaldehyde accumulations in artichoke tissue were measured. O_2 and CO_2 concentrations within the packages were measured using a WITT gascontrol 100-model MAPY 4.0 (WITT-GASETECHNIK GmbH & Co KG, Germany).

Overall appearance was evaluated by assigning a score to each artichoke quarter, from 5 (excellent) to 1 (very bad) using as a reference a photographic scale associated to brief descriptions (Amodio *et al.*, 2007). Samples in the bags were weighed at each storage period and the weight loss was calculated as percent of the initial fresh weight.

Colour in CIE L*a* b* scale was measured on the external surface of the outer bract and on the cutting surface, elaborating the images acquired with a Spectral scanner (DV SRL, Italia); Hue angle (h°) and Chroma were calculated from the primary L*, a*, and b* values.

Ethanol and acetaldehyde accumulation in artichoke tissues were measured only on the last sampling date with the method of Mateos *et al.* (1993) using a gas chromatograph Shimadzu GC-14A equipped with a FID detector (temperature 150°C); separation was carried out isothermally at 80°C on a capillary column 5% CBWX 20M on Carbograph 1AW20 80/120, 6'x 1/8" x 0.0085 (Alltech). Ethanol and acetaldehyde were identified and quantified by comparison with standard curves, and expressed respectively as umol ethanol g^{-1} and nmol acetaldehyde g^{-1}.

The experiment was organized in a Split-plot design with the packaging condition as the main factor and the time of storage as the secondary factor. The most conservative degrees of freedom were used to determine the effect of time, and packaging x time interaction. At each storage time the effect of packaging treatments was tested performing a one-way ANOVA. Means were separated using the Tukey test. For the O_2 and CO_2 concentrations within the packages and ethanol and acetaldehyde contents, standard deviations (STD) were calculated.

3. Results and Discussion

Packaging treatment and storage time significantly influenced all the quality parameters with few exceptions (a* value of bract cut surface was not influenced by packaging and b* value of the receptacle was not affected by time of storage). Also interactions were found to be significant for all attributes except for a* value in bract cut surface (Table 2).

The evolution of gas composition within the packages is shown in figure 1. All the micro-perforated films maintained gas levels within the safety range (O_2 higher than 3% and CO_2 lower than 15%). For samples stored in PP+PA MF2 and PP MF2 gas evolution showed the same trend: the O_2 level decreased in the first hours, due to the high respiration rate of cut artichokes, reaching a steady state at 12% and 10% respectively for PP+PA MF2 and PP MF2; CO_2 concentration increased slightly for both films, reaching a steady state at 11.5% for PP+PA MF2 and 12.5 for PP MF2. In PLA MF1 bags, O_2 concentration increased progressively, reaching a maximum of 14.5% after 216 h, while CO_2 concentration decreased sharply just after sealing, falling from 10 to 6%, and then rapidly increased, reaching at equilibrium a concentration of about 7.5%. In non-micro-perforated PLA packages, anaerobic conditions (0% O_2 and 16% CO_2) developed after 17 h; then the CO_2 level increased further, reaching a maximum of 29% after 120 h of storage.

All the samples stored in micro-perforated bags showed a better or comparable visual quality than CTRL samples, allowing the produce to keep marketability until the end of the experiment without significant differences among them (Fig. 2). On the contrary, artichokes stored in non-perforated PLA resulted heavily damaged: black spots appeared on outer bracts, a blackening of cut bracts and receptacle was observed and off-odours were perceived. After 2 and 4 days of storage, the injuries did not show up until removal from low O_2 atmosphere and exposure to

Table 2 - Results of ANOVA test for treatment and time of storage on quality attributes of fresh-cut artichoke quarters during storage in different atmospheres

Parameter	Packaging	Time	Packaging x Time
Appearance score			
Bract cut surface and receptacle cut surface	****	****	****
External bracts	****	****	****
Color			
External surface			
L*	****	****	****
a*	****	****	****
b*	***	****	****
Chroma	***	****	****
Hue Angle	**	***	**
Bract cut surface			
L*	****	****	****
a*	ns	****	ns
b*	****	****	****
Chroma	****	****	****
Hue Angle	*	****	**
Receptacle cut surface			
L*	***	****	*
a*	****	****	****
b*	***	ns	****
Chroma	****	*	****
Hue Angle	****	****	***
Weight loss	****	****	****

Within each row, each factor (Treatment and Time) and their interaction are significantly different for P≤0,05 (*); P≤0,01 (**); P≤0,001 (***); P≤0,0001 (****), or not significant (NS).

