Effects of modified atmosphere packaging with a silicon gum film as a window for gas exchange on *Agrocybe chaxingu* storage

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Abstract

The edible mushroom *Agrocybe chaxingu* was stored in packages with or without silicon gum film windows in three different modified atmosphere systems (5% O₂, with 5%, 10% and 15% CO₂) at a temperature of 3 ± 1°C. The results showed that there were significant differences between the packages with and without the silicon gum film windows on O₂, CO₂, and ethylene concentrations, respiration rate, ascorbic acid content, electrolyte leakage and sensory characteristics. Compared to the packages without the silicon gum film windows, the packages with the windows were more effective for quality keeping of the stored mushrooms. This window kept the gas compositions of the packages at levels which avoided anaerobic respiration and resulting off-odors. Among three different modified atmosphere systems, the packages with the silicon gum film window with initial gas concentrations of 5% O₂ and 10% CO₂ were the most effective for maintaining mushroom quality.

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Keywords: Modified atmosphere packaging; Silicon gum film; Window; *Agrocybe chaxingu*; Storage

1. Introduction

*Agrocybe chaxingu*, a valuable edible mushroom grown extensively in China, has high nutritive components, such as protein, amylose, essential amino acids and mineral elements (Zhang et al., 2005), and its consumption has been increasing substantially in recent years in China (Deng and Wu, 2005). Generally, mushrooms have a short shelf life of 3–4 days at ambient temperatures because of the absence of a cuticle to protect them from physical or microbial attack or water loss (Martine et al., 2000). Like other mushroom species, *A. chaxingu* is also highly perishable, and only be stored for 10 days at 3°C with vacuum pre-cooling technology (Shi et al., 2001).

Modified atmosphere packaging (MAP) is one of a number of technologies available to control product deterioration, providing an appropriate protective atmosphere around the product (Cliffe-Byrnes and O’Beirne, 2005; Zhang et al., 2001, 2006). However, MAP often results in development of undesirable fermentation because of insufficient permeability of the packaging film. Hypoxic atmospheres have to be avoided in packaging products because the shift to fermentation might cause formation of ethanol, acetaldehyde, and off-flavors and odors (Day, 2001; Jacxsens et al., 2000).

In order to improve MAP conditions, improved permeability of packaging films has been studied and developed successfully (Fonseca et al., 1999; Garcia et al., 1998; Lee et al., 2003; Van der Steen, 2002). Because respiration rates differ greatly among different vegetable products, packaging films with a wide range of permeabilities for O₂ and CO₂ are necessary to meet preservation requirements (Jacxsens et al., 1999). Other methods to improve the permeability of packages have also been studied and developed using a silicone membrane system (Vigneault et al., 1992). Gariepy et al.
(1986) used a silicone membrane system for long term storage of celery under controlled atmosphere conditions. Reederer et al. (1989) fitted silicone membranes of varying surface areas on containers to maintain the atmosphere for controlling storage rot of carrot. Stewart et al. (2005) found that silicone membrane systems with modified atmospheres were effective for storing bananas, maintaining the fruit with a harvest-fresh appearance, good color and excellent marketability after 42 days storage.

So far, there have been few reports on MAP with permeable films as a window for gas exchange to store mushrooms. In this study, a silicon gum film with high permeability for O$_2$, CO$_2$ and other gases was used as a window material in packages to store A. chaxingu mushrooms in three different initial modified atmosphere systems (5% O$_2$, with 5%, 10% and 15% CO$_2$) at a temperature of $3\pm 1\, ^\circ\text{C}$. The storage effects were investigated by evaluating physical, chemical, physiological and sensory characteristics.

2. Materials and methods

2.1. Plant material

A. chaxingu used in this study was purchased 3 h after harvesting, at Qingshan market, Wuxi City, Jiangsu Province, China, and then brought to the laboratory immediately and treated within 2 h.

2.2. Equipment

The gas supply system for MAP consisted of bottled N$_2$, O$_2$ and CO$_2$, a 10 L mixing cylinder and a vacuum pump to remove air in the gas supply system. The gas composition established in the packages was checked using a gas analyzer (CYES-II, Xuelian Analytical Instrument Co. Ltd., Shanghai, China) with a system accuracy of $\pm 0.5\%$. The modified atmosphere packaging equipment (ADFM-V3000, air controlled atmosphere packing machine, Hengzhong Packing Co., Lianyungang City, Jiangsu Province, China) was connected to the mixing cylinder of the gas supply system.

