Journal of Applied Microbiology 2000, **89**, 624–632

Effect of packaging conditions on the growth of micro-organisms and the quality characteristics of fresh mushrooms (*Agaricus bisporus*) stored at inadequate temperatures

E. González-Fandos[1], M. Giménez[1], C. Olarte[1], S. Sanz[1] and A. Simón[2]

[1]Area de Tecnología de los Alimentos. Universidad de La Rioja, and [2]Centro de Investigación y Desarrollo Agrario, Logroño, Spain

194/2/2000: received 21 February 2000, revised 30 May 2000 and accepted 7 June 2000

E. GONZÁLEZ-FANDOS, M. GIMÉNEZ, C. OLARTE, S. SANZ AND A. SIMÓN. 2000. Mushrooms were packed in two polymeric films (perforated and non-perforated PVC) and stored at 17 °C and 25 °C. The carbon dioxide and oxygen content inside the packages, aerobic mesophiles, *Pseudomonas* spp., faecal coliforms, *Escherichia coli*, anaerobic spores and major sensory factors (colour, texture, development stage and presence of moulds) were determined. The non-perforated packages had the highest contents of CO_2 (6–7%), the lowest contents of O_2 (0·013–0·17%) and the most desirable quality parameters (texture, development stage and absence of moulds). *Pseudomonas* spp. counts were around 1 logarithmic unit lower in mushrooms packaged in non-perforated film as the O_2 concentrations were lower than in perforated film. The mushrooms themselves were inoculated with an enterotoxin A-producing strain of *Staphylococcus aureus*, packaged in overwrapped trays and stored at 17 and 25 °C. *Staphylococcus aureus* did not grow in the samples stored at 17 °C. Only slight growth was observed in mushrooms packaged with non-perforated film after 1 day at 25 °C. No enterotoxin was detected in any package. Faecal coliform counts were < 2 log cfu g^{-1}. *Escherichia coli* was not isolated in any of the samples. At 25 °C, counts of anaerobic spores of around 2 log cfu g^{-1} were detected in those mushrooms packaged in non-perforated film.

INTRODUCTION

In recent years, there has been an increase in the consumption of fresh mushrooms. These products have a short shelf-life of 1–3 days at ambient temperature and 8–10 days under refrigeration conditions (Burton 1989); refrigeration is essential to their quality and safety. It is well known that the chill chain reduces spoilage. However, distribution and sale often occur at ambient temperature.

New technologies such as modified atmosphere packaging have been developed in order to delay quality loss and to extend storage life of mushrooms (Burton 1988; Hotchkiss and Banco 1992). The modified atmosphere packaging (MAP) method changes the mixture of gases surrounding a respiring product to a composition other than that of air. Modified atmosphere packaging delays 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 mushrooms 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 counts exceed 10^6 cfu cm^{-2} (Wong and Preece 1982; Soler-Rivas *et al*. 1999).

The gas composition of a storage atmosphere may reduce both microbial and physiological spoilage (López-Briones *et al*. 1993). Up to 2·5% CO_2 seems to benefit colour, but López-Briones *et al*. (1992) demonstrated that CO_2 concentrations higher than 5% enhance discoloration during storage. Carbon dioxide also exhibits a marked effect on

Correspondence to: Dr E. González-Fandos, Departamento de Agricultura y Alimentación, Area de Tecnología de los Alimentos, Universidad de La Rioja, Avda La Paz 105, 26004 Logroño, Spain (e-mail: elena.gonzalez@daa.unirioja.es).

mushroom development (Burton *et al*. 1987). Lopez-Briones *et al*. (1992) suggested that modified atmospheres should contain 2·5–5% CO_2 and 5–10% O_2. Microperforated films have been developed to create modified atmospheres with moderately low, but acceptable, oxygen levels (Burton 1988).

There has been concern that MAP might increase risks associated with pathogenic micro-organisms, mainly when unacceptably high temperatures are reached (Hotchkiss and Banco 1992). *Staphylococcus aureus* has been isolated from uninoculated mushrooms and staphylococcal enterotoxins have been detected (Hardt-English *et al*. 1990). *Staphylococcus aureus* is a poor competitor and does not grow well in the presence of other micro-organisms; it therefore seldom causes food poisoning in a raw product (Bergdoll 1990). This means that favourable conditions are necessary for its growth in raw products. *Staphylococcus aureus* grows both aerobically and anaerobically, but generally grows more slowly under anaerobic conditions. In contrast, cell survival may be improved under anaerobic compared with aerobic conditions (ICMSF 1996).

The effect of other organisms on the growth of *Staph. aureus* is very complex. The effectiveness of competitors in controlling growth and enterotoxin production depends on the ratio of competitor to *Staph. aureus* cells, the type of competitor, storage temperature and growth substrate. The effect of competitors in inhibiting the growth of *Staph. aureus* is very variable, being organism- and strain-dependent (ICMSF 1996).

The aim of this study was to evaluate the shelf-life, microbiological and quality characteristics of mushrooms packaged in two PVC films when stored at 17 and 25 °C. In addition, the potential of *Staph. aureus* to grow and produce enterotoxin in fresh packaged mushrooms, stored in the conditions described above, was investigated.

MATERIALS AND METHODS

Collection of mushrooms

Mushrooms (*Agaricus bisporus*) of the Fungisem H-25 strain were collected from Champra S.A. (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 prior refrigeration is a common step in industrial processing performed to reduce respiration.

