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Fresh Cut Lettuce (*Lactuca sativa* L.) Quality as Affected by Packaging Material

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Summary

The use of fresh cut vegetables has continued to increase in salad bars and fast food restaurants. Their storage life is influenced by their continued respiration and the biochemical activity associated with senescence. Enzymatic browning is one of the main causes of quality losses during postharvest storage. The combined use of modified atmosphere packaging and low temperature reduces respiration and delays senescence, thereby extending storage life. The objective of this work was to assess the suitability of a range of polymeric films for modified atmosphere packaging on butterhead, crisphead, latin and leafy lettuce types minimally processed during storage at 4 ºC for 9 days. Multilayered polyolefin RD-106, PD-900 and PD-961EZ films with different gas permeability (oxygen permeability from 3000 to 11500 cm^3 m^2 24 h, 1 atm at 22.7 ºC, carbon dioxide permeability from 9800 to 30000 cm^3 m^2 24 h, 1 atm at 22.7 ºC) were tested. Phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) activities, and the content of phenolic compounds were measured to relate them to overall visual quality, leaf edge browning and leaf surface browning. The variability in enzymatic activity was affected by both the type of lettuce and the permeability of the packaging material for O_2 and CO_2. A low chlorogenic acid concentration and the best organoleptic quality was achieved with the packaging material PD 900. The leafy lettuce type had the highest concentration of chlorogenic acid without quality losses by enzymatic browning. Leafy lettuce packed with multilayered polyolefins mantained an acceptable quality for consumption after 9 days of storage, independent on the polymeric film used as packaging material.

Zusammenfassung

Einfluss des Verpackungsmaterials auf die Qualität von frischgeschnittenem Salat (*Lactuca sativa* L.). Die Verwendung von frischgeschnittenem Gemüse in Salattheken und Fast Food Restaurants nimmt weiterhin zu. Seine Lagerfähigkeit ist abhängig von der Atmung und den biochemischen Aktivitäten in Zusammenhang mit der Seneszenz. Enzymatisch bedingtes Verbräunen ist einer der Hauptgründe für Qualitätsverlust während der Lagerung. Die Kombination von Verpackung in modifizierter Atmosphäre und niedrigen Temperaturen reduziert die Atmung und den Alterungsprozess und verlängert so die Lagerfähigkeit. Gegenstand dieser Untersuchung war es zu beurteilen, inwiefern sich eine Reihe von Polymerfolien für die Verpackung von geringfügig bearbeiteten Kopf-, Blatt- und Pflücksalaten in modifizierter Atmosphäre mit anschließender Lagerung bei 4 ºC über 9 Tage, eignen. Die vielschichtige Polyolefinfolien, RD-106, PD-900 und PD-961EZ mit unterschiedlicher Durchlässigkeit (Sauerstoffdurchlässigkeit zwischen 3000 und 11500 cm^3 m^2 24 h, 1 atm bei 22.7 ºC, CO_2-Durchlässigkeit zwischen 9800 und 30000 cm^3 m^2 24 h, 1 atm bei 22.7 ºC) wurden untersucht. Phenylalaninammoniumlyase- (PAL), Polyphenoloxidase- (PPO) und –peroxidase- (POD) Aktivitäten und der Gehalt an phenolischen Bestandteilen wurden gemessen und in Beziehung zu den gesamten visuellen Qualitätsmerkmalen, sowie Blattrand- und Blattoberflächenverbräunung gestellt. Die Variabilität der Enzymaktivität wurde durch den Salattyp und die Durchlässigkeit des Verpackungsmaterials für O_2 und CO_2 bedingt Eine geringe Chlorogensäurekonzentration und die beste organoleptische Qualität wurde mit dem Verpackungsmaterial PD 900 erzielt. Die Pflücksalate wiesen die höchsten Chlorogensäurekonzentrationen, ohne dass Qualitätsverluste durch enzymatisches Verbräunen auftraten. Pflücksalate, die in vielschichtige Polyolefine verpackt wurden, behielten ihre akzeptable Verzehrqualität auch nach neuntägiger Lagerung.

Key words. enzymatic browning – leafy vegetable – minimally processed – polymeric films – polyolefin – storage

Introduction

Fresh cut vegetables have become popular due to theirs increased use in salad bars, supermarkets and fast food restaurants. Many mechanical processes are involved in their preparation which injure the tissues. Wounding induces alterations in many physiological processes which often make the minimally processed product more perishable than the unprocessed fresh product, diminishing their shelf life (BOLIN and HUXSOLL 1991).

