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Behaviour of *Listeria monocytogenes* in packaged fresh mushrooms (*Agaricus bisporus*)

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Aims: The aim of this study was to evaluate the potential of *Listeria monocytogenes* to grow in mushrooms packaged in two different types of PVC films when stored at 4°C and 10°C.

Methods and Results: Mushrooms were packed in two polymeric films (perforated and nonperforated PVC) and stored at 4°C and 10°C. The carbon dioxide and oxygen content inside the packages, aerobic mesophiles, psychrotrophs, *Pseudomonas* spp., *Listeria monocytogenes*, faecal coliforms, *Escherichia coli*, anaerobic spores and major sensory factors were determined. The mushrooms packaged in nonperforated film and stored at 4°C had the most desirable quality parameters (texture, development stage and absence of moulds). *Listeria monocytogenes* was able to grow at 4°C and 10°C in inoculated mushrooms packaged in perforated and nonperforated films between 1 and 2 log units during the first 48 h. After 10 d of storage, the populations of *L. monocytogenes* were higher in mushrooms packaged in nonperforated film and stored at 10°C.

Conclusions: MAP followed by storage at 4°C or 10°C extends the shelf life by maintaining an acceptable appearance, but allows the growth and survival of *L. monocytogenes*.

Significance and Impact of the Study: According to this study additional hurdles must be studied in order to prevent the growth of *L. monocytogenes*.

INTRODUCTION

Since mushrooms have a very short life (Burton 1989) modified atmosphere packaging (MAP) has been developed in order to delay quality losses and to extend their storage-life (Burton 1988; Hotchkiss and Banco 1992). The modified atmosphere packaging method changes the mixture of gases surrounding a respiring product for a composition different than the composition of air. Modified atmosphere packaging delays the development and senescence of the product and can also affect the types and growth rates of micro-organisms present (Day 1992). Different films have been used to create modified atmospheres, with polyvinylchloride (PVC) being one of the most commercially used (Robertson 1993). The importance of modified atmosphere packaging and the chill chain in maintaining the quality of mushroom has been discussed at length by Saray *et al*. (1994).

Mushroom quality is defined by a combination of parameters, including whiteness, texture, development stage and microbial counts (Gormley 1975). Colour change is one of the most important parameters used to evaluate mushroom quality. In addition to enzymatic browning, several authors have suggested that some surface discoloration is due to the activity of bacteria. *Pseudomonas* has been associated with brown stains when its count exceeds 10^6 cfu cm^{-2} (Wong and Preece 1982; Soler-Rivas *et al*. 1999).

There is a great deal of concern about the microbiological safety of foods as regards packaging. Using packaging with modified atmospheres, pathogen growth may occur or even be stimulated before spoilage becomes evident (Hotchkiss and Banco 1992). As most MAP foods are stored at refrigeration temperatures, *Listeria monocytogenes* is of particular concern because of its ability to grow in low O_2

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atmosphere and at low temperatures (Beuchat *et al*. 1986; Beuchat and Brackett 1990).

Listeria monocytogenes occurs on fresh vegetables and has at the same time a psychrotrophic character (Doyle 1990). *Listeria monocytogenes* can survive and grow at refrigeration temperatures (4°C and 10°C) on packaged fresh cut produce (Farber *et al*. 1998). Moreover, this pathogen can grow on modified atmosphere-packaged fresh-cut vegetables, although the results depend on the type of vegetables and the temperatures (Carlin and Nguygen 1994). Van Netten *et al*. (1989) found that 10% of the mushrooms tested were positive for *Listeria monocytogenes*. There are several studies on the potential growth of *Listeria monocytogenes* in several vegetables, but there is a lack of information on the potential growth of this pathogen in mushrooms.

Reliance on microbial competition as one of the barriers that controls *Listeria monocytogenes* growth in food requires a detailed knowledge of the food, its microflora and how the food is handled.

The marketing temperature recommended for the MAP of vegetables is 3°C (Day 1992), but these products are often stored at 10°C, an abusing temperature.

The aim of this study was to evaluate the potential of *Listeria monocytogenes* to grow in mushrooms packaged in two different types of PVC films when stored at 4°C and 10°C. In addition, the shelf life, microbiological and quality characteristics of mushrooms packaged and stored in the conditions described above, were investigated.

MATERIALS AND METHODS

Collection of mushrooms

Mushrooms (*Agaricus bisporus*) of the Fungisem H-25 strain were collected from Champra SA (a local producer in La Rioja, Spain) and inoculated the next day. The mushrooms were selected from the second flush, diameter 3 or 4 cm and maturity 1 or 2 according to the scale described by Guthrie (1984). Immediately after picking, the mushrooms were transported to the laboratory where they were stored in a 4°C cooler for 24 h prior to packaging. This previous refrigeration is a common step in industrial processing in order to reduce the respiration.

Preparation of *Listeria monocytogenes* inoculum

The *Listeria monocytogenes* serotype 1/2a strain CECT 932 was grown in Brain Heart Infusion Broth (Oxoid) for 18 h. The culture was then transferred to a sterile centrifuge bottle and centrifuged at 10 000 *g* for 10 min at 4°C. The supernatant was decanted and the pellet resuspended in 0·1 mol l^{-1} potassium phosphate buffer (pH 7·0) by vortexing. The washing step was repeated twice. The suspension of washed cells was diluted in potassium phosphate buffer to obtain the appropriate cell concentration for the inoculation of the mushrooms.

Mushroom contamination

The samples were inoculated by dispensing a 25 μl drop of the appropriate *Listeria monocytogenes* cell suspension on the cap. The drop was spread using a sterile disposable loop.