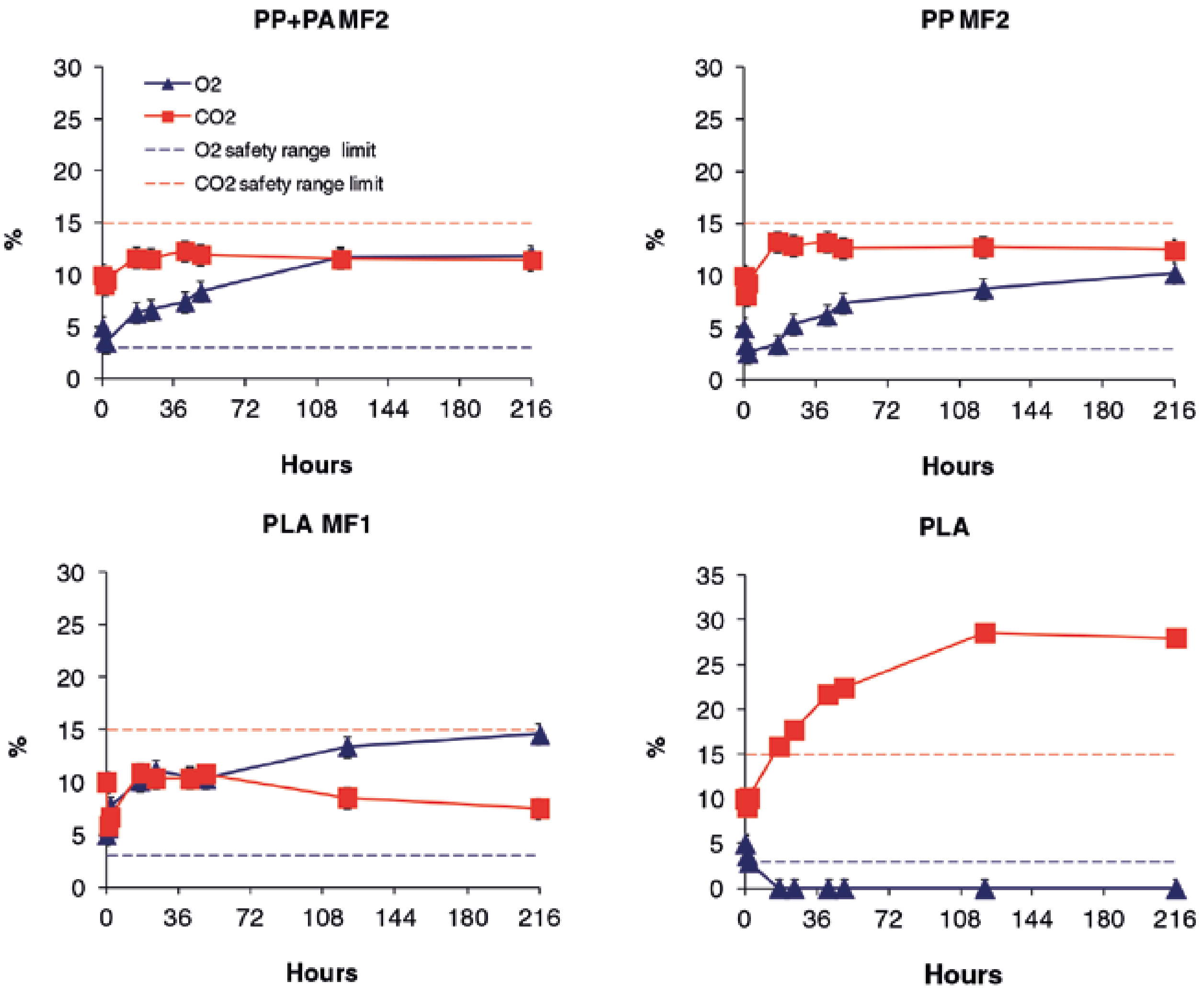

Fig. 1 - O_2 and CO_2 concentrations within different packages used for artichoke quarters stored at 4°C: PP+PAMF2 = polypropylene + polyamide with two lines of micro-perforations; PP MF2 = polypropilene with two lines of micro-perforations; PLA MF1= polylactid acid with one line of micro-perforation; PLA= polylactic acid without micro-perforation. Mean values of three replicates ± STD.