Preparation of *Staphylococcus aureus* inoculum

The enterotoxin A producer, *Staphylococcus aureus* strain ATCC 13565, 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 fluid 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 inoculation of the mushrooms.

Mushroom contamination

The samples were inoculated by dispensing a 25 μl drop of the appropriate *Staph. aureus* 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, Madrid, Spain, and sealed using a hot plate (Resinite Maem SA, Madrid, Spain). Film I was a non-perforated film (12 μm in thickness) with O_2 permeability of 25·000 $cm^3\ m^{-2}\ 24\,h^{-1}$ and water steam permeability of 200 g m^{-2} $24\,h^{-1}$ at 25 °C. Film II was a microperforated film (12 μm in thickness). The conditions of the packaging and batches inoculated with *Staph. aureus* are summarized in Table 1.

Packaged mushrooms were stored at 17 and 25 °C for up to 5 days, and samples were taken on days 0, 1, 2, 3 and 5.

Two experiments were carried out. The following determinations were made in each experiment: microbiological analyses, gas determination, colour, texture and other qual-

Table 1 Packaging conditions of different mushroom batches

Batch	Package	*Staphylococcus aureus* inoculation	Storage temperature (°C)
A	PVC non-perforated	yes	17
B	PVC non-perforated	no	17
C	PVC non-perforated	yes	25
D	PVC non-perforated	no	25
E	PVC perforated	yes	17
F	PVC perforated	no	17
G	PVC perforated	yes	25
H	PVC perforated	no	25

ity characteristics (development stage, mould presence and unpleasant odours). All the analyses were performed in duplicate.

Gas determination

Carbon dioxide and oxygen were determined using a O_2 and CO_2 head space gas analyser, Checkmate model 9900 (PBI-Dansensor, Ringsted, Denmark).

Colour determination

Colour was determined using a HunterLab MiniScan/EX colorimeter (Hunterlab, Reston, VA, USA) with a diaphragm of 8 mm diameter, calibrated with a white tile (X = 81·1, Y = 86·0 and Z = 91·8). 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, Bucks, 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 cap opening on a 7-point scale (Guthrie 1984). Mould presence was scored visually. Unpleasant odours were also evaluated.

Microbiological analyses

Mushrooms (25 g) were weighed aseptically and homogenized in a Stomacher (IUL, Barcelona, Spain) for 2 min with 225 ml sterile peptone water (0·1% w/v). Further decimal dilutions were made with the same diluent. The total number of aerobes was determined on Plate Count Agar (Merck) following the pour plate method, incubating at 30 °C for 72 h (ICMSF 1978). Faecal coliforms were determined by the MPN method for a three tube series using Brilliant Green Lactose Broth (BGBL, Difco) incubated at 44 °C for 48 h; when gas was formed, sub-cultives were made onto Levine agar (Merck) and incubated at 37 °C for 48 h. The plates were then examined for suspect *Escherichia coli* colonies (ICMSF 1978). *Staphylococcus aureus* was enumerated by plating onto Baird-Parker agar (Merck) following the surface plate method. The incubation conditions used were 37 °C for 24 h. Suspect colonies were subjected to the coagulase (Merck) and DNAse tests (Merck) (ICMSF 1978). Anaerobic spores were determined on 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 37 °C for 48 h.

Enterotoxin determination

Staphylococcal enterotoxins were extracted from 20 g samples by the procedure of Freed *et al.* (1982) and were then detected using the ELISA sandwich technique of Fey *et al.* (1984). The reagents for the ELISA test were obtained from W. Bommeli, Bern, Switzerland.

All analyses were performed in duplicate.

Statistical analysis

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

RESULTS AND DISCUSSION

Atmosphere within the packages

The kinetics of O_2 and CO_2 changes within the packages depended on the film permeability (Fig. 1). The atmosphere within the microperforated package for the two storage temperatures became highly modified in the first 24 h.

Significant differences in CO_2 and O_2 concentrations were found only between mushrooms packaged with perforated and non-perforated films. The CO_2 concentration increased sharply after 1 day in non-perforated film, and after the atmosphere composition reached an equilibrium of 6–7%. These carbon dioxide levels are considered as phytotoxic by Lopez-Briones *et al.* (1992). Oxygen consumption corresponded to CO_2 production as long as the mushrooms remained aerobic. In perforated packages, the increase in CO_2 was lower (0·85%) and the O_2 level remained around 20%. These results are in agreement with those reported by other authors (Burton and Twyning 1989; Lopez-Briones *et al.* 1993; Martin and Beelman 1996). Oxygen concentration was below 1% in non-perforated film after 1 day stored at 17 or 25 °C. Beit-Halachmy and Mannheim (1992) reported that oxygen levels of 1·5–