2.3. Modified atmosphere packaging

The polystyrene (PS) packaging trays (18 cm length $\times$ 12 cm width $\times$ 4 cm depth) were divided into two groups. One group had a window (0.9 cm $\times$ 1.0 cm, the preliminary test showed this size of window was optimum) cut into it at the central part on the side of each tray and the other was kept unchanged as the control. The window was covered and with high permeability film (FC-8 silicon gum film, Lanzhou Physical & Chemical Research Institute of the Academy of Sciences of China, Lanzhou, China), which was made by spreading 50 $\pm$ 5 g silicon gum on 1 m$^2$ cloth to achieve an O$_2$ permeability of $4.08 \times 10^{-9}$ mol s$^{-1}$ m$^{-2}$ Pa$^{-1}$ and CO$_2$ permeability of $12.24 \times 10^{-9}$ mol s$^{-1}$ m$^{-2}$ Pa$^{-1}$ at $20\, ^\circ\text{C}$ and 90% RH. There were three combinations of modified atmosphere systems in this study: 5% O$_2$, with 5%, 10% and 15% CO$_2$. For each modified atmosphere, 42 trays were packaged, including 21 trays (three replicates with seven time measurements for each treatment) each with and without the FC-8 silicon gum film windows. In each tray, approximately 115 g of mushrooms was packaged and sealed with 35 $\mu$m thick polypropylene (PP) membrane. The storage temperature was $3\pm 1\, ^\circ\text{C}$ with a relative humidity of 95–100% inside the packages and of about 85% in the storage room, for 20 days.

2.4. Gas analysis

The headspace gas concentrations of O$_2$ and CO$_2$ in the sealed trays during storage were measured in each package before opening using a gas analyzer (CYES-II, Xuelian Analytical Instrument Co. Ltd., Shanghai, China) at 12 and 24 h on the 1st day and then at every 4 days.

The headspace gas concentration of ethylene was analyzed every 4 days, using a gas chromatograph (Shimadzu GC-2010, Japan) equipped with a flame ionization detector (FID) and a DB-1 column. Nitrogen was used as a carrier gas, and the flow rate was 10 mL min$^{-1}$.

The headspace gas concentrations of ethanol were analyzed at the 3rd day and then at every 4 days, using a gas chromatograph (Agilent GC-6890A, USA) equipped with a flame ionization detector and a PP-20000 column. Nitrogen was used as a carrier gas, and the flow rate was 2 mL min$^{-1}$.

2.5. Sensory quality

A panel of 10 trained judges evaluated the sensory quality characteristics of all the mushrooms from each tray. Before the experiment started, the typical characteristics of the mushrooms and the possibilities of deterioration were explained. All sensory tests (general appearance, firmness and odor) were performed in a special taste room with separated boxes. General appearance was judged under normal light. The sensory characteristics, such as firmness and odor were evaluated under IR light to exclude the influence of the visual characteristics. General appearance was evaluated on a scale of 1–9, where 1 represents excellent without any rotten areas, 3 represents good without any rotten areas, 5 represents fair and limit marketability with 1–2 0.5 mm$^2$ rotten areas, 7 represents poor and limit usability with 3–6 0.5 mm$^2$ rotten areas, and 9 represents very poor and inedible with more than 7 0.5 mm$^2$ rotten areas. Firmness was evaluated by pressing the mushroom between the thumb and index finger, on a scale of 1–9, where 1 represents very firm and turgid, 3 represents firm, 5 represents moderately firm, 7 represents soft and 9 represents very soft. Off-odors, mainly because of fermentation, were evaluated on scales of 1–9, where 1 represents none, 3 represents slight, 5 represents moderate, 7 represents moderate severe and 9 represents severe. The cut-off score was...
set at score 5. Below this score, the sample was acceptable (Jacxsens et al., 2001).

2.6. Physical and chemical analysis

2.6.1. Respiration rate

A static method was used to assess respiration rate. Before assessment, the mushrooms (115 ± 5 g) was taken out from the packages and exposed to ambient conditions in a storage room (3000 L) for 1 h so that the CO₂ accumulated in the tissue diffused into air, and then the sample was put into a gas-tight container 260 mm in diameter with 10 mL 0.4N NaOH in a Petri dish, and the jars were placed at 3 °C. The Petri dish was taken out after 30 min and the NaOH titrated with 0.2N oxalic acid (C₂H₂O₄) immediately. The change of concentration of CO₂ was used to estimate respiration rates (Yang and Zhang, 2000).