Low temperature and modified atmosphere packaging reduce respiration, water losses and delay senescence, thereby extending the storage life of vegetables. Temperature, oxygen and carbon dioxide concentrations vary according to the type of vegetable, variety, origin and season (BALLANTYNE et al. 1988). For minimally processed lettuce, quality and shelf life vary depending with cultivar, maturity, processing grade, and storage conditions (COUTURE et al. 1993).

The equilibrium gas composition of the atmosphere inside the package depends on the gas diffusion properties of the packaging material and the respiratory activity of the product (KADER et al. 1989). High barrier package films and films with excessive permeability are known to have adverse effects on processed vegetable. In general, optimal conditions for equilibrium modified atmosphere can be achieved with the use of a film with intermediate permeability (BENNINK 1997).

Enzymatic browning of fruits and vegetables after minimal processing is one of the most important causes of quality losses, resulting in consumers rejection (ARTÉS et al. 1998). Increases in phenylalanine ammonia lyase (PAL) activity and in soluble phenolic content have been reported for processed lettuce (KE and SALVEIT 1989a). The phenolic compounds are oxidized by polyphenoloxidase enzyme (PPO) producing polymers that contribute to tissue browning (KE and SALVEIT 1989a, TOMÁS-BARBERÁN and ESPIN 2001). Wounding also increases peroxidase (POD) activity and lignin formation (MADER and FUSSL 1982, TOMÁS-BARBERÁN et al. 1997a, TOMÁS-BARBERÁN and ESPIN 2001).

The objective of this work was to assess the suitability of a range of polymeric films for modified atmosphere packaging of minimally processed lettuce, using four lettuce types (butterhead, crisphead, latin and leafy lettuce) during 9 days of storage at 4 ºC.

Material and Methods

Plant material

'Regina' (butterhead lettuce), 'Coolguard' (crisphead lettuce), 'Gallega' (latin lettuce) and 'Grand Rapids' (leafy lettuce) cultivars were harvested at commercial maturity in the experimental field of the Horticulture Department, Buenos Aires University, during Spring time. These lettuce types are the most used for the different fresh cut salads.

Lettuce processing

For each plant, wrapper leaves were discarded and individual leaves were cut perpendicularly to the midrib into segments between 0,5 to 1 cm width. The processed material was dipped in a 50 ppm chlorine solution for 1 min at 5 °C and then centrifuged at 2800 rpm for 15 sec to remove surface moisture. About 100 g of processed lettuce was weighted and packed into bags made with different polymeric films and stored at 4 ºC.

Treatments

The four lettuce types were packed with the most common and available packaging materials:

1. Microperforated polypropylene SM-250Y, 8 perforations of 1.7 mm per square inch (Control);
2. Multilayered polyolefin RD-106;
3. Multilayered polyolefin PD-900 and
4. Multilayered polyolefin PD-961EZ.

The main characteristics of the packaging materials are presented in Table 1.

Measurements

Enzymatic activity and organoleptic quality were assessed after processing, and after 4 and 9 days of storage period. Before the measurements, the bags containing the processed lettuce were weighted using an Acculab scale (S=0.01 g).

PAL, PPO and POD activities. PAL was extracted and PAL activity was determined as described by KE and SALTVEIT (1986). PAL activity was expressed as μmol of *t*-cinnamic acid g^{-1} fresh weight h^{-1}. PPO was extracted and PPO activity was determined according to COUTURE et al. (1993), and it was expressed as μmol

Table 1. Ranges of gas permeability and water vapour transmissivity of the packaging materials included in this study.

Packaging material	Gas permeability$^{\phi}$ (cm^3 m^2)		Water vapour transmissivity* (g 100 square inch)
	O_2	CO_2	
RD 106	8500–11500	26000–30000	1.6–2.1
PD 900	3000	9800	0.65
PD 961 EZ	6000–8000	19000–22000	0.90–1.10

$^{\phi}$ measured during 24 h, at 1 atm and 22.7 ºC
* measured during 24 h, at 22.7 ºC and 100 % RH

of quinone g^{-1} fresh weight min^{-1}. POD was extracted as described by COUTURE et al. (1993), while POD activity was measured according to LOAIZA-VELARDE et al. (1997). POD activity was expressed as μmol of guaiacol g^{-1} fresh weight min^{-1}.