Packaging

Groups of 12 mushrooms were placed in polystyrene trays of 140 × 230 mm. The trays were overwrapped with two different PVC films provided by Borden España SA and sealed using a hot plate (Resinite Maem SA, Madrid). Film I was a nonperforated film (12 μm in thickness) with O_2 permeability of 25·000 cm^3 m^{-2} per 24 h and water steam permeability of 200 g m^{-2} per 24 h at 25°C. Film II was a microperforated film (12 μm in thickness). The conditions of the packaging and batches inoculated with *Listeria monocytogenes* are described in Table 1.

Packaged mushrooms were stored at 4°C and 10°C for up to 10 d, and samples were taken on day 0, 1, 2, 3, 6, 8 and 10.

Two experiments were carried out. The following determinations were made in each experiment: microbiological analyses, gases determination, colour, texture and other quality characteristics (development stage, mould presence and unpleasant odours).

Gases determination

Carbon dioxide and oxygen were determined using an O_2 and CO_2 head space gas analyser, Checkmate model 9900 (PBI- Dansensor, Denmark). Determinations were performed in duplicate.

Table 1 Packaging conditions of different mushroom batches

Batch	Package	*Listeria monocytogenes* inoculation	Storage temperature (°C)
A	PVC perforated	no	10
B	PVC perforated	yes	10
C	PVC perforated	no	4
D	PVC perforated	yes	4
E	PVC nonperforated	no	10
F	PVC nonperforated	yes	10
G	PVC nonperforated	no	4
H	PVC nonperforated	yes	4

Colour determination

Colour was determined using a HunterLab MiniScan/EX colorimeter with a diaphragm of 8 mm diameter, calibrated with a white tile ($X = 81{\cdot}1$, $Y = 86{\cdot}0$ and $Z = 91{\cdot}8$). For each batch, three different points were measured on the caps of each of eight mushrooms. The parameter considered was L^* (luminance), obtained as the mean of all the determinations.

Texture determination

Texture was measured for each cap using a compression press with an Instron Universal Testing Machine (Instron Model 1140, UK), with a displacement speed of 50 mm min^{-1}. The slope of the graph is considered to be the force (expressed in Newtons) needed to obtain a constant deformation of 1 mm. For each batch, the texture of eight mushrooms was determined.

Other quality characteristics

A development stage was assigned to each mushroom, based on the extent of the cap opening on a 7-point scale (Guthrie 1984). Mould presence was scored visually. Unpleasant odours were also evaluated.

Microbiological analyses

Twenty-five grams of mushrooms were weighed aseptically and homogenized in a Stomacher (IUL, Barcelona, Spain) for 2 min with 225 ml of sterile peptone water (0·1% peptone plus 0·5% sodium chloride). Further decimal dilutions were made with the same diluent. The total number of mesophilic micro-organisms was determined on Plate Count Agar (PCA, Merck) following the pour plate method, incubating at 30°C for 72 h (ICMSF 1978). Psychrotrophs were determined on Plate Count Agar (Merck) with an incubation temperature of 7°C for 10 d, following the pour plate method (ICMSF 1978). Faecal coliforms were determined by the MPN method for a three tube series using Brilliant Green Bile Lactose Broth (BGBL, Difco) incubated at 44°C for 48 h; when gas was formed, subcultures were made onto Levine agar (Merck) and incubated at 37°C for 48 h. The plates were then examined for suspected *Escherichia coli* colonies (ICMSF 1978). *Listeria monocytogenes* was enumerated by plating onto Oxford agar (Merck) following the surface plate method. The incubation conditions used were 30°C for 48 h (Mossel *et al*. 1995). Suspected colonies grown on Oxford agar were identified by using API *Listeria* strips (BioMérieux, Marey Létoile, France). Anaerobic spores were determined using PCA following the pour plate method and incubated under anaerobic conditions at 30°C for 72 h after a heat treatment at 80°C for 10 min to destroy vegetative cells. *Pseudomonas* spp. were determined in King's B medium (King *et al*. 1954), with an incubation temperature of 25°C for 48 h.

All analyses were performed in duplicate.

Statistical analysis

Analysis of variance was performed using the SYSTAT program for Windows; Statistics version 5·0 (Evanston, Illinois, 1992). Tuckey's test for comparison of means was performed using the same program. Plate count data were converted to logarithms prior to their statistical treatment.

RESULTS AND DISCUSSION

Atmosphere within the packages

The kinetics of the O_2 and CO_2 changes within the packages depended on the film permeability and temperature (Fig. 1). The atmosphere within the nonperforated packages for the two storage temperatures became highly modified in the first 24 h. Increasing the storage temperature (10°C) of the mushrooms packaged in nonperforated film increased the CO_2 production, since temperature is one of the most important factors in determining the respiration rate of fresh vegetables postharvest (Kader 1987).

Significant differences in CO_2 and O_2 concentrations were found between mushrooms packaged with perforated and nonperforated films. The CO_2 concentration increased sharply after 1 d in nonperforated film, and after the atmosphere composition reached an equilibrium of 4–5·5% at 4°C and 5·5–6·5% at 10°C. CO_2 levels above 5% are considered as phytotoxic by Lopez-Briones *et al*. (1992). O_2 consumption corresponded to CO_2 production as long as the mushrooms remained aerobic. O_2 and CO_2 concentrations reached equilibrium levels at approximately the same time. In perforated packages, the increase in CO_2 was lower (0·1–0·3% at 4°C and 0·3–1% at 10°C) and the O_2 level remained around 20%. These results are in agreement with those reported by other authors (Lopez-Briones *et al*. 1993; Martin and Beelman 1996; Tano *et al*. 1999). O_2 concentration was 5·5–7·8% in nonperforated film after 1 d stored at 4°C. This level was higher than the 1·5–2% considered by Beit-Halachmy and Mannheim (1992) to be related to the start of anaerobic respiration and the accumulation of unpleasant odours. It should be noted that *Clostridium botulinum* can grow at O_2 levels of 2% (Sugiyama and Yang 1975). Lower O_2 concentrations, 1–2%, were observed in nonperforated film after 1 d stored at 10°C due to the higher respiration rate of mushroom at 10°C. Furthermore, in a previous work, O_2 concentrations were below 1% when