2.6.2. Ascorbic acid

Ascorbic acid was determined by the indophenol titration method (Association of Vitamin Chemists, 1966; Favell, 1998). A 10 g sample was ground in a mortar with the same quantity of 2% oxalic acid. One percent oxalic acid solution was used to wash the paste into a 100 mL volumetric flask and made to volume. Ten milliliters filtered solution was titrated with 2,6-dichlorophenol indophenol solution until the distinct light rose pink color persisted for more than 5 s.

2.6.3. Soluble solids content

Measurements of the percentage of soluble solids were made with an ABBE Bausch and Lomb refractometer (2W AJ, Shanghai Optical Instruments Factory, Shanghai, China) on juice squeezed from undamaged pieces of tissue cut from the mushrooms for each treatment. These observations were obtained initially and every 4 days during storage.

2.6.4. Electrolyte leakage

Electrolyte leakage was used to assess cell membrane permeability according to the procedure described by Kaya et al. (2002). Mushroom discs (3 mm thick, 3 mm diameter, 5 g total) were immersed in 50 mL distilled water for 1 h to remove surface contamination, then were taken out and immersed in another 50 mL distilled water and incubated at ambient temperature (20 ± 3 °C). Conductivity of the suspending solution was measured at 3 h and after boiling for 30 min with an electrical conductivity meter (DDB-303A, Leici Instrument Co., Shanghai, China). Relative electrolyte leakage (%) was calculated as the ratio of the electrolyte leakage after 3 h of submersion to the total value.

2.6.5. Protein content

The nitrogen content of the mushrooms A. chaxingu was determined by the Micro-Kjeldahl method (AOAC, 1984) and the amount of total protein was calculated from percent nitrogen content using a conversion factor of 6.25.

2.7. Statistical analysis

All experiments were conducted in triplicate and the average values with standard deviation were used in the analysis. All data were evaluated by multi-factorial analysis of variance (ANOVA) using SPSS (Windows XP) with window and gas composition as main effects. To determine differences between treatments, Duncan tests were applied and significant differences were established at $P < 0.05$.

3. Results and discussion

3.1. Gas concentrations

The O₂ concentration decreased rapidly in the first day for all treatments, and then remained relatively stable during the following storage days (Fig. 1a). The O₂ concentrations in packages with the silicon gum film windows were at least higher than 1.1% during the whole storage period, and the O₂ concentrations in packages without the silicon gum film windows were lower than 0.9% after 1 day of storage. The CO₂ concentrations increased in the packages without the silicon gum film windows and exceeded 25.3% at the end of storage.

![Fig. 1. Changes of O₂ (a) and CO₂ (b) concentrations (mean ± S.D. for three replicates) in packages for Agrocybe chaxingu stored at 3 °C for 20 days. (■) 5% O₂, 5% CO₂, W; (□) 5% O₂, 5% CO₂, NW; (▲) 5% O₂, 15% CO₂, W; (△) 5% O₂, 15% CO₂, NW; (●) 5% O₂, 10% CO₂, W; (○) 5% O₂, 10% CO₂, NW. W, packaged with silicon gum film windows; NW, packaged without silicon gum film windows.](image)
Table 1
The change of ethanol concentrations in packages and protein contents of *Agrocybe chaxingu* over 20 days storage

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Ethanol concentration (µL L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 days</td>
</tr>
<tr>
<td>NW + 5% O₂ + 10% CO₂</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>W + 5% O₂ + 15% CO₂</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Protein content (g 100 g DW⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 days</td>
</tr>
<tr>
<td>NW + 5% O₂ + 10% CO₂</td>
<td>24.8 ± 1.3</td>
</tr>
<tr>
<td>W + 5% O₂ + 15% CO₂</td>
<td>24.8 ± 1.3</td>
</tr>
</tbody>
</table>

Note: Values were mean ± S.D. for three replicates. DW, dry weight; W, packaged with silicon gum film windows; NW, packaged without silicon gum film windows.

Table 2
Mean squares and mean values for ethylene concentrations in packages of *Agrocybe chaxingu* over 20 days storage