Phenolic compounds content. Phenolic compounds were extracted as described by KE and SALTVEIT (1988), and quantified as chlorogenic acid concentration.

Organoleptic quality evaluation. Several quality parameters of minimally processed lettuce were evaluated. Overall visual quality was evaluated using a scale of 9 to 1, where 9=excellent and 1=unusable, a score of 6 was considered as the limit of commercial acceptability. Leaf surface browning and leaf edge browning were evaluated using a scale of 1 to 5, where 1=none and 5= severe (LÓPEZ-GÁLVEZ et al. 1996).

Experimental design and statistic analysis

The processed lettuce material was randomly separated into four batches (packaging materials), with three replications for each one. Results were subjected to analysis of variance and means comparised by Tukey test (5 %). Non-parametric methods were used for organoleptic quality variable analysis.

Results and Discussion

Fresh weight loss

All minimally processed lettuce packed with SM 250 films had weight losses as high as 31 %, therefore SM 250 was regarded as control treatment, i.e. simulating the non-packaging conditions the lettuce would have. Losses were below 1 % when the other materials (RD-106, PD-900 and PD-961EZ) were used (Fig. 1). These materials appeared suitable for this type of packaging according to the tolerance limit of 3 % weight loss indicated for lettuce (NAMESNY-VALLESPIR 1993).

PAL activity

Initial PAL activity measured after lettuce processing ranged between 0.183 μmol of *t*-cinnamic acid g^{-1} fresh weight h^{-1} for latin lettuce and 0.300 μmol of *t*-cinnamic acid g^{-1} fresh weight h^{-1} for leafy lettuce (Fig. 2).

Until the 4th day of storage, the enzymatic activity increased for butterhead, latin and leafy lettuce but not for crisphead lettuce. PAL activity increase was not detected for the latter probably due to a high enzymatic activity reached during the first 3 days of storage at 4 °C, as reported by LÓPEZ-GALVEZ et al. (1996). At the 9th day of storage, the enzymatic activity decreased or remained stable depending on the lettuce type and packaging material. Leafy lettuce had the highest PAL activity during storage while crisphead lettuce had the lowest. This is in agreement with other authors (LÓPEZ-GALVEZ et al. 1996; TOMÁS-BARBERÁN et al. 1997b). Low CO_2 transmission rate packaging materials induced PAL activity due to pH modification as earlier reported (SIRIPHANICH and KADER 1985; KE and SALTVEIT 1989b). In this trial, fresh cut lettuce packed with any of the three multilayered polyolefin films had higher PAL activities than the control without significant differences between them.

PPO activity

Initial values of PPO activity ranged between 6.410 μmol of quinone g^{-1} fresh weight min^{-1} for leafy lettuce and 8.434 μmol of quinone g^{-1} fresh weight min^{-1} for butterhead lettuce (Fig. 3).

PPO activity tended to increase during the first 4 days of storage in leafy and latin lettuce. PPO was constant or even decreased until the 9th day of storage, depending on the variety and the packaging material. Fresh cut latin and butterhead lettuce packaged with PD-961 had the lowest PPO activity, significantly different from the other packaging materials. The lowest enzymatic activity for crisphead lettuce was achieved with RD-106 and PD-900 and for leafy lettuce with RD-106 and PD-961 differing significantly in both cases when compared with the other packaging materials.