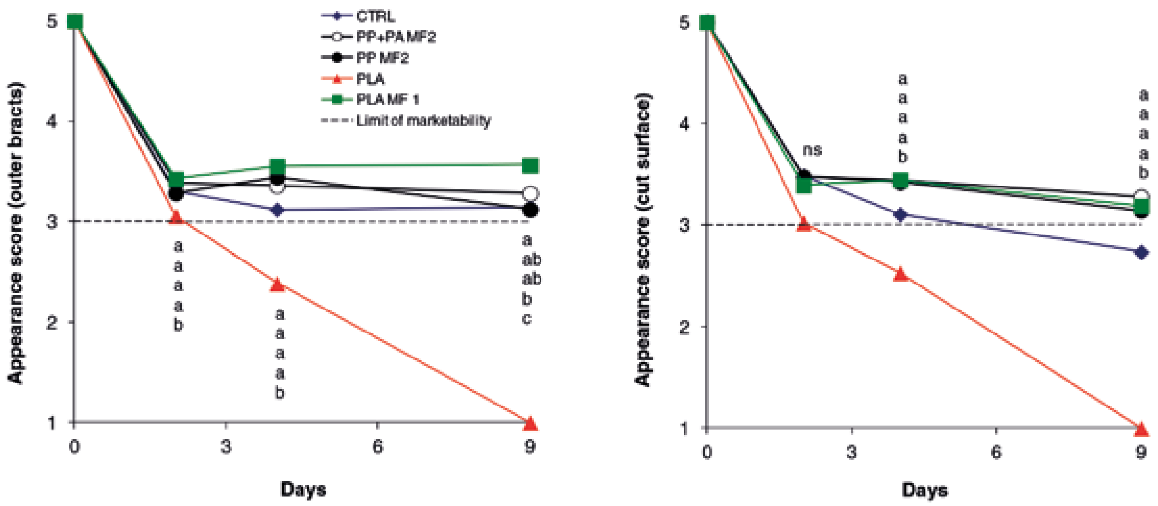

Fig. 2 - Changes over time of overall appearance in fresh-cut artichoke quarters packaged in active modified atmosphere (5% O_2+10% CO_2) and stored at 4°C: PP+PAMF2= polypropylene + polyamide with two lines of micro-perforations; PP MF2= polypropilene with two lines of micro-perforations; PLA MF1= polylactid acid with one line of micro-perforation; PLA= polylactic acid without micro-perforation. CTRL represents samples stored in air in macro-perforated bags. At each storage sampling different letters indicate significant differences (P<0.05).

air, when in few minutes they became completely inedible, with the onset of off-odours. Black spots and blackening could be due to non-enzymatic browning, as suggested by Lattanzio (2003) on artichokes: chlorogenic acid, the most representative phenolic compound of artichoke heads, in the absence of O_2 forms colourless complexes with Fe^{2+}, but after exposure to air the complexed Fe^{2+} is quickly oxidized to Fe^{3+} to give coloured compounds. In the same way, also enzymatic reactions could have taken place after exposure to O_2. At the last sampling, after 9 days of storage in extreme atmosphere condition, black spots and blackening began to appear in the produce when still inside the PLA package, suggesting a possible necrosis of the tissues.

Colour data (data not shown) confirmed the deleterious effect of the extreme gas conditions reached within the PLA bags, indicating a severe browning (drop on L and b*, increase of a* and higher variation of Hue Angle) and a better retention on colour parameters for samples stored in micro-perforated films, also compared to CTRL samples.

Weight loss (Fig. 3) was greater in CTRL samples and in samples stored in PLA bags (7.5, 5.7 and 3.7% for CTRL, PLA and PLA MF1 respectively), than in samples stored in PP MF2 and PP+PA MF2 (below 1%). Weight loss in non micro-perforated PLA bags was significantly higher than in bags of the same material but with micro-perforations. Most probably the deleterious atmosphere condition within these bags played a more important role on weight loss than the possible higher transpiration which the presence of micro-perforations might have generated.

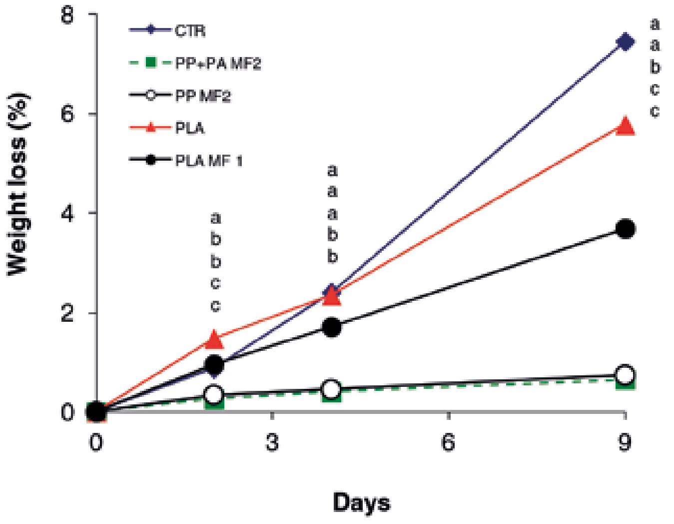

Fig. 3 - Weight loss over time of cut bract and external surfaces of fresh-cut artichoke quarters packaged in active modified atmosphere (5% O_2 + 10% CO_2) and stored at 4°C: PP+PAMF2 = polypropylene + polyamide with two lines of micro-perforations; PP MF2= polypropilene with two lines of micro-perforations; PLA MF1= polylactid acid with one line of micro-perforation; PLA= polylactic acid without micro-perforation. CTRL represents samples stored in air in macro-perforated bags. At each storage sampling different letters indicate significant differences (P<0.05).