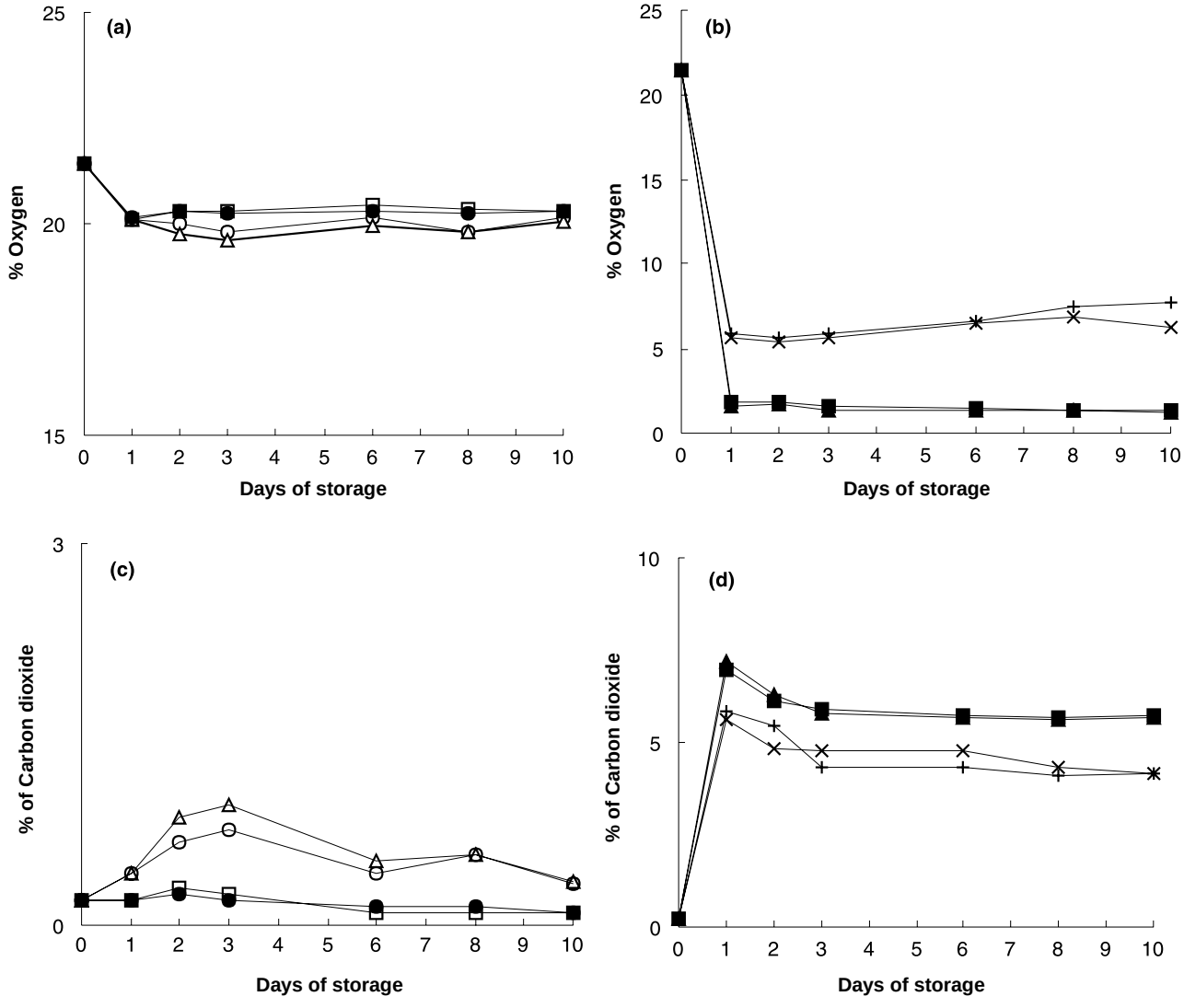

Fig. 1 Oxygen and carbon dioxide concentrations in fresh mushrooms overwrapped with PVC films: (a, c) perforated film; (b, d) nonperforated film. (a) Oxygen concentrations in Batch A (○), Batch B (△), Batch C (□) and Batch D (●). (b) Oxygen concentrations in Batch E (▲), Batch F (■), Batch G (×) and Batch H (+). (c) Carbon dioxide concentrations in Batch A (○), Batch B (△), Batch C (□) and Batch D (●). (d) Carbon dioxide concentrations in Batch E (▲), Batch F (■), Batch G (×) and Batch H (+) (see Table 1). The data are the mean values of two experiments

mushrooms were packaged in nonperforated film and stored at 17°C and 25°C (Gonzalez-Fandos *et al*. 2000). Storage at lower temperatures reduced the atmosphere changes due to the influence of temperature on the respiration rate of mushrooms.

These facts suggest the importance of the storage temperature and the O_2 level in order to control *Clostridium botulinum* growth. *Clostridium botulinum* proteolytic strains do not grow at 10°C or lower. However, *Clostridium botulinum* nonproteolytic strains are psychrotrophic and can multiply and produce toxin at temperatures as low as 3·3°C, thus it is important to control the O_2 levels (Sugiyama and Yang 1975; ICMSF 1996; Varnam and Evans 1996).