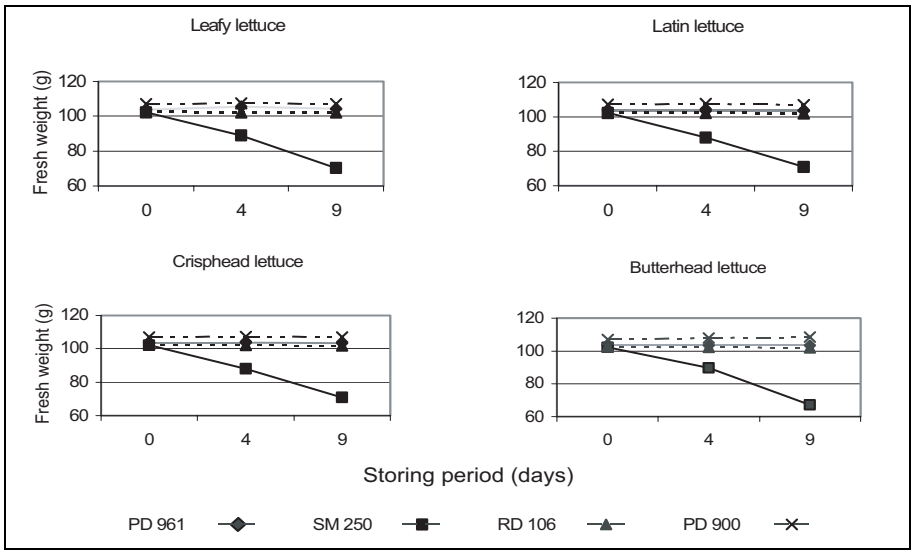


Fig. 1. Fresh weight (g) of each lettuce type according to the polymeric film employed as packaging material during storage period: PD 961, SM 250, RD 106, PD 900. SEM (standard error of mean) for every lettuce type: SEM leafy=3.06, SEM latin=2.48, SEM crisphead=3.02 and SEM butterhead=3.29.

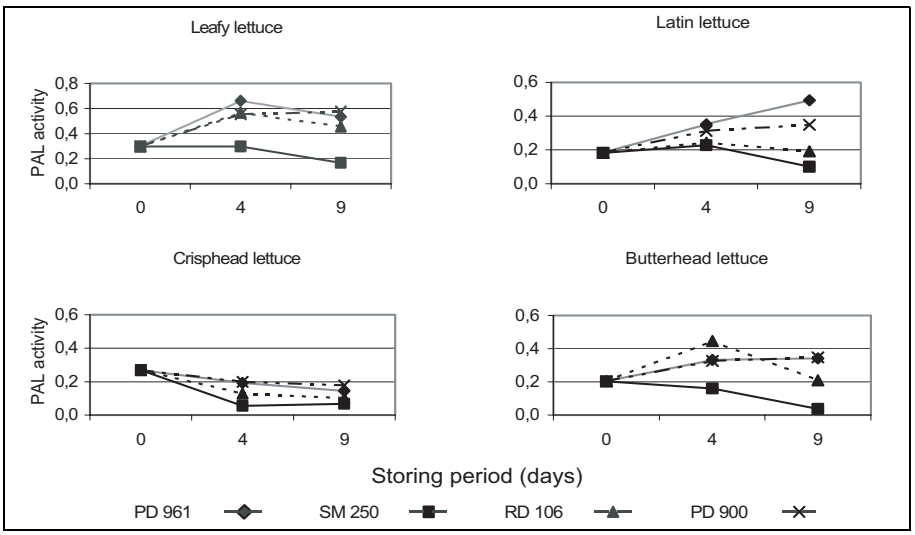


Fig. 2. PAL activity (μmol t-cinamic acid g^{-1} fresh weight h^{-1}) of each lettuce type according to the polymeric film employed as packaging material during storage period: PD 961, SM 250, RD 106, PD 900. SEM (standard error of mean) for every lettuce type: SEM leafy=0.048, SEM latin=0.033, SEM crisphead=0.023 and SEM butterhead=0.034.

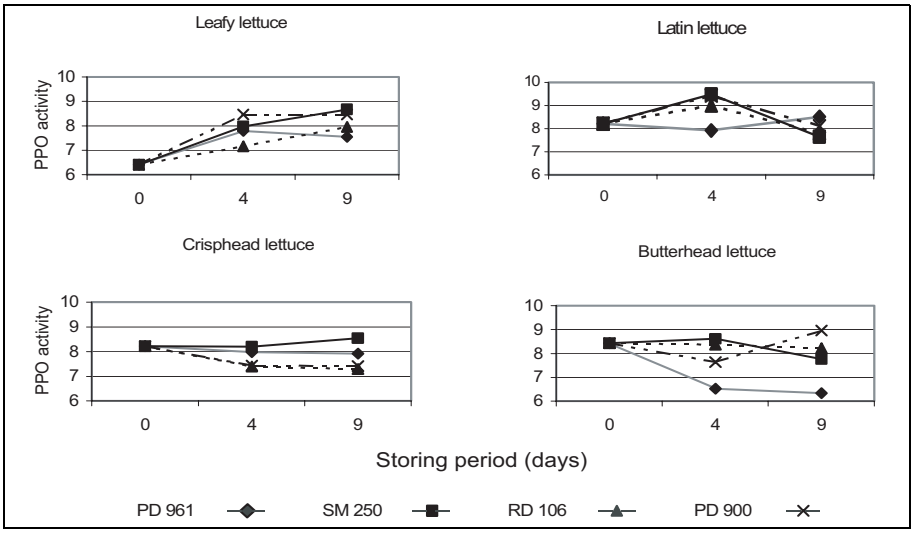


Fig. 3. PPO activity (μmol quinone g^{-1} fresh weight min^{-1}) for each lettuce type according to the polymeric film employed as packaging material during storage period: PD 961, SM 250, RD 106, PD 900. SEM (standard error of mean) for every lettuce type: SEM leafy=0.258, SEM latin=0.189, SEM crisphead=0.132 and SEM butterhead=0.256.