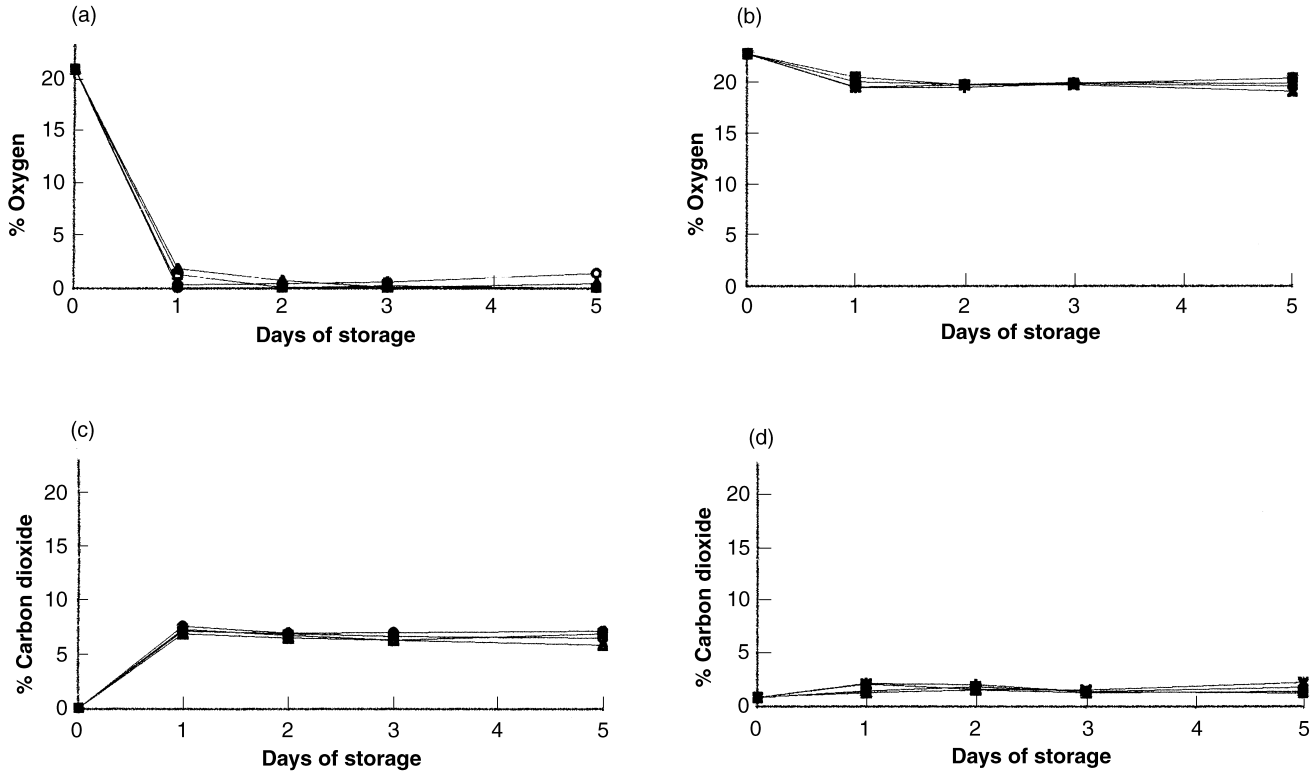

Fig. 1 Oxygen and carbon dioxide concentrations in fresh mushrooms overwrapped with PVC films: (a, c) non-perforated film; (b, d) perforated film. Batch A (○), Batch B (△) Batch C (□), Batch D (●), Batch E (▲), Batch F (■), Batch G (×) and Batch H (+) (see Table 1). The data are the mean values of two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

2% led to the start of anaerobic respiration and the accumulation of unpleasant odours. It should be noted that *Clostridium botulinum* can grow at these oxygen levels (Sugiyama and Yang 1975)

The product's microbial load may also affect O_2 uptake and CO_2 evolution, as reported by Beit-Halachmy and Mannheim (1992). No significant differences in mesophile counts were found between mushrooms packaged in the same type of film, even when different storage temperatures and *Staph. aureus* inoculation were investigated (Fig. 2). This explains the fact that the oxygen and carbon dioxide evolution was similar in packages with the same type of film.

Colour

Results are shown in Fig. 3. In every sample, the original colour of the caps became relatively darker during storage (day 0 mean L* value = 92·34, day 5 mean L* = 84·8). 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 fair.

The luminance values of the inoculated packages are not shown as these values were very close to those of the non-inoculated packages. Until day 3, no significant differences were observed in colour between mushrooms stored at 17 °C and those stored at 25 °C. Both abusive storage temperatures had a negative influence on the colour compared with other studies at lower temperatures (Simon and Gurria 1998). The day 5 colour was darker in packages stored at 25 °C, especially in those of perforated film (L* = 80·4).

Some authors have reported that concentrations of CO_2 lower than 2·5% reduce brown discoloration and concentrations higher than 5% enhance browning (Lopez-Briones *et al.* 1992). In contrast, no significant differences were found here between mushrooms packaged in perforated films, where the CO_2 concentration was around 7%, and those packaged in non-perforated films with a CO_2 concentration around 2%. This could be explained by the fact