The gas composition of the storage atmosphere reduced the microbial load. Mesophiles and psycrotrophs counts were around 0·5 log units higher in mushrooms packaged in perforated film. Significant differences were found between mushrooms packaged in the same type of film and stored at 4°C and 10°C (Figs 2 and 3). Lopez-Briones *et al*. (1993) have also reported that the gas composition may reduce the microbial spoilage.

Colour

Results are shown in Fig. 4. In every sample, the original colour of the caps became relatively darker during the

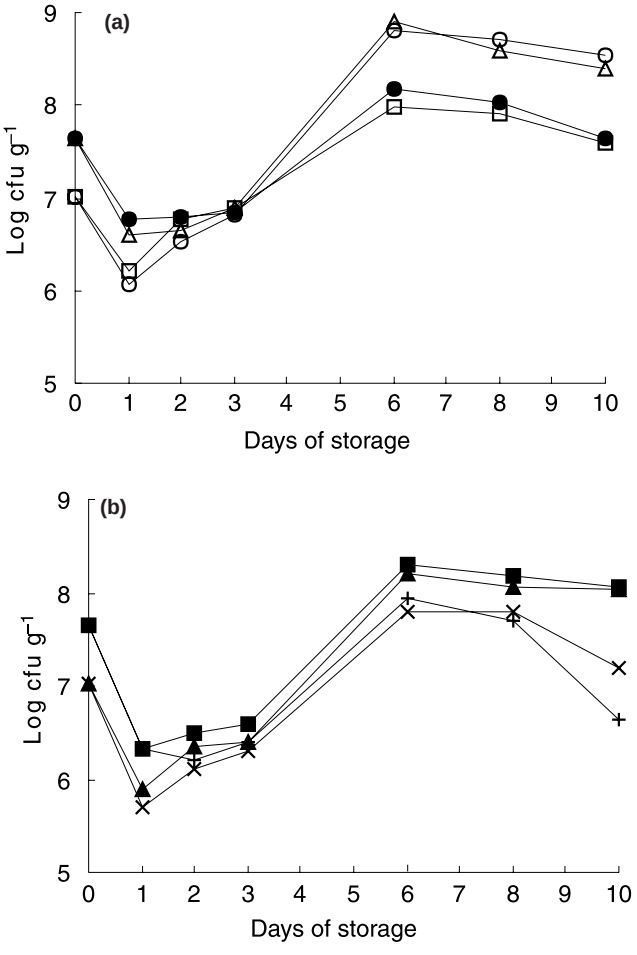

Fig. 2 Effect of packaging on mesophile counts of mushrooms overwrapped with PVC films. (a) Perforated film: Batch A (○), Batch B (△), Batch C (□) and Batch D (●). (b) Nonperforated film: Batch E (▲), Batch F (■), Batch G (×) and Batch H (+) (see Table 1). The data are the mean values of two experiments

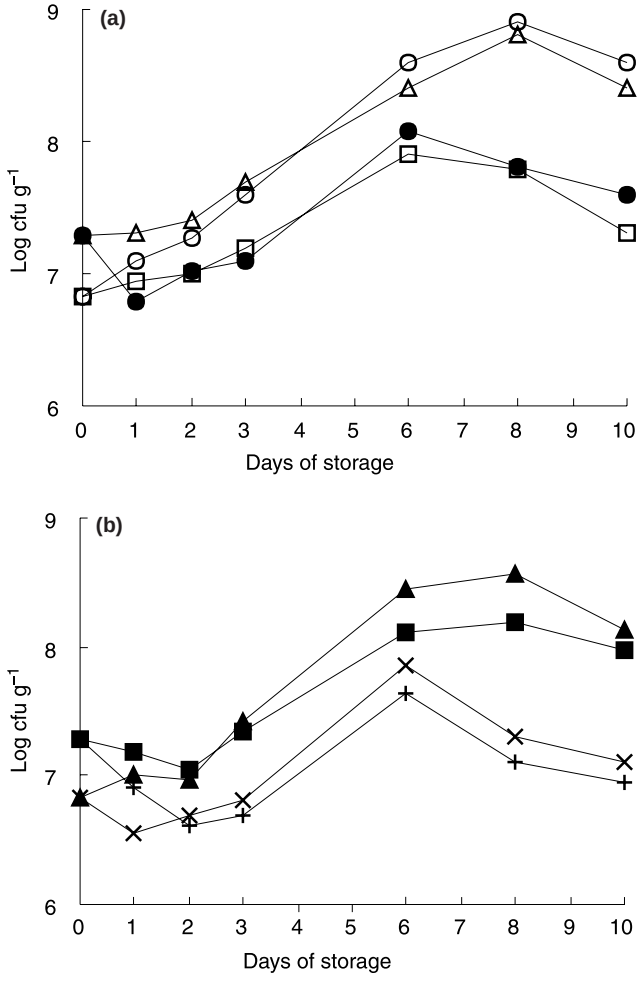

Fig. 3 Effect of packaging on psychrotrophs of mushrooms overwrapped with PVC films. (a) Perforated film: Batch A (○), Batch B (△), Batch C (□) and Batch D (●). (b) Nonperforated film: Batch E (▲), Batch F (■), Batch G (×) and Batch H (+) (see Table 1). The data are the mean values of two experiments

storage (Day 0 mean L* value = 91·52, Day 10 mean L* = 87·15). The luminance values (L*) of the inoculated packages are not shown, since these values were very close to those of the noninoculated packages.