After 9 days of storage, butterhead lettuce packed with PD 961 had a low enzymatic activity. The lowest activity for leafy lettuce was observed with both PD 961, and RD-106. Latin and crisphead did not differ significantly in enzymatic activity regardless the packaging material used.

PPO activity reduction or inhibition during storage would be related to an O_2 concentration decrease and a CO_2 concentration increase in the package atmosphere as reported by KE and SALTVEIT (1989a), since CO_2 is a competitive inhibitor of PPO enzyme (MURR and MORRIS 1974).

POD activity

Initial POD activity ranged between 1.27 μmol of guaiacol g^{-1} fresh weight $min^{-1}$for crisphead lettuce and 4.79 μmol of guaiacol g^{-1} fresh weight min^{-1} for leafy lettuce (Fig. 4).

At the 4th day of storage, crisphead lettuce had the lowest POD activity values. Crisphead and butterhead lettuce had significantly lower enzymatic activity when packed with PD-900 compared with the other packaging materials. The lowest enzymatic activity for latin lettuce was achieved with PD-961 films. No differences between packaging materials were observed for leafy lettuce.

Leafy and latin lettuce presented a higher POD activity than crisphead and butterhead lettuce at the 9th day of storage. Fresh cut lettuce in PD-900 bags had a significantly lower enzymatic activity than with the other packaging materials. For crisphead lettuce, the enzymatic activity did not differ significantly among packaging materials.

In this trial, a remarkable increase in POD activity during the first 4 days of storage for all lettuce types was generally observed. At 9 days of storage, POD activity depended on both lettuce type and package permeability. KE and SALTVEIT (1989b) reported an enzymatic activity increase during the storage, especially after the 8th day.

Phenolic compound evolution

The initial values of chlorogenic acid ranged between 67.2 μg g^{-1} fresh weight for crisphead lettuce and 392.4 μg g^{-1} fresh weight for leafy lettuce (Fig. 5). TOMÁS-BARBERÁN et al. (1997b) found different initial concentrations of phenolic contents for crisphead, romaine and butterhead lettuce with the lowest values for crisphead lettuce, too.

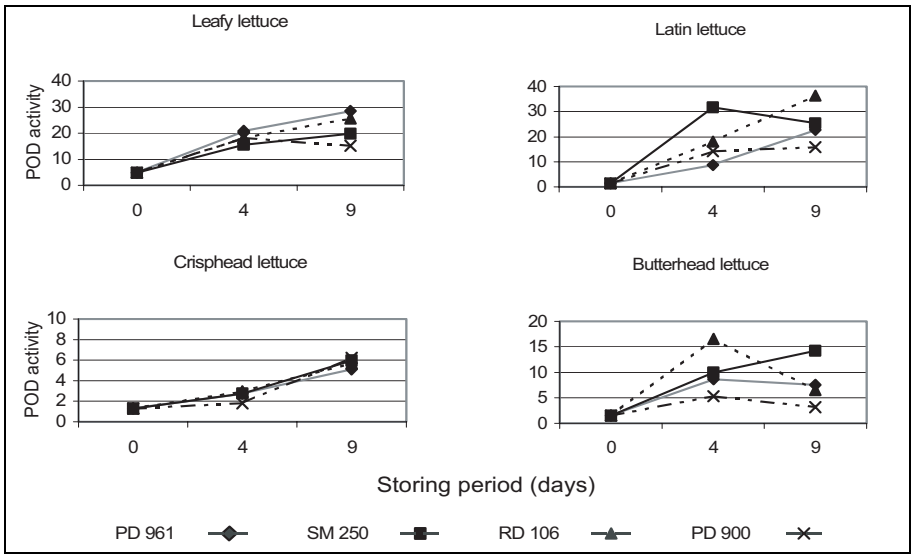


Fig. 4. POD activity (μmol guaiacol g^{-1} fresh weight min^{-1}) for each lettuce type according to the polymeric film employed as packaging material during storage: PD 961, SM 250, RD 106, PD 900. SEM (standard error of mean) for every lettuce type: SEM leafy=2.472, SEM latin=3.690, SEM crisphead=0.600 and SEM butterhead=1.551.

