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Effect of controlled atmosphere and storage temperature on the weight loss and cap colour of fresh mushrooms (*Agaricus bisporus*)

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ABSTRACT

The effects of O_2, CO_2 concentrations and storage temperature on the quality of fresh mushrooms (Agaricus bisporus) were investigated. Eight different gaseous atmospheres were used in the combinations of 3, 12, 16.5 and 21% of O_2 and 0, 3, 6 and 12% of CO_2. The storage temperatures for each gas condition were 1°C, 6°C and 12°C. Measurements extended over a storage period of 9 days. After storage under controlled atmosphere, the influence of O_2, CO_2 and temperature on the weight loss and cap colour was analysed. The results showed that the weight loss of fresh mushrooms in all gas combinations increased with an increase in temperature and storage period. A model for colour loss following an exponential law described the effect of temperature and gas composition on the quality of mushrooms. Oxygen concentration has a less pronounced effect on the cap colour of mushroom as compared to CO_2 concentration and temperature. In order to maintain an extended lifetime of colour under controlled atmosphere, mushrooms should be stored at < 6°C. At higher temperatures, CO_2 concentration must be maintained at the highest level as compared with the control.

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1 INTRODUCTION

Mushrooms are highly perishable crops (Peppelenbos *et al.*, 1993), hence there is a need to increase their shelf-life if they are to be presented to the consumer as fresh produce. After harvest, they are prone to spoilage because of respiration, biochemical changes and microbial activity, which depend on ambient conditions. Quality change occurs and leads to breaking of veil, expansion of cap, elongation of stem, shriveling and darkening of gills, which make mushrooms unattractive to consumers and lower the value of the commodity in the market. Excessively high relative humidity causes unattractive elongation of stem and a shiny surface unless the temperature is low. Slow handling of mushrooms at high temperature results in brown discoloration of cap and wilting of entire structure (Sawant *et al.*, 1998).

Controlled atmosphere storage is a technology to extend the shelf-life of fresh agricultural and horticultural produce. This technology involves storing a fruit or vegetable in a modified atmosphere usually consisting of reduced O_2 and elevated CO_2 concentration compared to air (Kader, 1986). Kinetic theory suggests that the deterioration of product quality can occur due to fermentation which can be set off by low oxygen and high carbon dioxide conditions. One way of reducing quality loss is by altering the atmosphere under which the mushrooms are stored. This means the creation of a controlled atmosphere or modified atmosphere packaging. The concentrations of oxygen and (or) carbon dioxide in normal air can be altered and maintained at the new levels in order to increase the shelf-life of the product. However, there is little consensus in literature on the optimum controlled atmosphere conditions to increase the shelf-life of mushrooms. Sveine *et al*. (1967) investigating the storage life of mushrooms reported that high CO_2, low O_2 and low temperature prevented cap opening. Murr and Morris (1975) reported that browning was slowed down due to tyrosinase inhibition by the high CO_2 concentrations. Lopez-Briones *et al.* (1992) suggested that storage atmospheres should contain 2.5% to 5% CO_2 and 5% to 10% O_2. For measuring the effect of O_2 and CO_2 concentrations on the quality of mushrooms, colour is one of the most important parameters (Mac Canna and Gormley, 1968). The most common measure for evaluating the development stage is the percentage of mushrooms with opened caps. Because consumers require mushrooms at the button stage or at least with a stretched veil, loss of whiteness should be minimal. Moreover, consumers also take into accountthe weight of the mushrooms. When the mushrooms lose water, not only do they lose weight, but they also shrivel. So, the percentage of weight loss was also taken into account.

In the present study, the effects of O_2 and CO_2 concentrations giving rise to an extended storage life at three different temperatures (1°C, 6°C and 12°C) evaluated. The objective of this study was to evaluate weight loss data and the modelling of cap colour changes, in relation to O_2/CO_2 concentrations and storage temperature of fresh mushrooms.

2 MATERIALS AND METHODS

2.1 Sample

Cultivated mushrooms (*Agaricus bisporus*) were bought from a local grower in Leuven, Belgium as fresh mushrooms. The mushrooms were sorted for sizes and appearance in a cool room at 1°C ± 0.5°C before further analysis. Diseased, damaged, open veiled and extremely large (cap diameter > 40mm) or small mushrooms (cap diameter < 25mm) were discarded.

2.2 Experimental design and procedure

In order to study the effect of O_2 and CO_2 concentration at different storage temperatures, 15 mushrooms (equivalent to 500g) were placed into each jar and weighed; the weight of the mushrooms was recorded before each jar was tightly sealed. Following, the jars were connected to one of the gas combinations with different concentrations of O_2 (%), CO_2 (%); the remainder of the gaseous mixture (%) consisted of N_2. An overview of all the O_2-CO_2 combinations used is given in Table 1. A given controlled atmosphere was established by flushing the jars with a given gas combination as described by Que *et al.* (2015). Each combination was used used for experilentsat three different temperatures: 1°C ± 0.5°C, 6°C ± 0.5°C and 12°C ± 0.5°C.

Table 1: The different gas combinations used in the experiment

Nature of gas	Gas combination (%)							
O_2	21	12	3	16.5	12	21	3	21
CO_2	0	0	0	3	6	6	12	12

2.3 Measurement of quality characteristics

The measurements extended over a period of 9 days, whereby measurements were performed at day 0 and subsequently after 2, 4, 7 and 9 days of storage. For each gas combination, the colour change and weight loss of the mushrooms were estimated.