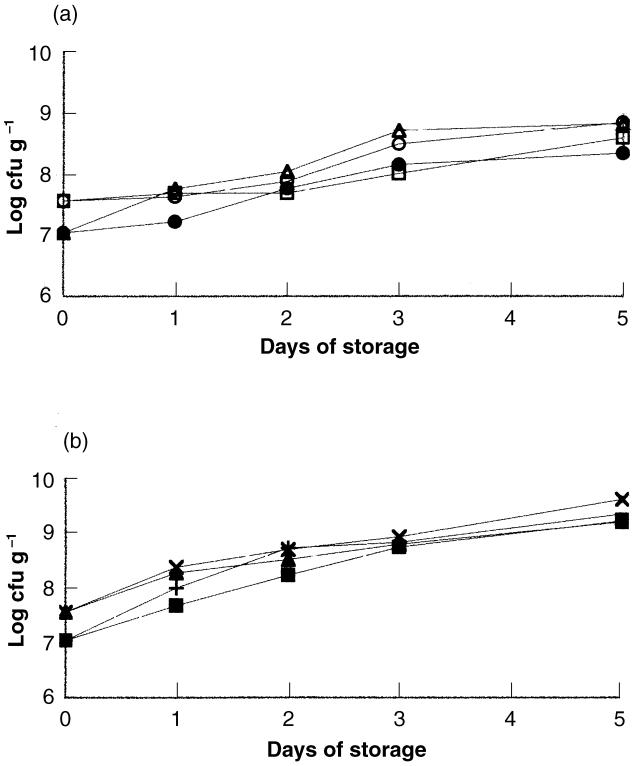

Fig. 2 Effect of packaging on mesophile counts of mushrooms overwrapped with PVC films: (a) non-perforated film; (b) perforated film. Batch A (○), Batch B (△) Batch C (□), Batch D (●), Batch E (▲), Batch F (■), Batch G (×) and Batch H (+) (see Table 1). The data are the mean values of two experiments

that CO_2 is not the only factor involved in the brown discoloration of mushrooms; *Pseudomonas* spp. are also often involved.

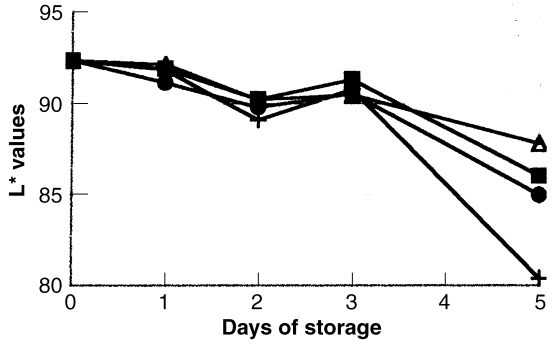

Fig. 3 Influence of packaging film and storage temperature on colour of mushrooms overwrapped with PVC film. Batch B (△), Batch D (●), Batch F (■), and Batch H (+) (see Table 1). The data are the mean values of two experiments

Texture

Significant differences in texture were found after 2 days between mushrooms packaged with perforated and non-perforated films. The mushrooms packaged in the non-perforated film had better textural characteristics (mean 16·47 N) than the others (mean 12·24 N). Storage temperature and inoculation of *Staph. aureus* did not significantly affect the texture values.

Changes to texture were delayed in non-perforated film as the respiration rate decreases and development is retarded, as pointed out by Murr and Morris (1975). Moreover, texture losses decrease when the carbon dioxide concentration increases (Lopez-Briones *et al.* 1992).

Development stage

Results are shown in Fig. 4. For the perforated films, after 2 days of storage at 17 °C, 33% of the mushrooms had partially broken veils (category 3 and 4 according to the Guthrie scale); the corresponding percentage for 25 °C was 50%. After 5 days, the packages contained 75% of mushrooms with opened veils (category 6–7) at 17 °C and 100% at 25 °C.

For the non-perforated films, development was retarded; after 5 days of storage, only 25% of these had completely broken veils (category 5) at 17 °C, with 33% at 25 °C. This was caused by the raised CO_2 levels (Burton and Twyning 1989). Moreover, CO_2 acts as a regulator for mycelial growth and mushroom morphogenesis (Flegg *et al.* 1985).

Presence of moulds

The presence of moulds in mushrooms packaged with perforated film was observed in 80% of the samples after 2 days and in 100% after 5 days. In mushrooms packaged with non-perforated film, moulds were only detected after 3 days of storage, with the percentage in each case depending on the batch (10–40%). The temperature did not significantly affect the parameter.

Odour

Unpleasant odours were detected in mushrooms packaged with non-perforated film, although they disappeared after a short period. These unpleasant odours were also found by Beit-Halachmy and Mannheim (1992) with O_2 levels of 1·5–2%.

Pseudomonas

The evolution of *Pseudomonas* spp. is shown in Fig. 5. Mesophile counts (Fig. 2) were similar to *Pseudomonas*