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Ethylene concentration (µL L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8 days</td>
</tr>
<tr>
<td>Mean squares</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Window (WI)</td>
<td>1</td>
<td>1.5901*</td>
</tr>
<tr>
<td>Gas composition (GC)</td>
<td>2</td>
<td>0.0057</td>
</tr>
<tr>
<td>WI × GC</td>
<td>2</td>
<td>0.0015</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Window</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W</td>
<td>0.21a</td>
<td>8.51a</td>
</tr>
<tr>
<td>NW</td>
<td>0.81b</td>
<td>85.42b</td>
</tr>
<tr>
<td>Gas composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% O₂ + 5% CO₂</td>
<td>0.51c</td>
<td>46.91c</td>
</tr>
<tr>
<td>5% O₂ + 10% CO₂</td>
<td>0.48c</td>
<td>44.07c</td>
</tr>
<tr>
<td>5% O₂ + 15% CO₂</td>
<td>0.54c</td>
<td>49.92c</td>
</tr>
<tr>
<td>W + gas composition</td>
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<td></td>
</tr>
<tr>
<td>W + 5% O₂ + 5% CO₂</td>
<td>0.21 ± 0.03a</td>
<td>8.58 ± 0.39a</td>
</tr>
<tr>
<td>NW + 5% O₂ + 5% CO₂</td>
<td>0.81 ± 0.06b</td>
<td>85.24 ± 4.51b</td>
</tr>
<tr>
<td>W + 5% O₂ + 15% CO₂</td>
<td>0.26 ± 0.02a</td>
<td>10.22 ± 0.48a</td>
</tr>
<tr>
<td>NW + 5% O₂ + 15% CO₂</td>
<td>0.82 ± 0.07b</td>
<td>89.62 ± 4.35b</td>
</tr>
<tr>
<td>W + 5% O₂ + 10% CO₂</td>
<td>0.17 ± 0.02a</td>
<td>6.73 ± 0.35a</td>
</tr>
<tr>
<td>NW + 5% O₂ + 10% CO₂</td>
<td>0.79 ± 0.07b</td>
<td>81.41 ± 3.67b</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate that means are significantly different (*P* < 0.05). W, packaged with silicon gum film windows; NW, packaged without silicon gum film windows; d.f., degree of freedom.

* Main effects and interactions are significant at *P* < 0.05.
Fig. 2. Changes in scores (mean ± S.D. for three replicates) of appearance (a), firmness (b) and odor (c) for Agrocybe chaxingu packaged and stored at 3 °C for 20 days. (■) 5% O₂, 5% CO₂, W; (□) 5% O₂, 5% CO₂, NW; (▲) 5% O₂, 15% CO₂, W; (△) 5% O₂, 15% CO₂, NW; (●) 5% O₂, 10% CO₂, W; (○) 5% O₂, 10% CO₂, NW. Symbol (*) represents the scores above 5 suggesting unacceptable quality. W, packaged with silicon gum film windows; NW, packaged without silicon gum film windows.

3.2. Sensory evaluation

Fig. 2a shows the changes in general appearance of stored A. chaxingu. The scores for both packages increased with increasing storage time. There were significant differences (P < 0.05) between the packages with and without the silicon gum film windows after the 8th day. The scores of the packages with the silicon gum film windows increased slowly with storage time, and were under the acceptable limit of 5.0 at the end of storage. The scores of the packages without the silicon gum film windows increased rapidly from the 8th day, and all of them exceeded 5.0 after the 16th day.

Fig. 2b shows the changes in firmness and the trend of firmness for both packages was similar to that of general appearance shown in Fig. 2a. The scores of the packages with the silicon gum film window increased slowly with storage time, and were under 5.0 at the end of storage. The scores of packages without the silicon gum film windows increased relatively slowly before the 8th day, but increased rapidly from day 8 until the end of storage, and all of them were above 5.0 after 16 days. The significant differences (P < 0.05) in scores of firmness were observed between the packages with and without the silicon gum film windows after 12 days storage.

The low O₂ level, high CO₂ level (Fig. 1) and high ethylene level (Table 2) in the packages without silicon gum film windows resulted in physiological injury and senescence, which resulted in the mushrooms having fragile caps and soft and spongy stems. The mushrooms were unacceptable after 16 days storage.

The score changes for odor during storage are shown in Fig. 2c. There were significant differences (P < 0.05) between the packages with and without the silicon gum film windows from the 4th day. The scores of the packages with the silicon gum film windows increased slowly with a prolonging of storage time, and all of them were under 3.0 at the end of storage. It indicated that there was little odor produced and accumulated in these packages during the storage period. In the packages without the silicon gum film windows, however, the scores increased, and all of them exceeded 5.0 at 12 days and exceeded 7.0 at the end of storage. This suggests that anaerobic fermentation reactions occurred, resulting in the detected odors. Some studies have shown that MAP can have a damaging effect, causing anaerobic respiration (Beit-Halachmy and Mannheim, 1992; Varoquaux et al., 1999). According to Lopez-Briones et al. (1992), working with common mushrooms, fermentation did not occur at a minimum O₂ concentration of 1–2%. In our study, the O₂ concentration in the packages without the silicon gum film windows was under 1.0% after the 1st day, it decreased with increasing storage time and reached the lowest level (0.5%) at 16 days, and the CO₂ concentration increased strongly with increasing storage time and reached the highest levels (27.3%) at the end of storage. All of these factors might contribute to increased anaerobic respiration and accumulation of metabolites that were perceived as off-odors.