2.3.1 Weight loss

The weight loss during storage was determined by monitoring the weight of the content of the jars before and after storage for each gas mixture, temperature and storage period. The weight loss was expressed as the percentage of the initial weight at day 0. The results were averaged.

2.3.2 Colour

The quality of mushrooms during storage was assessed by measurement of the cap colour using a Minolta colorimeter CR-300 (KONICA Minolta Inc., Japan). Sixty fresh mushrooms were randomly taken to evaluate the cap colour on day 0. The colour measurement was done at a random location on the cap of each mushroom. These mushrooms were then used, at random, to fill the jars and this for each gas combination and for each of the chosen storage temperatures. Two measurements were performed at random locations on the cap of each mushroom, on 15mushrooms from each jar, and on two jars from each gas combination so that 60 observations of each gas combination were collected on any given day. The results were averaged.

2.3.3 Colour model development

A general colour model was estimated by fitting the model to the experimental data by non-linear regression using the following model structure.

$$\frac{dX_i}{dt} = -k_i X_i \quad \text{with } X_i\ (t=0) = X_{io} \qquad (1)$$

The equation for a first order degradation process (Equation 1) was integrated for constant temperature conditions (constant k).

$$X_i = X_{io}\exp(-k_i t) \qquad (2)$$

In this equation, the output variable X denotes the measured colour (i = L, a, b), X_{io}, is the colour of the initial mushrooms, k_i, the apparent rate constant and, t, time of storage.

The temperature dependency of the apparent rate constant, k_i, was modelled by an Arrhenius type model (Arrhenius, 1889).

$$k_i = k_i^{ref} \exp\left[-\frac{Ea_i}{R}\left(\frac{1}{T} - \frac{1}{T_i^{ref}}\right)\right] \text{with i = L, a, b} \quad (3)$$

In this equation, k_i^{ref}, is the rate constant at a predefined reference temperature T_i^{ref}, Ea_i, is the apparent activation energy, R, the universal gas constant and T, the absolute temperature.

The apparent rate constants, k_i, according to the Arrhenius' model for colour measurements were estimated. However, the rate constants k_i are not temperature dependent at each O_2/CO_2 combination, but k_i depend on O_2 and CO_2 concentration and on temperature

$$k_i = f(O_2, CO_2, T) \qquad (4)$$

The dependency of the apparent rate constant, k_i, on O_2, CO_2 and temperature is modelled as follows:

$$k_i = \beta_{io} + \beta_{i1}*O_2 + \beta_{i2}*CO_2 + \beta_{i3}*T + \beta_{i12}*O_2*CO_2 + \beta_{i13}*O_2*T + \beta_{i23}*CO_2*T \qquad (5)$$

The coefficient β_{i12}, β_{i13}, and β_{i23} are interaction-effect coefficients for interactions between pairs of independent variables.

2.4 Statistical analysis

Experimental data on weight loss were expressed by mean and standard error. The mathematical models will be developed to describe the transient course of quality change under controlled atmosphere during storage time. The model procedure was developed using Statistical Analysis Systems, version 6.11 (SAS Institute, Inc., Cary, NC, USA) and Matlab software (The Mathworks Inc., Natick, Massachusetts).

3 RESULTS

3.1 Effects of gas combination and storage temperature on the weight loss of the mushrooms

The effect of eight different gas combinations on the weight loss of fresh mushrooms at three different temperatures for 2, 4, 7 and 9 days storageis shown in Figure 1.

From the comparison between temperatures, all gas combinations showed that the weight loss of fresh mushrooms increased with the increase in temperature and storage period. The mushrooms stored at temperature of 6°C exhibited a weight loss of 1.3% at the end of 9 days of storage. Mushrooms subjected to temperature of 12°C exhibited a more severe weight loss (about 3.6%) at the end of the storage period. The weight loss at temperature of 1°C was far much lower than that at 6°C and 12°C.

At a storage temperature of 1°C, no significant difference between all gas combinations during the nine-day period was observed. Storage at 6°C and 12°C, with the gas combinations of 21%O_2 + 0%CO_2, 3%O_2 + 0%CO_2 and 12%O_2 + 6%CO_2 resulted in a higher weight loss as compared to the other gas combinations. The gas combination of 3% O_2 + 12% CO_2 gave the best results in that it had the least weight loss, but no significant difference with that of 21%O_2 + 12%CO_2 was noticeable. The weight loss seemed to decrease with increasing CO_2 concentration.