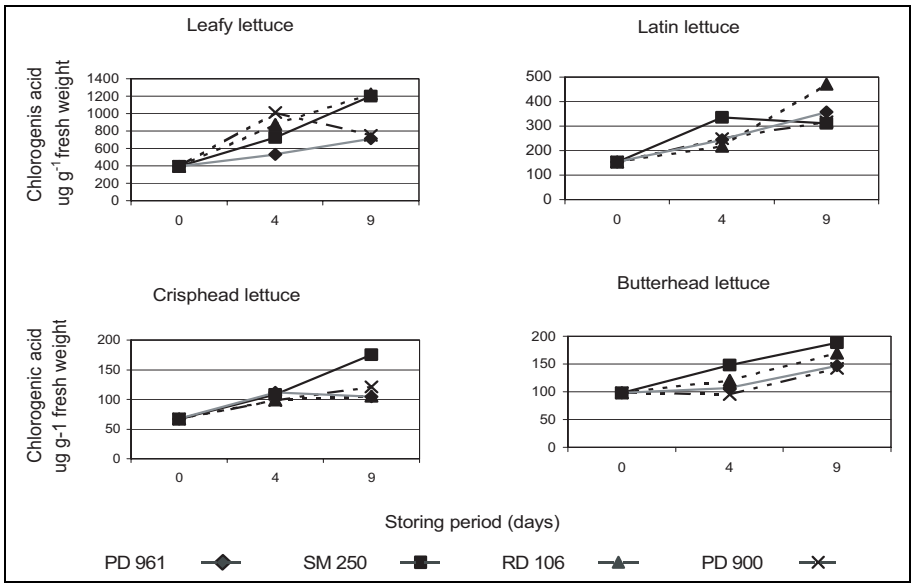


Fig. 5. Chlorogenic acid content (μg g^{-1} fresh weight) for four lettuce types during storage according to the polymeric film employed as packaging material: PD 961, SM 250, RD 106, PD 900. SEM (standard error of mean) for each lettuce type: leafy=92.64, SEM latin=30.32, SEM crisphead=9.31 and SEM butterhead=9.82.

In general, an increase in chlorogenic acid concentration was observed for all lettuce types and packaging materials during the storage. After 4 days of storage, the lowest concentrations of chlorogenic acid for leafy and butterhead lettuce were observed with PD-961 and PD-900, respectively, for crisphead with PD-900 and RD-106, without significant differences between both and for latin lettuce with RD-106.

After 9 days of storage, significant differences were observed between packaging materials for leafy, latin and butterhead lettuce, achieving the lowest concentration of chlorogenic acid with PD-961, SM-250 and PD-900, respectively. The lowest concentration of chlorogenic acid for crisphead lettuce was achieved with either RD-106 or PD-961, without significant differences between them.

Fresh cut lettuce packed with SM-250 (regarded as control) had high concentrations of chlorogenic acid due to the free diffusion of gases through the microperforations, with an increase of enzymatic activity, except for latin lettuce. Fresh cut lettuce packed with PD-900, the material with the highest CO_2/O_2 relation, finished the storage period with the lowest chlorogenic acid contents. Probably, the general enzymatic activity was reduced by the relatively high CO_2 concentration in the package.

Chlorogenic acid, a natural antioxidant, was considerably higher for leafy lettuce. Most natural antioxidants, which take part in daily diet are phenolic compounds found in all kind of fruits and vegetables, in fresh as well as derived products (Amiot et al. 1997; Tomás-Barberán and Espin 2001). Castañer et al. (1999) recommend the consumption of mix-salads with high proportion of green tissues because of the high amount of phenolic compounds in photosynthetic tissues, being higher in romaine than in baby lettuce.