Until Day 6, no significant differences were observed in colour between mushrooms stored at 4°C and those stored at 10°C. The Day 10 colour was darker in packages stored at 10°C, especially in those of perforated film (L^* = 81·94). In a previous work, the negative effect of abusing storage temperatures (17°C and 25°C) was observed (Gonzalez-Fandos *et al*. 2000). Gormley (1975) categorized mushrooms with L* values ≥ 86 to be of good quality and 80–85 to be of fair quality. The luminance values measured at the end of the storage period showed that the degree of whiteness could be considered as good except in mushrooms packaged in perforated film after 10 d of storage at 10°C.

Some authors have reported that concentrations of CO_2 up to 2·5% reduce brown discoloration and concentrations higher than 5% enhance browning (Lopez-Briones *et al*. 1992). In contrast, we did not find significant differences between mushrooms packaged in perforated film and stored at 4°C, where the CO_2 concentration was around 0·1%, and those packaged in nonperforated film with a CO_2 concentration around 4%. This fact could be explained because CO_2 is not the only factor involved in the brown discoloration of mushrooms, with *Pseudomonas* spp. often involved in the discoloration effect (Wong and Preece 1982).

However, after Day 6 significant differences were found between mushrooms packaged in perforated film stored at 10°C with CO_2 concentration of 0·3–0·5%, and those packaged in nonperforated film stored at 10°C with a CO_2

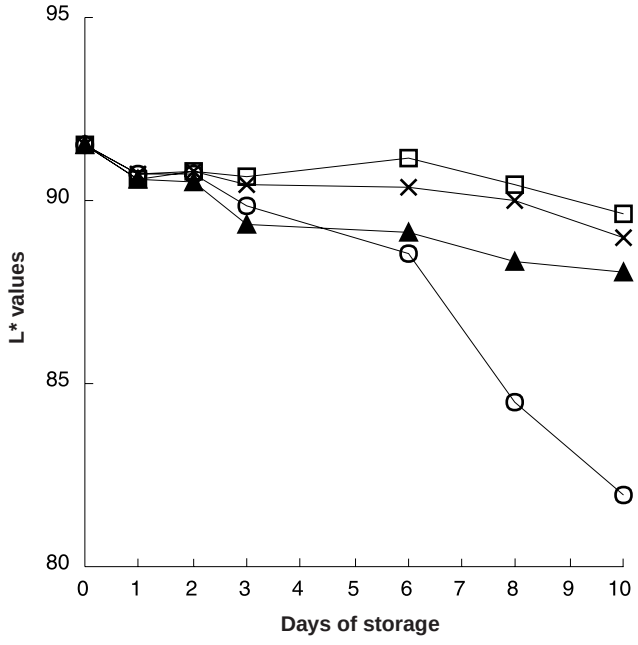

Fig. 4 Influence of packaging film and storage temperature on colour of mushrooms overwrapped with PVC film. Batch A (○), Batch C (□), Batch E (▲) and Batch G (×) (see Table 1). The data are the mean values of two experiments

concentration of 5–6%. Colour was better in mushrooms packaged in nonperforated film.

It can be concluded that storage at the lower temperature (4°C) maintained an acceptable colour during storage. On the other hand, the film type had a significant effect on maintaining an acceptable colour at 10°C.

Texture

Significant differences in texture were found after 2 d between the mushrooms stored at 4°C and 10°C. The mushrooms stored at 4°C after 2 d had better textural characteristics (mean 20·1 N) than the other ones storaged at 10°C (mean 16·5 N). Changes of texture were delayed at 4°C since the respiration rate decreases and the development is retarded. Inoculation of *L. monocytogenes* did not significantly affect the texture values.

No significant differences in texture were found between mushrooms packaged with perforated and nonperforated films at 4°C. This fact could be explained since the protective effect of CO_2 on the texture of mushrooms is evident at concentrations over 10% (Lopez-Briones *et al.* 1993). At 10°C nonsignificant differences were found after 2 d of storage, although better textural characteristics were observed in mushrooms packaged in nonperforated film (16·9 N) than those packaged in perforated film (12·9 N).

It must be noticed that a decrease in texture was specially evident in mushrooms stored at 10°C after 6 d.

Development stage

Results are shown in Fig. 5. For the perforated films, after 6 d of storage at 10°C, 50% of the mushrooms had partially broken veils (category 3 and 4 according to the Guthrie scale), the corresponding percentage for 4°C was 25%. After 10 d, the packages contained 92% of mushrooms with opened veils (category 6–7) at 10°C and 42% at 4°C.

The development of the mushrooms packaged in nonperforated film was retarded: after 10 d of storage, only 17% of them had completely broken veils (category 5) at 10°C, at 4°C 100% had their veil intact. This was caused by the higher CO_2 levels comparing to perforated film (Burton and Twyning 1989). Moreover, CO_2 acts as a regulator for mycelial growth and mushroom morphogenesis (Flegg *et al.* 1985). Mushroom development stage was retarded at 4°C, since the CO_2 concentration to prevent the breaking of the veil is higher when the temperature increases (Lopez-Briones *et al.* 1993).

However, Roy *et al.* (1995) reported that the development stage of mushrooms was influenced by O_2 concentration rather than CO_2 concentration, with optimum levels of 5–6% O_2. These reduced O_2 levels (5·5–7%) were observed by us in mushrooms packaged in nonperforated film and stored at 4°C. It must be pointed out that a delayed development stage was observed in these conditions.

After 10 d, only mushrooms packaged in nonperforated film were acceptable, those stored at 4°C being better.

Presence of moulds

The presence of moulds in mushrooms packaged in perforated film stored at 10°C was observed in 42% of the samples after 3 d and in 100% after 6 d. In the mushrooms packaged with nonperforated film stored at 10°C, moulds were only detected after 10 d in 67% of the samples.