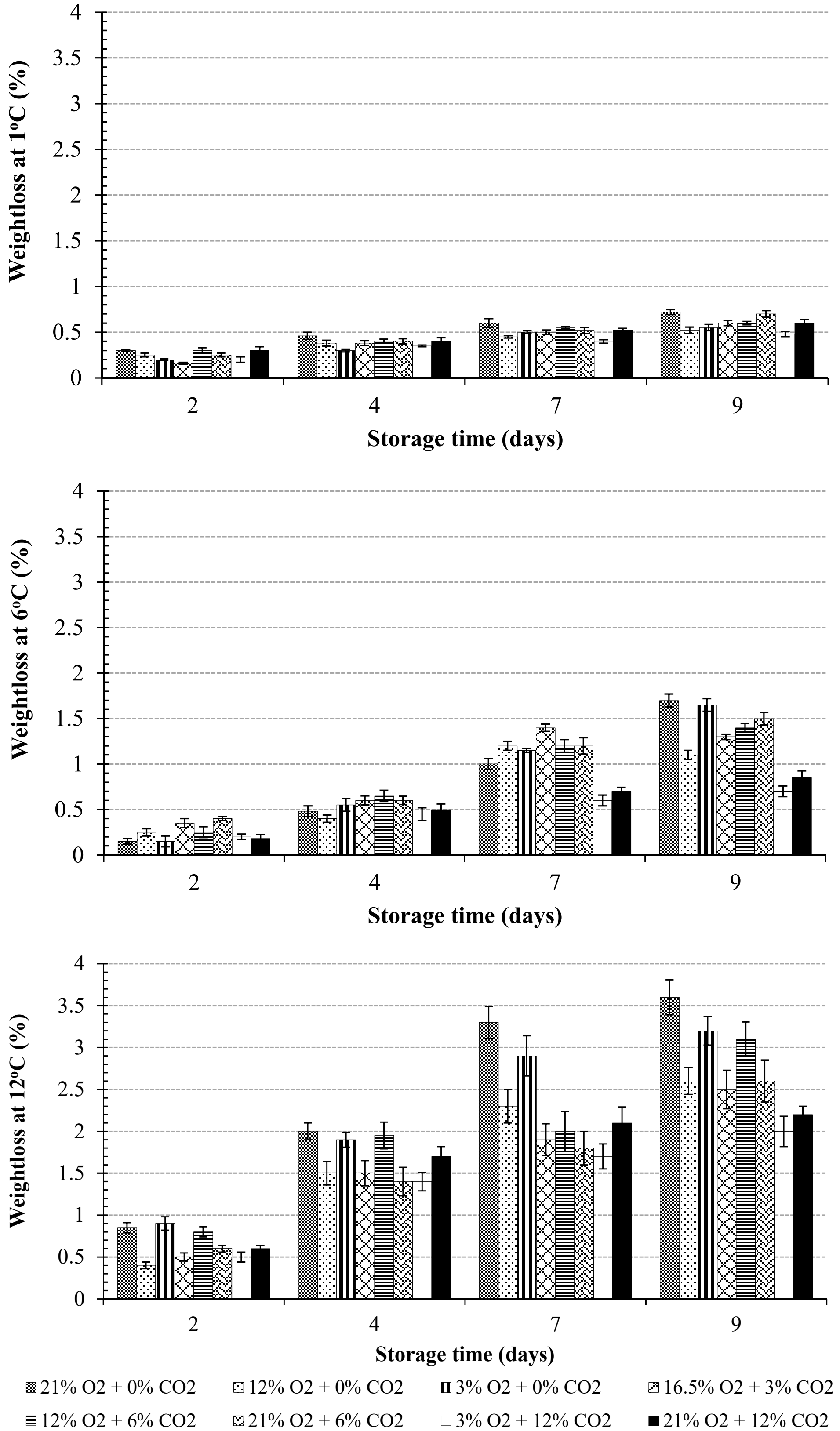


Fig. 1: Weight loss expressed as % of fresh mushroom's weight at three different temperatures

3.2 Effect of gas combinations and storage temperatures on the colour of the mushroom cap

The significant effects of controlled atmosphere conditions and storage temperatures on the colour were described using model Equation [2] and [5]. These effects were non-linear as indicated by the significant interaction between CO_2 concentration and storage temperature. The numerical estimates of the model parameters with their 95% confidence intervals for each of the three measured colour properties are given in Table 2, 3 and 4. In Figure 2, 3 and 4 the experimental data of each O_2 and CO_2 gas concentration, expressed as L*, a* and b* space colour, were plotted against storage time together with the simulated model values. The values plotted were the average of 180 measurements for gas combinations of 21%O_2+0%CO_2, 12%O_2+6%CO_2; 120 measurements for gas combinations of 3%O_2+0%CO_2, 21%O_2+12%CO_2, 3%O_2+12%CO_2 and 60 measurements for gas combinations of 12%O_2 + 0%CO_2, 16.5%O_2 + 3%CO_2 and 21%O_2+6%CO_2.

As some parts of the foregoing experimental work dealt with L* value (whiteness) changes under given gaseous atmospheres and since the mathematical model will also be extensively used in subsequent studies, a model parameter of whiteness and experimental data are presented here. Figure 2 showed the fitted model together with the experimental data of the measured L* value against storage time. The model was calculated for the three different temperatures at each gas concentration. The lines on the graph show the results of the model calculations. From the figures, it can be deduced that the model slightly underestimated the experimental data for the temperature of 12°C and overestimates for temperature of 1°C. Despite these small inadequacies, the model was still able to describe the experimental data at constant storage temperature.