3.3. Respiration rate

Fig. 3 shows the changes in respiration rates during storage for all treatments. The initial respiration rates were very high, reaching 223.2 ± 4.2 mg CO₂ kg⁻¹ h⁻¹. These high initial respiration rates were associated with harvest stress caused by the harvesting process just before storage. Similar results were obtained by Villaescusa and Gil (2003), who reported...
that Pleurotus mushrooms had initial high respiration rates followed by lower levels that lasted 10 days and then a subsequent rapid rise in respiration. For all the treatments in our experiments, the respiration rate dropped by 22.1–37.5% for the first 4 days. After the decline, the respiration rate of the mushrooms in the packages without the silicon gum film windows remained stable, and increased after 12 days. The respiration rate of the mushrooms in the packages with silicon gum film windows remained stable from 4 to 16 days, and increased after 16 days, 4 days later than the controls (Fig. 3). Significant differences ($P < 0.05$) were observed for the respiration rates of mushrooms among treatments during 12–20 days storage. These results indicated that the mushrooms in the packages with silicon gum film windows had lower respiration rates, and these would be beneficial in maintaining quality during storage. Furthermore, Fig. 3 also shows that the respiration rate of the mushrooms in the package with silicon gum film windows with 5% O$_2$ and 10% CO$_2$ initial gas concentrations decreased at the slowest rate (Fig. 4).

### 3.4. Ascorbic acid contents

Fig. 4 shows the ascorbic acid contents as a function of storage time. The ascorbic acid content in the fresh condition was not high, only 5.54 mg 100 g FW$^{-1}$, and it decreased gradually during storage for all treatments. Significant differences ($P < 0.05$) in ascorbic acid contents were observed among treatments after 4 days storage, and the differences were associated with the windows of silicon gum film in the first instance and the initial gas compositions as a secondary contribution. In this case, the fast change in O$_2$ and CO$_2$ concentrations, the anaerobic respiration (Fig. 2c) and high ethylene levels (Table 2) in the packages without silicon gum film windows are likely to cause cell injury and senescence, resulting in a more rapid decline in the ascorbic acid contents.

Among the packages with the silicon gum film window, the ascorbic acid contents of the mushrooms with 5% O$_2$ and 10% CO$_2$ initial gas concentrations decreased at the slowest rate (Fig. 4).

### 3.5. Soluble solids contents

Fig. 5 shows the change in soluble solids contents, which increased for most of the treatments and reached a peak at 8 days in the packages with the silicon gum film windows, but at 12 days in the controls, followed by a decrease in levels. Tao et al. (2006) reported that the soluble solids contents of Agaricus mushrooms reached the highest levels after about 5 days storage, and then declined. In our study, only the soluble solids contents of the mushrooms in the packages with the...
silicon gum film window under 5% O2 and 10% CO2 initial gas concentrations remained relatively stable although they peaked at 8 days. The changes varied from 5.8% to 6.1%, resulting in only a 0.3% overall change.

3.6. Electrolyte leakage

Relative cell membrane permeability was determined by measuring relative electrolyte leakage as shown in Fig. 6. This increased with increasing storage time and showed that membrane systems became more vulnerable to leakage. Similar results were observed by Tao et al. (2006) in stored Agaricus mushrooms. The relative cell membrane permeability of A. chaisingu mushrooms had significant differences (P<0.05) among treatments after 12 days of storage, and the differences largely resulted from use of the windows of silicon gum film, with the initial gas compositions providing a secondary contribution. The relative membrane permeability of mushrooms stored in the package with the silicon gum film window with 5% O2 and 10% CO2 initial gas concentrations was the lowest (16.77%), whereas it reached the highest levels (28.94%) in the controls at the end of the storage. These results indicated that storage of A. chaisingu for more than 8 days at low O2 (lower than 1.1%), high CO2 (higher than 15.9%) and high ethylene (higher than 21.08 µL L−1) would cause cell membrane injury.

4. Conclusion

The gas exchange between the package and surrounding atmosphere through the silicon gum film windows resulted in an in-package optimum gas composition for A. chaisingu of O2 above 1.1%, CO2 under 15.9% and ethylene under 21.08 µL L−1. The packages with the silicon gum film windows were more effective for maintaining mushroom quality. In this study, the initial gas concentrations also had some effects on the quality attributes during storage, and 5% O2 and 10% CO2 was the most effective initial gas concentration for maintaining high storage quality.

References