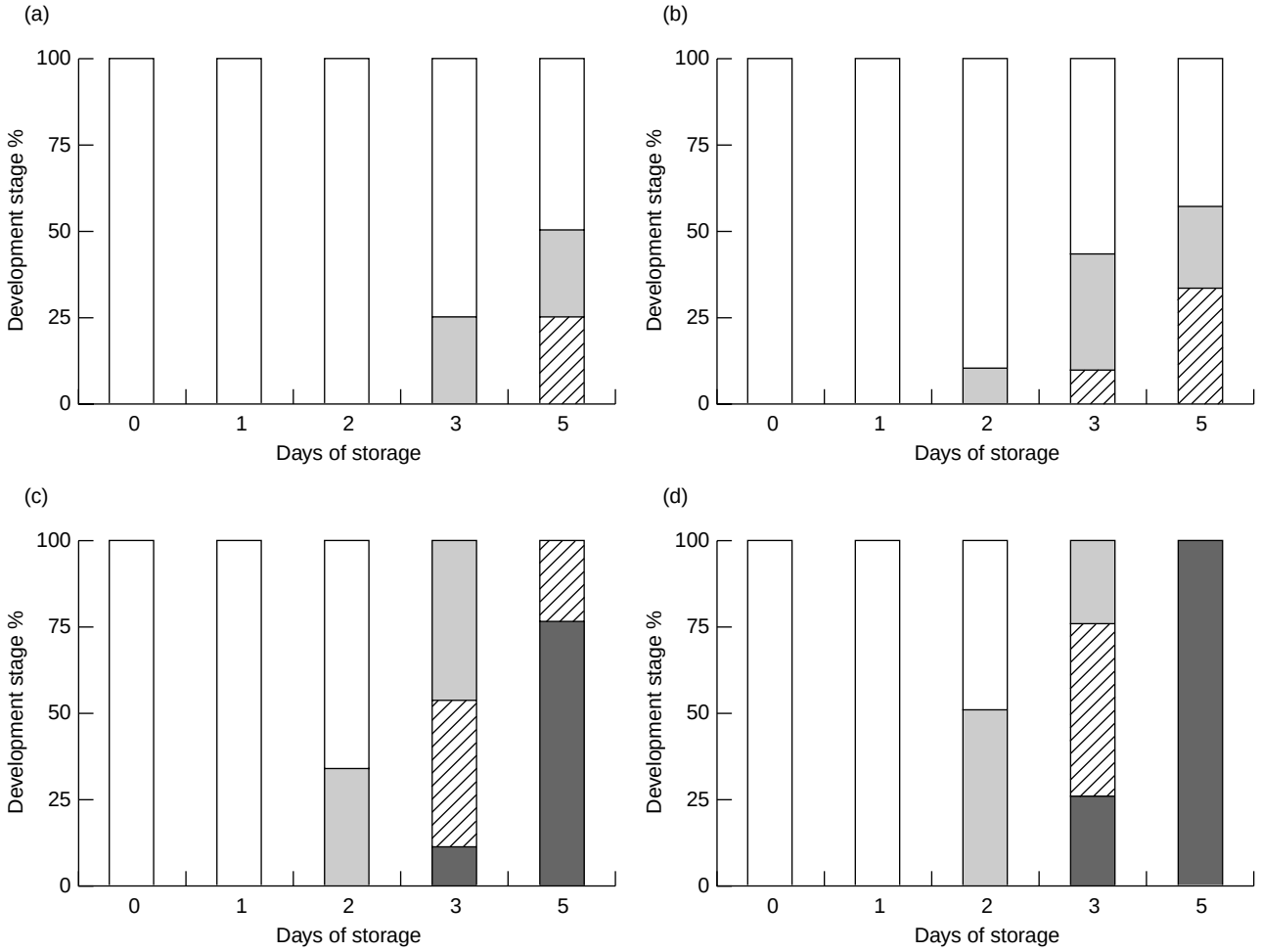

Fig. 4 Effect of packaging and storage temperature on the development stage of mushrooms overwrapped with PVC films, according to the Guthrie scale (Guthrie 1984). The development stage of each batch is expressed as the percentage of mushrooms in each category. (a) Batch B; (b) batch D; (c) batch F; (d) batch H. (□), 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

populations in every package, indicating that the microbial load of mushrooms was mainly *Pseudomonas*.

After 5 days of storage, the mushrooms packaged with perforated film had counts of fluorescent pseudomonad bacteria of 9·3 log cfu g^{-1} at 17 °C and 9·7 log cfu g^{-1} at 25 °C; these figures are 2·6–3·1 log cycles higher than the original count, respectively (6·6 log cfu g^{-1}).

After 5 days, the mushrooms packaged with non-perforated film had counts of *Pseudomonas* spp. of 8·8 log cfu g^{-1} at 17 °C (Fig. 5a) and 8·1 log cfu g^{-1} at 25 °C. This film generated atmospheres with higher CO_2 and lower O_2 concentration; as *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 with an air atmosphere. In addition, high O_2 concentrations enhance the growth of fluorescent pseudomonads.

It should be noted that mushrooms packaged in perforated film and stored at 25 °C had the lowest L* values. This could be explained by the fact that these packages had the highest *Pseudomonas* counts, this bacteria being associated with brown stains (Wong and Preece 1982).

Staphylococcus aureus

No *Staph. aureus* was detected in any of the uninoculated mushrooms so no enterotoxins were detected. Hardt-