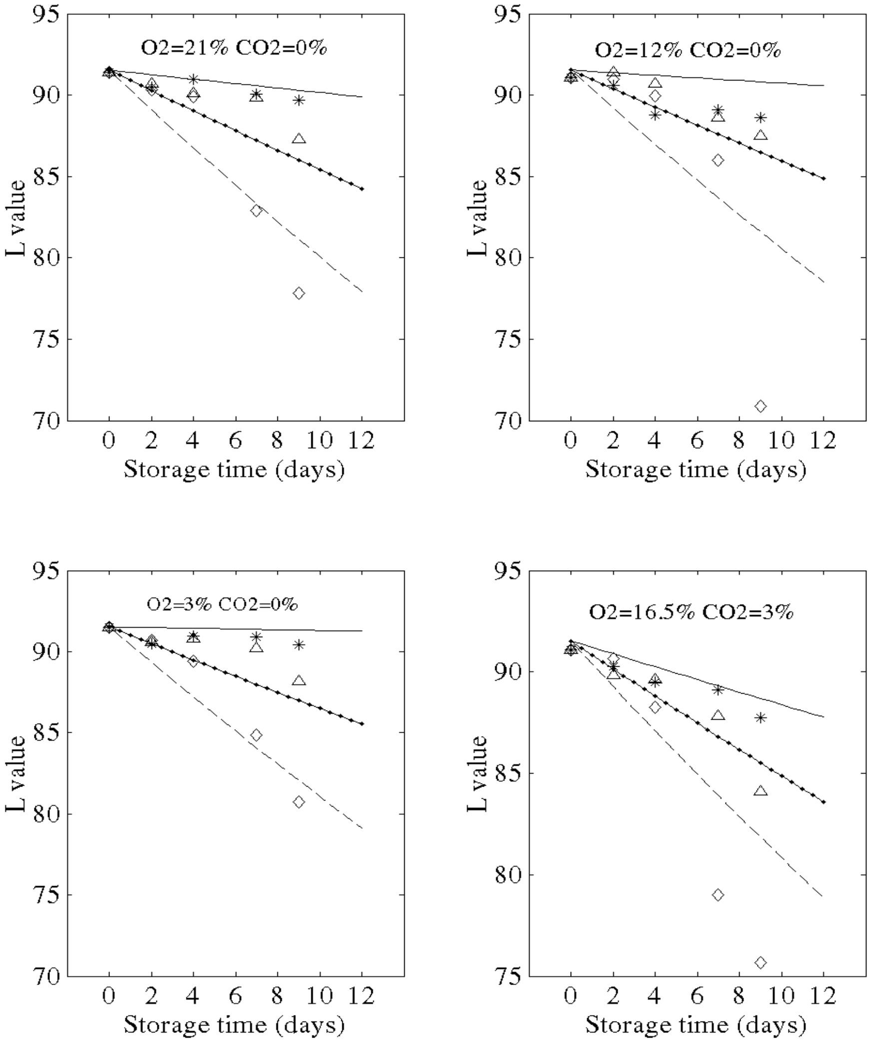
Table 2: Estimate for the model parameters whiteness (L*) of mushrooms (model Equation (2) and (5))

Explanatory variables	Model parameters	Estimates	95% Confidence intervals	
Initial L value	L_o	91.529	91.389	91.669
Intercept of k	β_{Lo}	-0.00104	-0.00162	-0.00046
Oxygen (%)	β_{L1}	0.00007	0.00005	0.00009
Carbon dioxide (%)	β_{L2}	0.00086	0.00080	0.00092
Temperature (°C)	β_{L3}	0.00108	0.00102	0.00113
Carbon dioxide*Temperature	β_{L23}	-0.00009	-0.00010	-0.00008

The result of this study showed no differences in brown discoloration between mushrooms stored at 3% O_2 and those stored at 21% O_2 at any temperature. Therefore, it can be assumed that the effect of O_2 was negligible, and differences in discoloration were probably due to the differences in CO_2 concentration.

In the case of low CO_2 concentration (0% or 3%), it was apparent that at the storage temperature of 1°C, brown discoloration was slowed down. At this temperature, whiteness can be kept for more than 9 days, as the model predicted. Discoloration increased with an increase in temperature (1°C, 6°C and 12°C). At 12°C and after 9 days of storage the whiteness of mushrooms decreased rapidly.

In contrast, at high CO_2 concentration, the brown discoloration was strong at the three temperatures (1°C, 6°C and 12°C) used in the experiment.


O2=21% CO2=0%
O2=12% CO2=0%
O2=3% CO2=0%
O2=16.5% CO2=3%
L value
Storage time (days)
95
90
85
80
75
70
0 2 4 6 8 10 12