Overall visual quality

At the 4th day of storage, the four lettuce types scored 6 or more in most treatments, according to the scale used (Fig. 6). For leafy and crisphead lettuce, the pack-

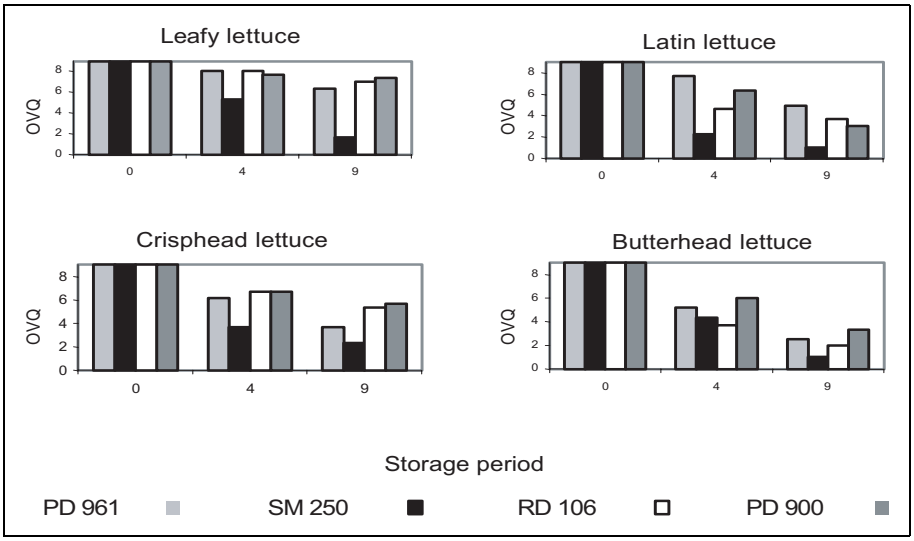


Fig. 6. Overall visual quality (OVQ) for each lettuce type during storage according to the polymeric film employed as packaging material: PD 961, SM 250, RD 106, PD 900, using a scale of 9 to 1 (9=excellent and 1=unusable).

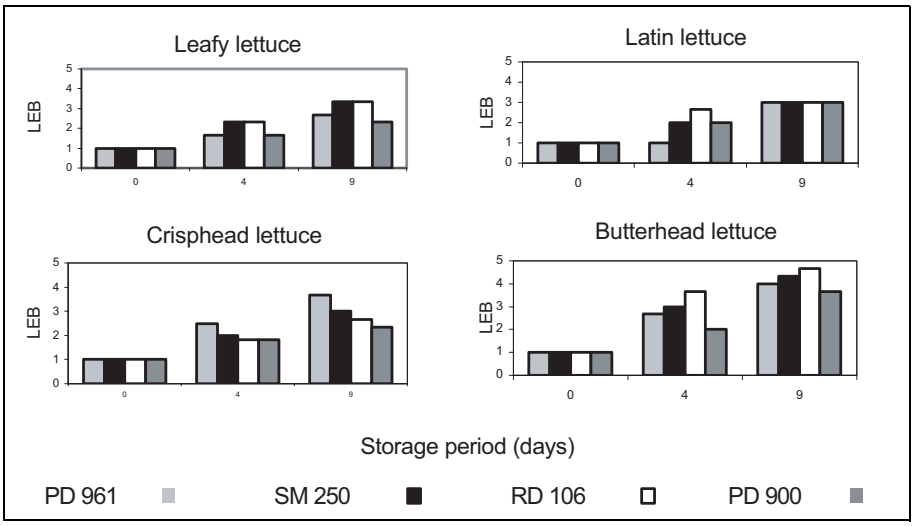


Fig. 7. Leaf edge browning (LEB) for each lettuce type during storage according to the polymeric film employed as packaging material: PD 961, SM 250, RD 106, PD 900, using a scale of 1 to 5 (1=none and 5=severe).

aging materials PD-961, RD-106 and PD-900 kept an acceptable commercial quality without significant difference among them. For latin lettuce, PD-961 and PD-900 kept a significantly better overall visual quality compared with other polymeric films. Butterhead packed with PD-900 was only slightly better.

After 9 days of storage, only the overall visual quality of leafy lettuce was maintained above the limit of commercial acceptability for packaging materials: PD-961, RD-106 and PD-900. These results indicate the importance of the genotype.

The characteristics of the SM-250 film led to a high dehydration rate for the four lettuce types, leading to a non-commercial quality of the product.