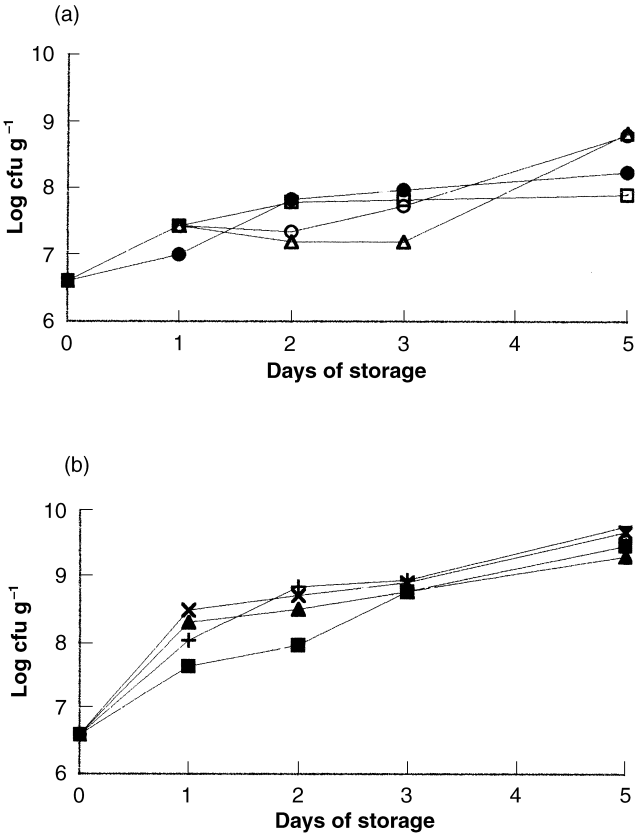

Fig. 5 Effect of packaging film, inoculation of *Staphylococcus aureus* and storage temperature on *Pseudomonas* growth. (a) Non-perforated film; (b) perforated film. Batch A (○), Batch B (△) Batch C (□), Batch D (●), Batch E (▲), Batch F (■), Batch G (×) and Batch H (+) (see Table 1). The data are the mean values of two experiments

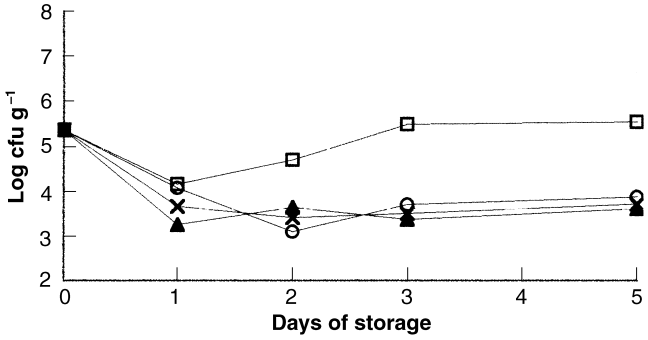

Fig. 6 Influence of packaging film and storage temperature on *Staphylococcus aureus* growth. Batch A (○), Batch C (□), Batch E (▲) and Batch G (×) (see Table 1). The data are the mean values of two experiments

English *et al.* (1990) isolated *Staph. aureus* from uninoculated mushrooms stored in sealed plastic bags, but the reason for this could lie in the sample origin, as these authors used mushrooms harvested in China.

Staphylococcus aureus did not grow in the packaged mushrooms stored at 17 °C (Fig. 6). This was in contrast to *Pseudomonas* spp., which increased by 2·6 log cycles.

Only slight growth was observed in the mushrooms packaged with non-perforated film after 1 day at 25 °C (Batch C). The decrease of storage temperature from 25 to 17 °C caused a greater inhibition of *Staph. aureus*. The higher growth of *Staph. aureus* in non-perforated packaging than in perforated films stored at 25 °C could be due to the lower growth of competitors such as *Pseudomonas* spp., as *Staph. aureus* can grow under anaerobic conditions while *Pseudomonas* spp. are aerobic bacteria and their growth is reduced under low O_2 concentrations. In addition, *Staph. aureus* survival may be improved under anaerobic compared with aerobic conditions (ICMSF 1996).

Brunner and Wong (1992) did not detect growth of *Staph. aureus* when inoculated mushrooms were stored at 25 °C in plastic bags, while the background flora increased by 2–3 log cycles. However, these authors observed *Staph. aureus* growth when mushrooms were stored at a higher temperature (37 °C). In contrast, Martin and Beelman (1996) observed growth of *Staph. aureus* in unventilated PVC-overwrapped mushroom packages at 25 °C; however, when the packages were ventilated, *Staph. aureus* growth was suppressed.

The inability of staphylococci to compete successfully with other food bacteria has been reported by Minor and Marth (1976). The inhibitory effect is dependent on factors which include the ratio of *Staph. aureus* to its competitors, the storage temperature and atmosphere, the intrinsic characteristics of the food such as pH, a_w and Eh and the ability of the flora to produce inhibitory or stimulatory compounds (Genigeorgis 1989).

No enterotoxins were detected in any of the samples. *Staphylococcus aureus* produces enterotoxin when present in food at levels exceeding approximately 6 log cfu g^{-1} (Mossel *et al.* 1995); such populations were not reached in the present experiments. Moreover, Noleto *et al.* (1987) reported that in the presence of *Pseudomonas aeruginosa*, enterotoxin A was produced in broth and in meat media generally when the number of *Staph. aureus* cells was equal to or greater than that of the competitors. Higher *Pseudomonas* spp. counts than *Staph. aureus* were found in the present experiments. Martin and Beelman (1996) observed *Staph. aureus* growth in inoculated mushrooms

packaged in unventilated PVC and stored at 25 °C, but they did not detect enterotoxin.

Lindroth *et al.* (1983) studied the growth of *Staph. aureus* in inoculated wild mushrooms stored at 15 and 21 °C for 3 days. These authors found that *Staph. aureus* grew to levels high enough to detect enterotoxin. Hardt-English *et al.* (1990) detected enterotoxin in non-inoculated mushrooms packaged in sealed bags which had been harvested in China. This could be explained by the fact that naturally present *Staph. aureus* could have been able to compete and produce enterotoxin.

Faecal coliforms and *Escherichia coli*

Faecal coliform counts were <2 log cfu g^{-1} in all the batches analysed. This could be related to the hygienic handling conditions. *Escherichia coli* was not isolated in any sample. Coliform growth could be inhibited by the high populations reached by other competitors.