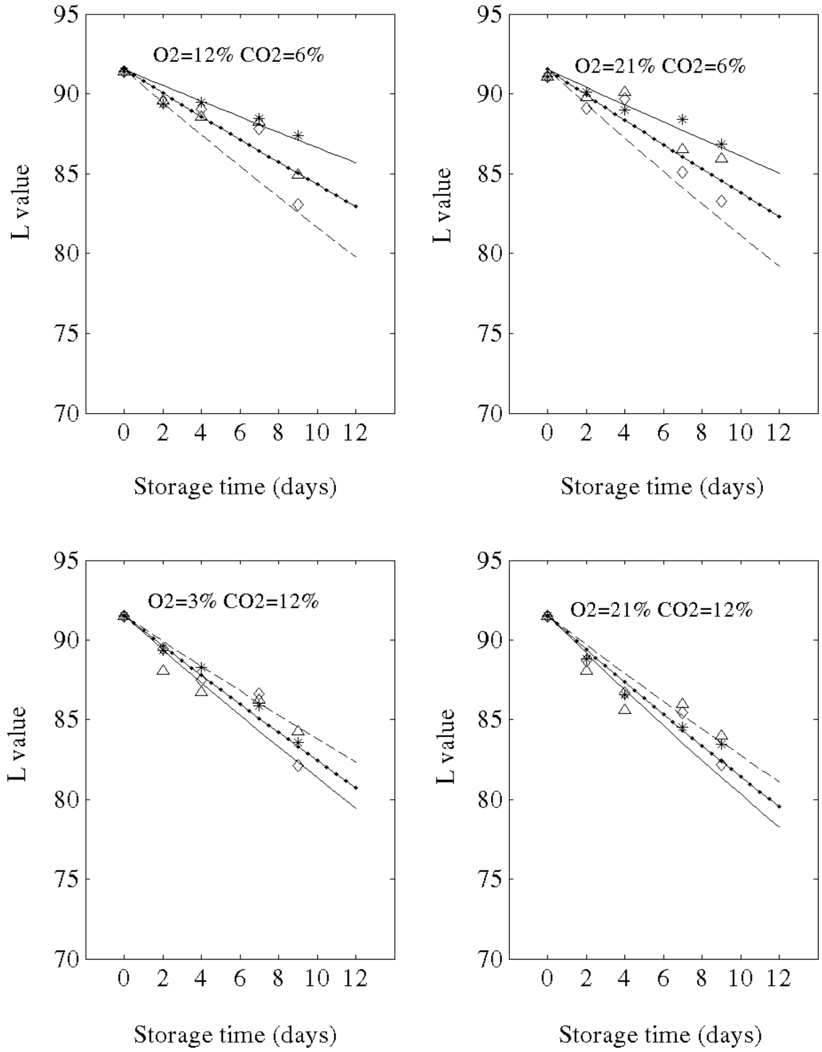


Fig. 2: Colour of mushroom caps, expressed as L value, plotted against storage time at temperatures of 1°C (——*), 6°C (—•—Δ) and 12°C (---- ◊) for the different eight gas combinations

In Figure 3, the experimental data of the a* values together with the fitted model are plotted for the three temperatures and for each of eight gas combinations of O_2 and CO_2. The overall view of the graphs wasthe same for all the gas combinations except for 3% O_2 + 12% CO_2 and 21% O_2 + 12% CO_2. With those controlled atmospheres, the model did not fit the experimental data well.

Table 3: Estimates for the model parameter redness (a*) of mushrooms (model Equation [2] and [5])

Explanatory variables	Model parameters	Estimates	95% Confidence intervals	
Initial a value	a_o	0.209	0.194	0.224
Intercept of k	β_{ao}	0.0694	0.0506	0.0881
Oxygen (%)	β_{a1}	-0.00158	-0.00183	-0.00132
Carbon dioxide (%)	β_{a2}	-0.0287	-0.0301	-0.0273
Temperature (°C)	β_{a3}	-0.0285	-0.0298	-0.0271
Carbon dioxide*Temperature	β_{a23}	0.0031	0.0030	0.0032