A clear accumulation of ethanol and acetaldehyde was found in artichokes stored for 9 days in PLA (Fig. 4), where complete anaerobic conditions developed. The synthesis of these volatile compounds caused by anaerobic respiration was demonstrated by several authors (Shaw, 1970; Woodward and Topping, 1972; Prasad and Stadelbacher, 1974). Ke *et al*. (1991) found strong correlations between off-flavour development and ethanol content, and to a lesser extent, acetaldehyde. Severe off-odours were produced by broccoli under anaerobic conditions developed after few days in MAP (Forney *et al*., 1991). Nichols and Patterson (1987) observed that in apples the injury due to ethanol and acetaldehyde accumulation in anaerobic conditions or high CO_2 atmospheres is post-anoxic: it occurs after removal from low O_2 exposures, when ethanol is oxidized to acetaldehyde.

4. Conclusions

All the tested micro-perforated materials made it possible to reach and maintain the atmosphere composition within a safety range (O_2>3% and CO_2<15%), with a posi-

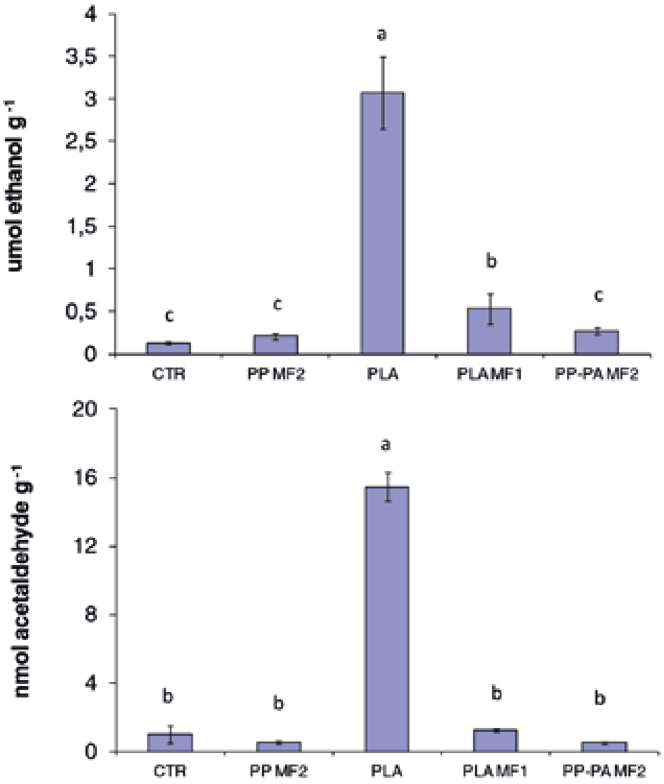

Fig. 4 - Ethanol and acetaldehyde accumulation, after 9 days of storage, in fresh-cut artichoke quarters packaged at 4°C in active modified atmosphere (5% O_2+10% CO_2), in different packaging conditions: PP+PAMF2 = polypropylene + polyamide with two lines of micro-perforations; PP MF2 = polypropilene with two lines of micro-perforations; PLA MF1 = polylactid acid with one line of micro-perforation; PLA = polylactic acid without micro-perforation. CTRL represents samples stored in air in macro-perforated bags. Mean values of three replicates ± STD.

tive effect on quality of stored fresh-cut artichokes. Artichokes packaged in PLA MF1 suffered a higher weight loss than produce stored with other packaging materials, which however did not seem to influence its overall appearance. On the contrary, the use of non micro-perforated films was not suitable for cut artichoke, probably due to its high transpiration rate: serious damage occurred to the produce in PLA bags, causing a drastic reduction in its quality. Extreme conditions in terms of CO_2 and O_2 concentrations may be very deleterious for packaged vegetables and the avoidance of these conditions within the packages must be the main objective when designing a MAP system for fresh-cut artichokes. MAP optimization for fresh-cut artichokes was possible with the auxilium of micro-perforation, which permitted establishment and maintenance of the desired gas conditions, regardless of the plastic material. The designed MAP allowed storage of cut artichokes in the absence of any stabilizing treatment for 9 days.

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