The temperature did not significantly affect the parameter. At 4°C the presence of moulds was only detected in mushrooms packaged in perforated film (17% of the samples).

Odour

Unpleasant odours were not detected in mushrooms packaged and stored at 4°C or 10°C. Unpleasant odours were found by us in a previous work at 17°C and 25°C (Gonzalez-Fandos *et al.* 2000). This fact indicates the importance of temperature.

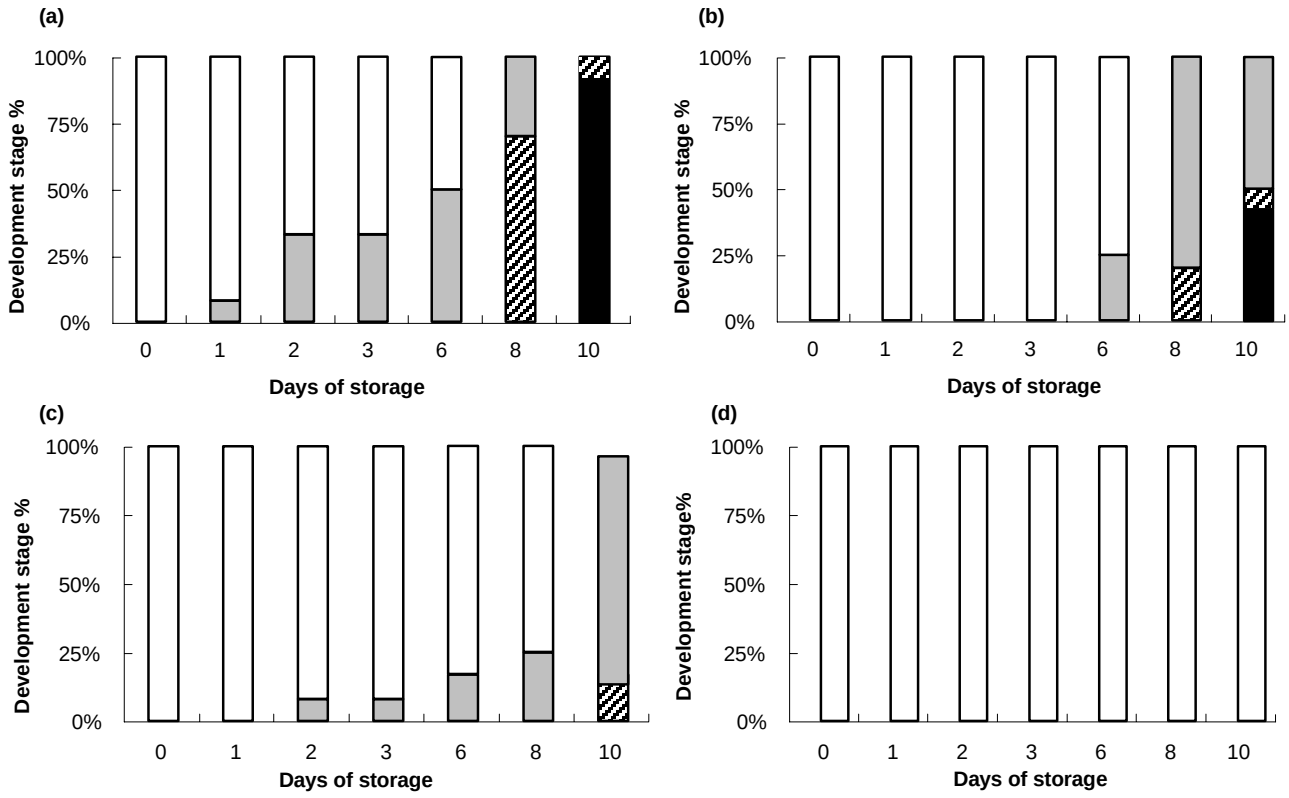

Fig. 5 Effect of packaging and storage temperature on the development stage of mushrooms overwrapped with PVC films, according to the Guthrie scale (Guthrie 1984). The development stage of each batch is expressed as the percentage of mushrooms in each category. (a) Batch A; (b) Batch C; (c) Batch E; and (d) Batch G (see Table 1). The data are the mean values of two experiments. □ Veil intact (category 1 and 2); ▒ veil partially broken (category 3 and 4); ▨ veil completely broken (category 5); ■ cap open (category 6 and 7). The data are the mean values of two experiments

Pseudomonas

The growth of *Pseudomonas* spp. is shown in Fig. 6. The psychrotroph evolution (Fig. 3) was similar to the *Pseudomonas* evolution in every package, indicating that the microbial load of mushrooms was mainly *Pseudomonas*, specially in perforated film.

After 8 d of storage, the mushrooms packaged with perforated film had counts of fluorescent pseudomonad bacteria of 7·8 log cfu g^{-1} at 4°C and 8·4 log cfu g^{-1} at 10°C; these figures are 1·3 and 1·9 log cycles higher than the original count, respectively (6·5 log cfu g^{-1}).

After 8 d, the mushrooms packaged with nonperforated film had counts of *Pseudomonas* spp. of 6·7 log cfu g^{-1} at 4°C and 7·2 log cfu g^{-1} at 10°C (Fig. 6). This film generated atmospheres with higher CO_2 and lower O_2 concentration. Since *Pseudomonas* spp. are aerobic bacteria, their growth is inhibited under low O_2 concentrations. Lopez-Briones *et al.* (1992) pointed out that CO_2 concentrations between 2·5% and 5% reduce the growth of micro-organisms, including pseudomonads, compared to an air atmosphere. In addition, high O_2 concentrations enhance the growth of fluorescent pseudomonads.

Listeria monocytogenes

No *L. monocytogenes* was detected in any of the uninoculated mushrooms, pointing out that there was no initial contamination in the mushrooms.