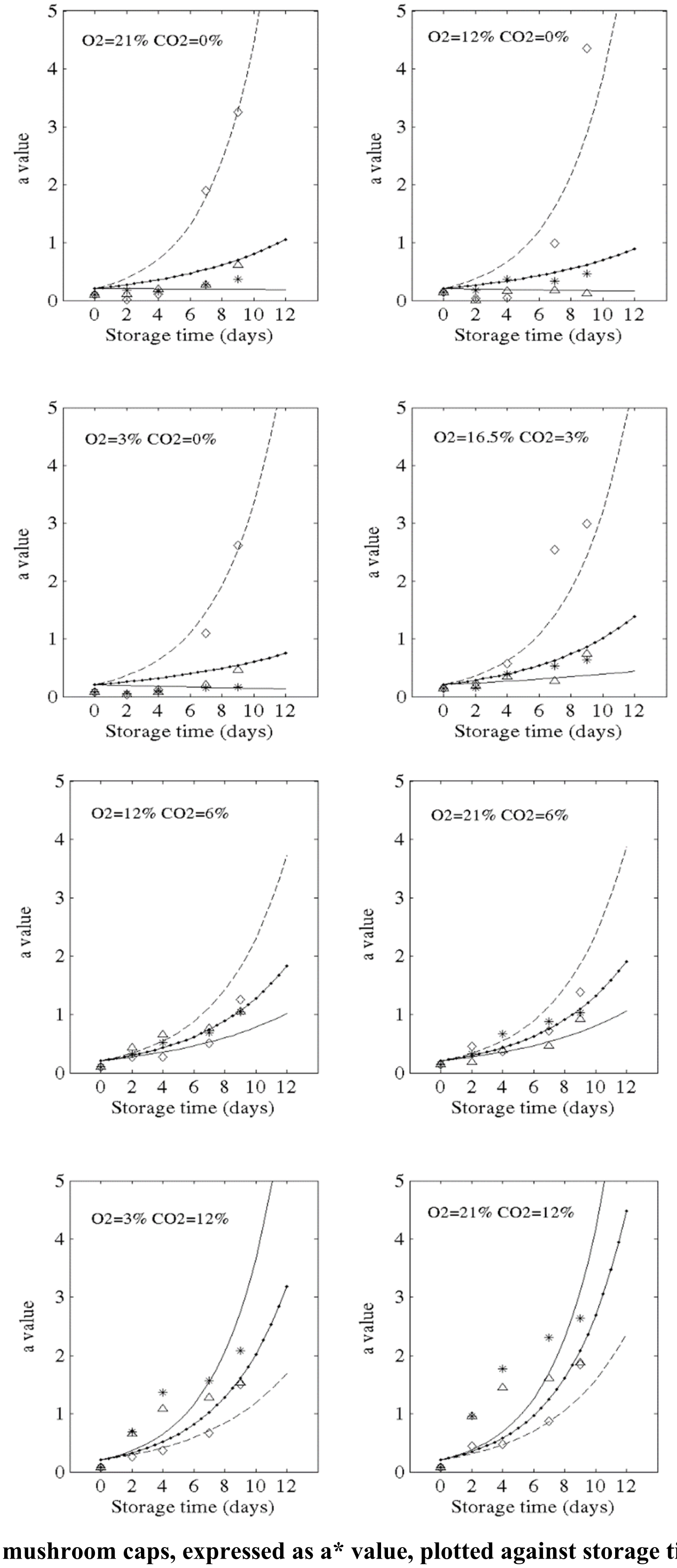


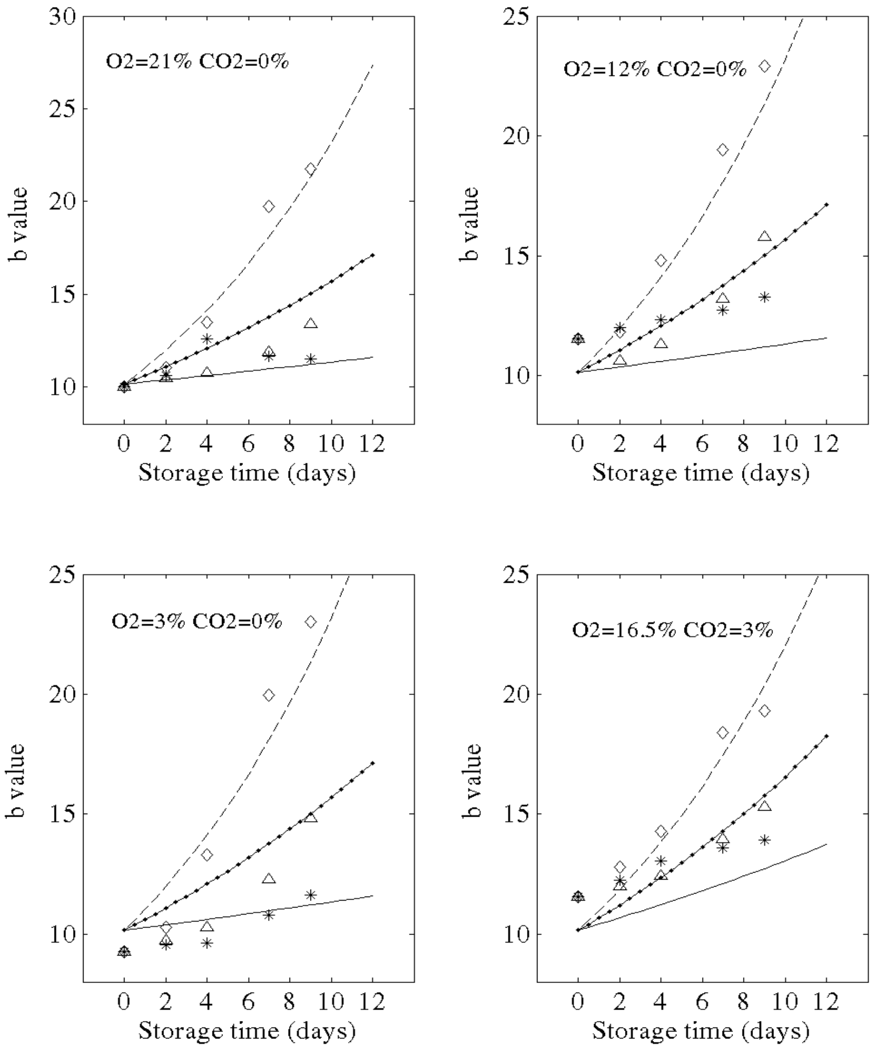
Fig. 3: Colour of mushroom caps, expressed as a* value, plotted against storage time at the temperatures of 1°C (——*), 6°C (—•—Δ) and 12°C (---- ◊) for the eight different gas combinations

The model, using the parameter estimates in Table 4, was used to predict yellowness (b*) changes under varying time-temperature conditions. For each gas combination, three temperatures were set up. At several time intervals during storage, samples were taken and yellowness (b* value) was measured. The experimental values and the model prediction are shown in Figure 4.

As the same colour parameters of mushroom, while L* value was expressed as whiteness. The a* and b* component, which measures the degree of redness and yellowness, increased with decreasing concentrations of CO_2 (12%, 6%, 3% and 0%) at three temperatures as shown in Figure 3 and 4. However at low storage temperature and CO_2 concentration < 6%, the effect of discoloration was negligible. For example, at 1°C, there was no difference in the a* and b* values of mushrooms stored at 0% CO_2 from those stored at 3% CO_2. These results also confirmed that no differences in whiteness were observed between mushrooms stored under 0% CO_2 and those stored under 3% CO_2.

Table 4: Estimate for the model parameters yellowness (b*) of mushrooms (model Equation (2) and (5))

Explanatory variables	Model parameters	Estimates	95% Confidence intervals	
Initial b value	b_o	10.1492	10.0868	10.2116
Intercept of k	β_{bo}	-0.0045	-0.0062	-0.0028
Carbon dioxide (%)	β_{b2}	-0.0053	-0.0055	-0.0051
Temperature (°C)	β_{b3}	-0.0065	-0.0067	-0.0063
Carbon dioxide*Temperature	β_{b23}	0.00058	0.00056	0.00060