Anaerobic spores

At 25 °C, higher counts of anaerobic spores (2 log cfu g^{-1}) were detected in the mushrooms packaged in non-perforated film than in those packaged in perforated film, as lower oxygen concentrations were detected in these packages (Fig. 7).

General acceptability

It can be concluded that at abuse storage temperatures, mushrooms packaged in non-perforated films had the most desirable quality parameters (texture, development stage and mould presence). Although 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 effective prevention of the potential growth of *Clostridium botulinum*. In addition, other psychrotrophs such as *Listeria* spp. have been isolated in vegetables, and although there are few data on the presence of this pathogen in mushrooms, further research is needed (Varnam and Evans 1996).

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, as temperatures of 60–85 °C are reached in the composting process (Fernandez *et al.* 1996), the probability of survival of this group of micro-organisms is low.

Staphylococcus aureus populations did not reach the dangerous levels (above 6 log cfu g^{-1}) associated with enterotoxin production. Only a slight growth was observed in mushrooms packaged with perforated film after 1 day of storage at 25 °C.

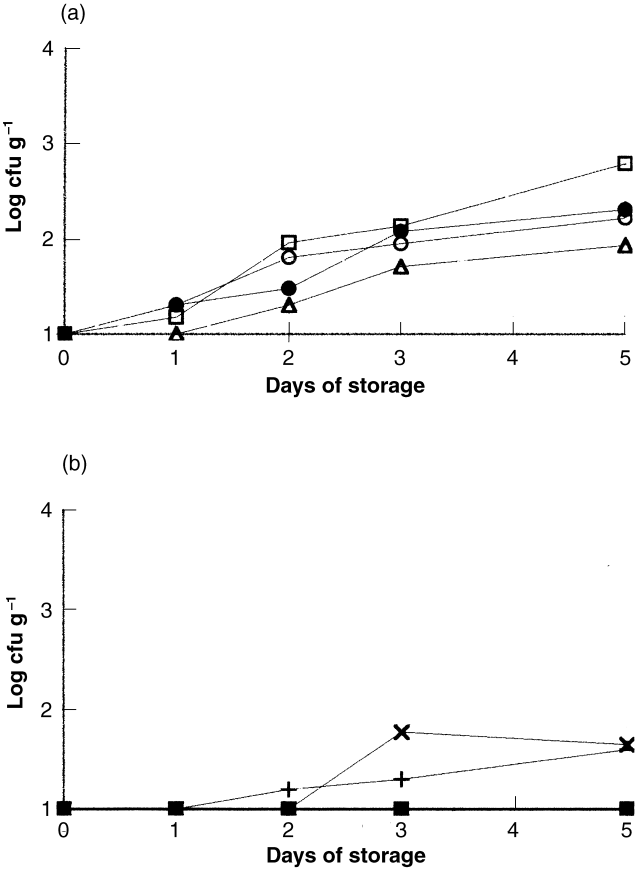

Fig. 7 Effect of packaging film, inoculation of *Staphylococcus aureus* and storage temperature on anaerobic spore growth. (a) Non-perforated film; (b) perforated film. Batch A (○), Batch B (△) Batch C (□), Batch D (●), Batch E (▲), Batch F (■), Batch G (×) and Batch H (+) (see Table 1). The data are the mean values of two experiments

REFERENCES

Beit-Halachmy, I.I. and Mannheim, C.H. (1992) Is modified atmosphere packaging beneficial for fresh mushrooms?. *Lebensmittelwissenschaft und Technologie* **225**, 426–432.

Bergdoll, M.S. (1990) Staphylococcal food poisoning. In *Foodborne Diseases* ed. Cliver, D.O. pp. 85–106. London: Academic Press.

Brunner, K. and Wong, A. (1992) *Staphylococcus aureus* growth and enterotoxin production in mushrooms. *Journal of Food Science* **57**, 700–703.

Burton, K.S. (1988) Modified atmosphere packaging. A new technology for extending mushroom storage-life. *The Mushroom Journal* **183**, 510.

Burton, K.S. (1989) The quality and storage life of *Agaricus bisporus*. *Mushroom Science XII. Proceedings of the 12th International Congress on the Science and Cultivation of Edible Fungi* Braunschwey, Germany, pp. 683–688.

Burton, K.S., Frost, C.E. and Nichols, R. (1987) A combination plastic permeable film system for controlling post-harvest mushroom quality. *Biotechnology Letters* **9**, 529–534.

Burton, K.S. and Twyning, R.V. (1989) Extending mushroom storage-life by combining modified atmosphere packaging and cooling. *Acta Horticulturae* **258**, 565–571.

Day, B.P.F. (1992) *Guidelines for the Good Manufacturing and Handling of Modified Atmosphere Packed Food Products* Technical Manual No. 34. Gloucestershire, UK: Campden & Chorleywood Food Research Association.

Fernandez, E.J., Castillo, J.E., Lopez, F.J. and Moreno, A. (1996) Compost para el cultivo de champiñón. *Hortoinformacion* **71**, 54–59.

Fey, H., Pfister, H. and Rüeg, O. (1984) Comparative evaluation of different enzyme-linked immunosorbent assay system for the detection of staphylococcal enterotoxins A, B, C and D. *Journal of Clinical Microbiology* **19**, 34–38.