Leaf edge browning

At the 4th day of storage, a light leaf edge browning was observed for all the lettuce types (Fig. 7). Fresh cuts of butterhead lettuce packed with PD-900 and latin lettuce packed with PD-961 showed a significantly lower leaf edge browning compared to the other treatments. Crisphead lettuce packed with RD-106 and PD-900 showed the lowest leaf edge browning with significant difference to PD-961. For fresh cut leafy lettuce no significant differences were observed between polymeric films.

After 9 days of storage, the browning degree was moderate for leafy, latin and crisphead lettuce, and moderate to severe for butterhead lettuce. Crisphead packed with RD-106 and PD-900 showed the lowest degree of leaf edge browning. PD-900 had a great performance for butterhead lettuce. No significant differences were observed among packaging materials for leafy and latin lettuces. It is remarkable that the use of PD-900 as packaging material produced the lowest degree of browning for every lettuce type, especially for leafy and crisphead lettuce.

Leaf surface browning

At the 4th day of storage, leafy, crisphead and latin lettuce did not present leaf surface browning. Butterhead lettuce presented light leaf surface browning in all packages, except for PD-900 (Fig. 8).

After 9 days of storage, crisphead and leafy lettuce had no browning with none of the packaging materials used, but browning was observed for leafy lettuce

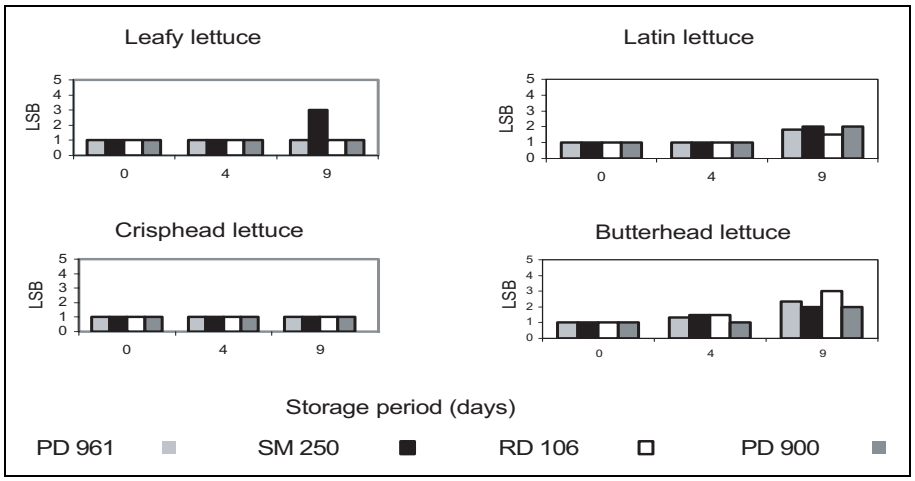


Fig. 8. Leaf surface browning (LSB) for each lettuce type during storage according to the polymeric film employed as packaging material: PD 961, SM 250, RD 106, PD 900, using a scale of 1 to 5 (1=none and 5=severe).

packed with SM-250 (control). The degree of browning was light to moderate for latin and butterhead lettuce. The lowest browning for latin was achieved with RD-106 and for butterhead lettuce packed with both PD-961 and PD-900.

Conclusions

The analysis of the different enzymatic activities (PAL, PPO and POD) and of the organoleptic quality traits assessed reflected the effect produced by the combinations of the lettuce type and packaging material.

To optimise the efficiency of the polymeric packaging, it is important to consider not only the packaging material to be used but also the lettuce type to be processed and packed. The concentrations of O_2 and CO_2 necessary to keep the quality of the processed lettuce leaves, will depend on the rate of O_2 and CO_2 permeability of the packaging materials and on the respiratory activity of the leaves. This may explain the variability measured in the enzymatic activity.

The packaging materials RD-106, PD-961 and PD-900 had a different behaviour in terms of the chlorogenic acid concentration reached during storage, depending also on the different lettuce type used. A low chlorogenic acid concentration and good organoleptic quality was achieved with the PD-900 film. The highest chlorogenic acid concentrations were measured in leafy lettuce with no quality loss by enzymatic browning. The PD-900 film had the best performance as packaging material, however, it presents commercial disadventages due to its opacity which underrates the visual quality of the product.

Even though the O_2 / CO_2 permeability of the packaging materials was different (3.3 for PD-900, 2.9 for PD-961 and 2.8 for RD-106), it was not enough to induce differences in the behaviour of the materials assessed.

Fresh cut leafy lettuce packed with any of the multilayered polyolefin tested maintained an acceptable commercial quality after 9 days of storage.

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