Listeria monocytogenes populations increased between 1 and 2 log units during the first 48 h (Fig. 7). The population remained relatively stable during days 3–8. After 8 d of storage, the population declined 1–1·5 log units except in mushrooms packaged in nonperforated film and stored at 10°C. The growth observed during the first two days corresponded to the lag phase of competitors (Figs 2, 3 and 6). After day 3, when growth of competitors was evident, limited or no growth of *L. monocytogenes* was observed.

Also Berrang *et al.* (1989) and Beuchat and Brackett (1990) observed a growth of *L. monocytogenes* at 4–5°C in fresh vegetables. However, Ringle *et al.* (1991) did not

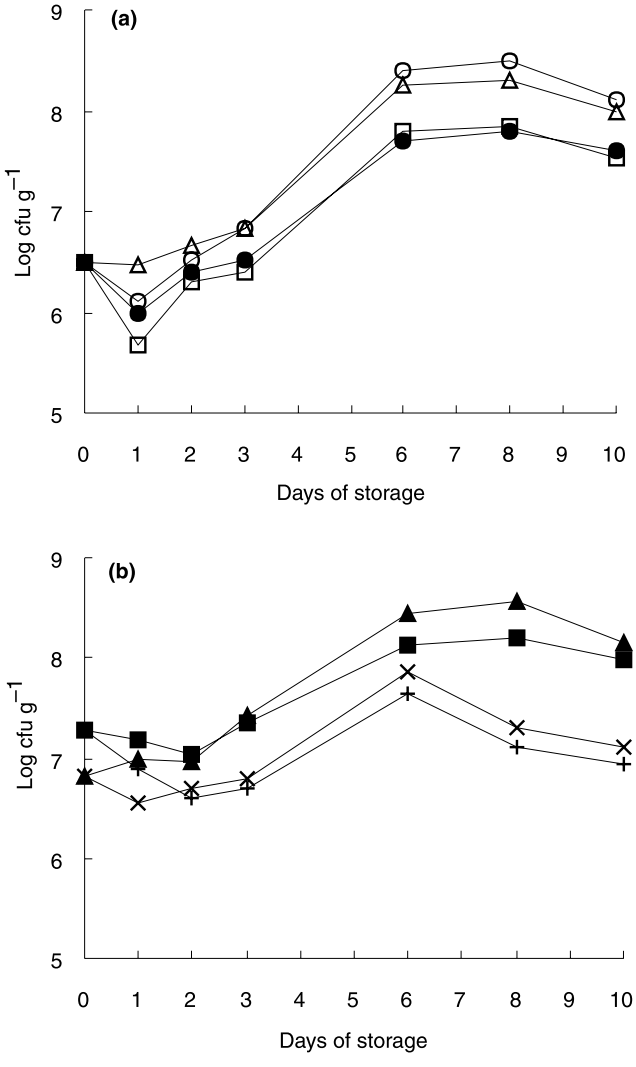

Fig. 6 Effect of packaging on *Pseudomonas* growth. (a) Perforated film: Batch A (○), Batch B (△), Batch C (□) and Batch D (●). (b) Nonperforated film: Batch E (▲), Batch F (■), Batch G (×) and Batch H (+) (see Table 1). The data are the mean values of two experiments

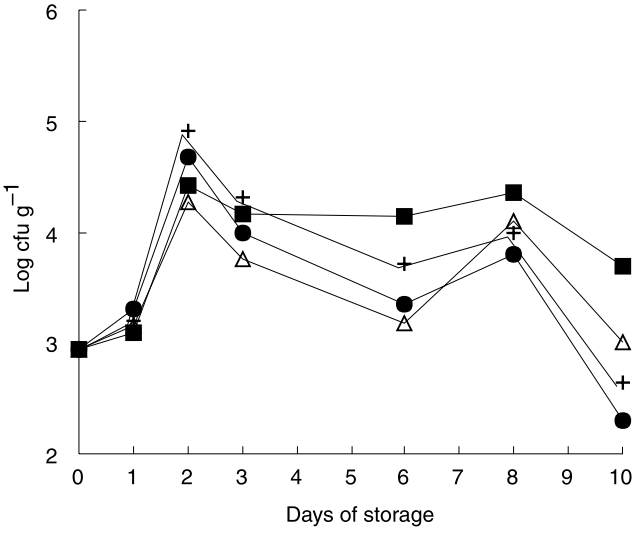

Fig. 7 Influence of packaging on *Listeria monocytogenes* growth. Batch B (△), Batch D (●), Batch F (■), Batch H (+) (see Table 1). The data are the mean values of two experiments

observe a growth of this pathogen at the same temperature. Discrepancies over the behaviour of *L. monocytogenes* at low temperatures may be explained by the type of vegetable studied, the competitors and the strain variation (Carlin and Nguygen 1994; Varnam and Evans 1996).

At 4°C Berrang *et al.* (1989) also reported a limited increase of about 1 log of *L. monocytogenes* on asparagus. A decline in *L. monocytogenes* after 9 d at 7°C was also observed by Jacxsens *et al.* (1999) in carrots.

The modified atmosphere created inside the nonperforated packages with an increase in the levels of CO_2 and a decrease in the O_2 concentration did not decrease the growth of *L. monocytogenes* since this microorganism is a facultative anaerobe (Beuchat *et al.* 1986). Amanatidou *et al.* (1999) observed that concentrations of 10% or 20% CO_2 did not reduce the growth rate of *L. monocytogenes*. Furthermore, Kallander *et al.* (1991) reported that *L. monocytogenes* growing on fresh produce was not inhibited by CO_2 up to 70%.