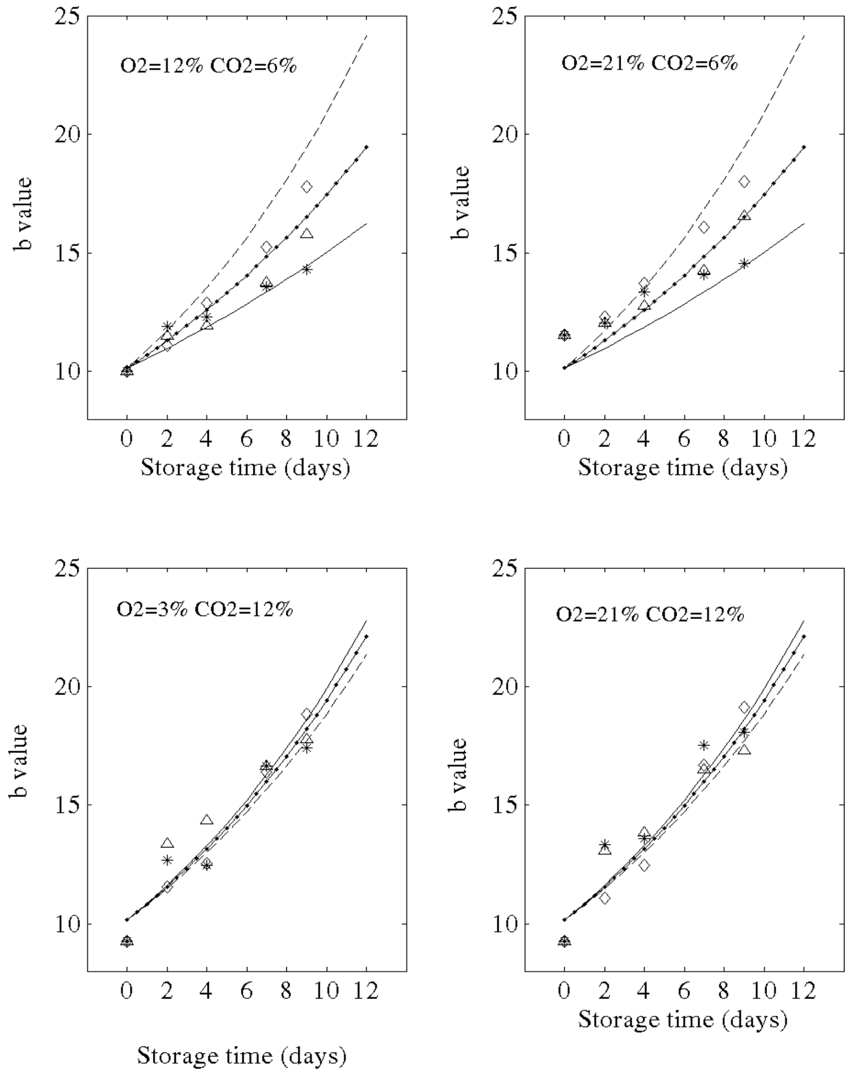


Fig. 4: Colour of mushroom caps, expressed as b* value plotted against storage time at temperatures of 1°C (——*), 6°C (—•— Δ) and 12°C (---- ◊) for the eight different gas combinations

4 DISCUSSIONS

4.1 Weight loss

The results with respect to weight loss (expressed as percentage of the fresh mushrooms' weight) of the present study areshown in Figure 1. After 9 days of storage, the weight loss varied from 0.7% at 1°C to about 3.6% at 12°C. This was lower than the reported weight loss of 5% to 7% after 5 days at 18°C (Nichols and Hammond, 1973). Higher weight losses were due to the higher temperature of storage, which increased water vapor transpiration and respiration rates of the mushrooms.

Irrespective of the presence of O_2 and CO_2 concentrations at different level, mushrooms stored at 1°C showed much less weight loss, especially from the seventh day onwards, as compatred to those stored at 6°C and 12°C. The weight loss is caused by the loss of water from the surface of the mushroom and is affected by temperature differences. These differences give rise to differences in water vapor pressure leading to evaporation and to the loss of carbon as a result of the formation of CO_2 during respiration. Moreover, mushrooms lack a protective epidermal structure to prevent excessive moisture loss, therefore, have high transpiration rates (Mohebbi *et al.*, 2012).

At 12°C, the percentage of weight loss under 12% CO_2 was lower than that of mushrooms under a CO_2 concentration of <6%. However, after 9 days of storage, the percentages of weight loss were similar for mushrooms under different gas combinations (Figure 1). These results indicated that the weight loss due to loss in respiratory carbon was pronounced at high temperature.

From the weight loss results, it can be concluded that, for mushrooms stored at low temperature (1°C), the effect of O_2 and CO_2 content was negligible. High CO_2 concentrations helped to reduce the weight loss over time at higher storage temperatures (6°C and 12°C).

4.2 Colour

The quality of mushrooms is influenced by the composition of the storage atmosphere. The results of this study have shown that O_2 and CO_2 concentrations and the temperature during controlled atmospheres storage have a major impact on the colour. Many factors (e.g. storage temperatures and gas composition) affect the discoloration of mushrooms during storage. A decrease in O_2 concentration and an increase in CO_2 concentration slowed down the discoloration rate as shown in Figures 2, 3 and 4. However, the influence of O_2 on the discoloration rate occurred at a much lower level than that of CO_2. These conclusions confirm the results of Lopez-Briones *et al.* (1992) on brown discoloration of mushroom. Increased CO_2 concentration has the benefit of inhibiting the growth of microorganisms. This is valid, up to a point, but when the mushroom tissue begins to degrade due to CO_2 toxicity, the resistance of the mushrooms to infection is compromised. This may be one of the reasons why mushrooms stored at 12°C developed more infections than those stored at 1°C and 6°C. At sufficiently low O_2 concentrations (less 2%), spores of *Clostridium sp*. may also germinate (Sugiyama and Yang, 1975). Furthermore, under near anaerobic conditions, enterotoxigenic *S.aureus* can grow and produce enterotoxin. Exama *et al.* (1993) also reported that biological reactions tend to increase by a factor of 2 to 3 for each increase in temperature by 10°C.