Flegg, P.B., Spencer, D.M. and Wood, D.A. (1985) *The Biology and the Technology of the Cultivated Mushrooms.* Chichester, UK: John Wiley & Sons.

Freed, R.C., Evenson, M.L., Reiser, R.F. and Bergdoll, M.S. (1982) Enzyme-linked immunosorbent assay for detection of staphylococcal enterotoxins in foods. *Applied and Environmental Microbiology* **44**, 1349–1355.

Genigeorgis, C. (1989) Present stage of knowledge on staphylococcal intoxication. *International Journal of Food Microbiology* **9**, 327–360.

Gormley, T.R. (1975) Chill storage of mushrooms. *Journal of the Science of Food and Agriculture* **26**, 401–411.

Guthrie, B. (1984) Studies on the control of bacteria deterioration of fresh washed mushroom *Agaricus bisporus brunescens*. M.S. Thesis. The Pennsylvania State University.

Hardt-English, P., York, G., Stier, R. and Cocotas, P. (1990) Staphylococcal food poisoning outbreaks caused by canned mushrooms from China. *Food Technology* **44**, 74–77.

Hotchkiss, J.M. and Banco, M.J. (1992) Influence of new packaging technologies on the growth of microorganisms in produce. *Journal of Food Protection* **55**, 815–820.

ICMSF (1978) *Microorganisms in Foods. 1. Their Significance and Methods of Enumeration.* International Commission on Microbiological Specifications for Foods (ICMSF) 2nd edn. Toronto: University of Toronto Press.

ICMSF (1996) *Microorganisms in Foods. 5. Characteristics of Microbial Pathogens.* International Commission on Microbiological Specifications for Foods (ICMSF). London: Blackie Academic & Professional.

Kautter, D.A., Lilly, T. and Lynt, R. (1978) Evaluation of the botulism hazard in fresh mushrooms wrapped in commercial polyvinylchloride film. *Journal of Food Protection* **41**, 120–121.

King, E.O., Ward, M.K. and Ranzy, E.D. (1954) Two simple media for the demonstration of pyocyanin and fluorescein. *Journal of Laboratory and Clinical Medicine* **44**, 301–307.

Lindroth, S., Strandberg, E., Pessa, A. and Pellinen, M.J. (1983) A study on the growth potential of *Staphylococcus aureus* in *Boletus edulis*, a wild edible mushroom, prompted by a food poisoning outbreak. *Journal of Food Science* **48**, 282–283.

López-Briones, G., Varoquaux, P., Chambroy, Y., Bouquant, J., Bureau, G. and Pascat, B. (1992) Storage of common mushroom under controlled atmospheres. *International Journal of Food Science and Technology* **27**, 493–505.

López-Briones, G., Varoquaux, P., Bureau, G. and Pascat, B. (1993) Modified atmosphere packaging of common mushroom. *International Journal of Food Science and Technology* **28**, 57–68.

Martin, S. and Beelman, R. (1996) Growth and enterotoxin production of *Staphylococcus aureus* in fresh packaged mushrooms (*Agaricus bisporus*). *Journal of Food Protection* **59**, 819–826.

Minor, T.E. and Marth, E.H. (1976) *Staphylococci and Their Significance in Foods.* New York: Elsevier.

Mossel, D.A.A., Corry, J.E.L., Struijk, C.B. and Baird, R.M. (1995) *Essentials of the Microbiology of Foods. A Textbook for Advanced Studies.* Chichester, UK: John Wiley and Sons Ltd.

Murr, D.P. and Morris, L.L. (1975) Effect of storage atmosphere on postharvest growth of mushrooms. *Journal of American Society of Horticulture Science* **100**, 298–301.

Noleto, A.L.S., Malbur, L.M. and Bergdoll, M.S. (1987) Production of staphylococcal enterotoxin in mixed cultures. *Applied and Environmental Microbiology* **53**, 2271–2274.

Robertson, G.L. (1993) *Food Packaging.* New York: Marcel Dekker.

Saray, T., Balla, C. and Horti, K. (1994) The importance of packaging and modified atmosphere in maintaining the quality of cultivated mushrooms (*Agaricus bisporus* L.) stored in chill chain. *Acta Horticulturae* **368**, 322–326.

Simon, A. and Gurria, A. (1998) Empaquetado de champiñón (*Agaricus bisporus*) con cuatro tipos de film plástico. *Alimentaria* **36**, 57–62.

Soler-Rivas, C., Arpin, N., Olivier, J.M. and Wichers, H.J. (1999) WLIP, a lipodepsipeptide of *Pseudomonas 'reactans'*, as inhibitor of the brown blotch disease of *Agaricus bisporus*. *Journal of Applied Microbiology* **86**, 635–641.

Sugiyama, H. and Yang, K.H. (1975) Growth potential of *Clostridium botulinum* in fresh mushrooms packaged in semipermeable plastic film. *Applied Microbiology* **30**, 964–969.

Varnam, A.H. and Evans, M.G. (1996) *Foodborne Pathogens.* London: Manson Publishing.

Wong, W.C. and Preece, T.F. (1982) *Pseudomonas tolaasii* in cultivated mushrooms (*Agaricus bisporus*) crops: numbers of the bacterium and symptom development on mushrooms grown in various environments after artificial inoculation. *Journal of Applied Bacteriology* **53**, 87–96.