The higher growth of *L. monocytogenes* in nonperforated packages than in perforated film could be explained because this pathogen may be more tolerant to adverse conditions when grown in an O_2 restricted environment (Buchanan and Klawitter 1990).

Other authors have reported that the use of specific modified atmosphere gases may inhibit the growth of spoilage micro-organisms without affecting *L. monocytogenes*, thus altering any protection provided by the microflora (Wimpfheimer *et al.* 1990; Hendricks and Hotchkiss 1997).

Buchanan and Bagy (1999) suggested that *Pseudomonas fluorescens* is capable of stimulating, inhibiting or not affecting the growth of *L. monocytogenes*, depending on the environmental conditions. In this study, the higher growth of *L. monocytogenes* corresponded to the conditions with a lower growth of *Pseudomonas* spp.

It must be highlighted that an increase of two logarithmic units of *L. monocytogenes* in food constitutes a sanitary risk (ICMSF 1994; Miller *et al.* 1997).

Faecal coliforms and *Escherichia coli*

Faecal coliforms counts were < 1 log cfu g^{-1} in all the batches analysed. This finding could be related to the hygienic handling conditions. *Escherichia coli* was not

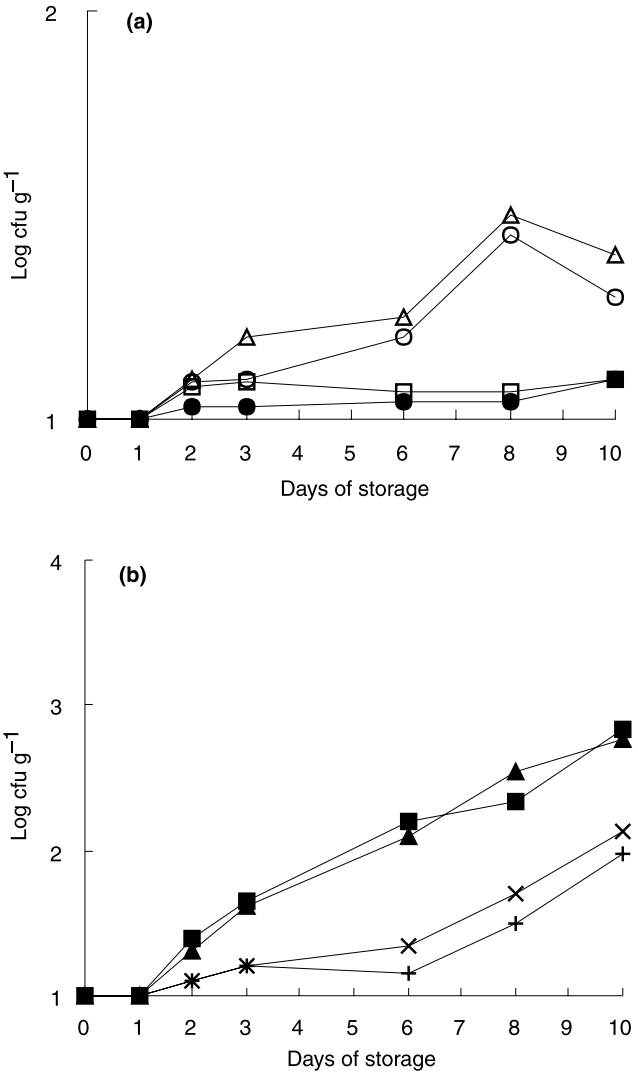

Fig. 8 Effect of packaging on anaerobic spore growth. (a) perforated film: Batch A (○), Batch B (△), Batch C (□) and Batch D (●). (b) Nonperforated film: Batch E (▲), Batch F (■), Batch G (×) and Batch H (+) (see Table 1). The data are the mean values of two experiments

isolated in any sample. Coliform growth could be inhibited by the high populations reached by other competitors.

Anaerobic spores

At 10°C after 10 d, higher counts of anaerobic spores (3 log cfu g^{-1}) were detected in the mushrooms packaged in nonperforated film than in those packaged in perforated film (1·5 log cfu g^{-1}), since lower O_2 concentrations were detected in these packages (Fig. 8).

At 4°C, no growth was observed in mushrooms packaged in perforated film. In nonperforated film, anaerobic spores reached populations of 2 log cfu g^{-1}.

General acceptability and safety

The most important factor in maintaining the quality and extending the shelf life of mushrooms is temperature. A modification of the atmosphere as an adjunct to low temperature can be more effective in order to extend their shelf life.

We can conclude that at 4°C, mushrooms packaged in nonperforated film had the most desirable quality parameters (texture, development stage and mould absence). Also, at 10°C, mushrooms packaged in nonperforated film delayed the undesirable evolution of quality parameters.

Althought Kautter *et al.* (1978) reported that the possibility of botulism resulting from mushrooms wrapped in PVC is minimal, additional research is needed in order to develop an effective prevention of the potential growth of *Clostridium botulinum*. However, the O_2 levels reached in nonperforated film stored at 4°C suggest the importance of controlling the storage temperature.

The low count of faecal coliforms is related to an adequate cultivation process. There are few studies on the influence of organic farming on the presence of faecal coliforms. However, since temperatures of 60–85°C are reached in compost process (Fernandez *et al.* 1996), the probability of survival of this group of micro-organisms is low.

Listeria monocytogenes was able to grow at 4°C and 10°C in mushrooms packaged in perforated and nonperforated films. MAP is not a reliable way to control the fate of this pathogen in mushrooms. Moreover, MAP followed by storage at 4°C or 10°C extends the shelf life by maintaining an acceptable appearance, but allows the growth of *L. monocytogenes*. Additional hurdles must be studied in order to prevent the growth of this pathogen.

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