Higher CO_2 concentrations are detrimental to colour (Lopez-Briones *et al.,* 1992). Unpleasant colour changes in mushrooms occur due to unsuitable concentrations of this gas, i.e. more than 6% CO_2, irrespective of the O_2 concentration. Storage of mushrooms in high CO_2 atmospheres causes an increase in enzymatic browning by exposure of both phenolic substrates and O_2 to polyphenol oxidase (Lopez-Briones *et al.,* 1993; Ares *et al.*, 2007). The resulting polyphenols then polymerize to form brown pigments. The excessive accumulation of CO_2 (>12%) can also cause physiological injuries to mushrooms resulting in severe browning (Nichols and Hammond, 1973; Lopez-Briones *et al.*, 1992). So, there exist limits in the CO_2 content that should not be exceeded. These limits are, however, dependent on the temperature of storage.

Combining the experimental results, the model predictions and the data from literature leads to the conclusion that, with respect to colour, the optimal concentration for CO_2 lies between 0 and 6% irrespective of the O_2 concentration. These values are dependent ontemperature. Mushrooms can be stored in normal atmosphere for at least 9 days at 1°C and 6°C while the gas combination of 12% O_2+ 6% CO_2 is the best condition for mushrooms stored at 12°C.

In order to know the exact concentrations necessary for optimal storage conditions, the storage time expressed as shelf-life was calculated on the basis of Equation 1 (Que *et al.*, 2015). The shelf-life of mushrooms was different under different storage conditions. For example, at temperatures < 6°C and 21% O_2 + 0% CO_2 (air) mushrooms were marketable for about 9 to 15 days, but at 12°C for the same controlled atmosphere conditions, shelf-life reduced to less than 3 days. The limit of shelf-life was derived from an acceptability test by consumers. Acceptability was fixed at a value of 0.5. A value above 0.5 was considered as an acceptable sample (Que *et al.*, 2015). From there, the L*, a* and b* values can be calculated by using Equation 2 and the models in Table 2, 3 and 4 show the limits of acceptable colour level for mushrooms.

Lopez-Briones *et al.* (1992) reported that mushroom with L* values > 80 would be acceptable to wholesales. Those with L*values < 70 would be rejected by consumers. As indicated by the data in Table 5, our findings show that the acceptability, based on L*, a* and b* values, is dependent on controlled atmosphere conditions and storage temperature. From the data, it appears that the consumers would accept mushrooms with L* value > 85, a* value < 1.2 and b* value < 16.

Table 5: Estimate of acceptability for colour level by consumers with probability 0.5

O_2 (%)	CO_2 (%)	Temperature (°C)	Model prediction			
			Shelf-life	L*	a*	b*
21	0	1	14.9	89.49	0.19	11.96
		6	9.3	85.79	0.74	15.25
		12	2.7	88.29	0.48	12.67
12	0	1	15.8	90.26	0.15	12.08
		6	10.3	85.82	0.72	15.86
		12	3.6	87.44	0.60	13.64
3	0	1	16.7	91.15	0.11	12.20
		6	11.2	85.94	0.68	16.49
		12	4.5	86.69	0.72	14.69
16.5	0	1	15.4	89.86	0.17	12.02
		6	9.8	85.79	0.73	15.55
		12	3.1	87.85	0.53	13.15
12	6	1	14.7	84.40	1.46	18.12
		6	10.5	84.00	1.39	17.95
		12	5.3	86.10	0.76	14.96
21	6	1	13.1	84.49	1.40	16.95
		6	9.7	83.98	1.40	17.25
		12	5.8	85.37	0.91	15.43
3	12	1	16.2	78.51	3.75	25.31
		6	11.3	82.25	2.71	21.18
		12	5.4	86.99	0.54	14.19
21	12	1	11.2	81.17	5.94	21.61
		6	10.1	82.09	2.79	19.66
		12	8.9	83.22	1.27	17.66
Average acceptability				85.70	1.26	16.08

5 CONCLUSIONS

Using different gas combinations at different storage temperatures results in differences not only in colour (L^*, a^*, b^* values) but also in weight loss. However, under these conditions the different quality parameters of mushrooms do not always seem to move along the same line. Hence, it is difficult to draw overall conclusions. In the present study, O_2 concentration had less effect on the quality than CO_2 concentration and temperature. High CO_2 concentrations caused an increase in enzymatic browning and tissue injury. Temperature was the variable with the most pronounced influence on the colour and the weight loss. The effect of gas composition was found to increase with an increase intemperature. Higher temperatures accelerated brown discoloration and weight loss and decreased consumer acceptability. This stresses the importance of refrigeration and suggests that the use of controlled atmosphere was more important when mushrooms were handled at temperatures above optimum temperature. Colour is an important quality factor for all consumers. The colour of mushrooms was modelled on the basis of an exponential law model taking into account storage temperature and gas composition. It can be concluded that in order to prolong shelf-life under controlled atmospheres, mushrooms should be stored at temperature < 6°C. At 12°C, gas combination of 12% O_2 + 6% CO_2 provided the best conditions with the shelf-life of less than 7 days.

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