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Effects of Storage Environmental Conditions on Weight Loss, Whiteness Change, and Microbial Activity of Mushrooms (*Agaricus bisporus*)

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The effects of storage temperature and high relative humidity (RH) on the weight loss, color change, and microbial activity of a mushroom ('Sylvan' hybrid white) were investigated. The experiment was performed at three temperature (5, 10 and 15°C) and four different relative humidity levels (91, 94, 97 and 99%). The weight loss of the tested samples had a highly correlated linear relationship with storage time at each RH level during storage. Both the storage temperature and RH levels in the experiment had signigicant effects ($p<0.05$) on the weight-loss rate of the tested samples. The loss of whiteness of mushrooms was not significantly affected ($p>0.05$) by RH ranges at the same temperature. No visible damage was caused by either bacteria or fungi in all samples during storage.

Key words: *mushroom, storage, refrigeration, high relative humidity.*

Upon harvesting, fruits and vegetables are deprived of their sources of water, minerals, and simple organic molecules which normally would be supplied to them from other parts of the plants. Since losses of respirable substances and water are not replaced, deterioration begins.[1] Several biological factors directly affect the postharvest changes of fruits and vegetables. These factors include maturity, transpiration, respiration, and pathogenic fungi and bacteria. The length of storage is also a function of environmental factors such as temperature, relative humidity, and various gases.[2-4]

Weight loss and quality deterioration of fruits and vegetables during refrigerated storage are a major function of moisture loss through transpiration. The types of surfaces and underlying tissues of fruits and vegetables have a marked effect on the rate of water loss. Different commodities loose moisture at different rates when held under the same environmental condition.[5]

Among environmental factors affecting postharvest changes of fruits and vegetables, temperature and relative humidity may be the most important factors. However, little information is available on the effects of temperature and high relative humidity (above 90%) on postharvest changes of fruits and vegetables during refrigerated storage.[6] High relative humidity(RH) near saturation is effective in reducing weight loss. However, high RH causes the condensation at the surface of the commodities and may increase the deterioration rate.

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Abbreviations: Aw, water activity; CFU, colony forming unit; Ea, activation energy; RH, relative humidity; SD, standard deviation.

A knowledge of the weight loss of stored produce and an understanding of the effects of such factors affecting the postharvest changes of the commodities should be useful in designing more efficient storage systems.

This study was performed to elucidate the effects of high RH levels on the quality parameters such as weight loss, microbial contamination, and browning of the mushrooms which have high transpiration rates among the vegetables, during refrigerated storage.

Materials and Methods

Weight loss, whiteness loss, and microbial activity of mushroom were investigated at three temperatures (5, 10, and 15°C), with four different RH levels (91, 94, 97, and 99%) at each temperature.

Experimental setup. Air-tight containers were specifically designed and fabricated in the laboratory using plexiglass. Two liters of distilled water or solutions of certified NaCl for biological work (Fisher Scientific, NJ, U.S.A.) was placed at the bottom of each container to assist in maintaining a desired RH. A one-way valve was used for ventilation to prevent a counter stream of air from entering from the outside of the container. Four storage containers were placed in an environmental chamber (Model C10632H-6M, Lunaire Environmental Inc., PA, U.S.A.) in which the temperature was controlled at test levels. To achieve the desired RH in the storage containers, distilled water and sodium chloride solutions of different concentrations were used. For 99% RH, distilled water was used. For 97, 94, and 91% RH, 5% (for 5°C test, 5.5%), 9.5 (for 5°C test, 10%), and 14% (for 5°C test, 15%) solutions of sodium chloride were used, respectively.[7] Dry, compressed

air was first humidified by passing through distilled water, and this pre-humidified air was then divided to four streams. These separated air streams were bubbled through three containers with different salt solutions and one container with distilled water (for 100% RH). Rate of air flow through each chamber was controlled by separate air flow meters (model FM-1100-VI, Matheson Instruments inc., PA, U.S.A.). The flow rate was 27.8 L/min of standard air through each chamber. Temperature of each chamber was recorded at every 15 min using copper-constantan thermocouple (diameter, 0.127 mm; gauge 30, Omega Engineering Inc., CT, U.S.A.) and KAYE Digistrip III data logger (Kaye Instruments Inc., PA, U.S.A.). RH of each chamber was recorded using Dew Point Hygrometer (Model 1200APS, General Eastern Instruments Co., MA, U.S.A.) and KAYE Digistrip III data logger.

Sample preparation. A white strain ('Sylvan' hybrid white) of the cultivated mushrooms (*Agaricus bisporus*), obtained from the Mushroom Test and Demonstration Facility of the Pennsylvania State University was used in this study. Mushrooms were brought to the laboratory on the day of harvest and carefully brushed to remove excess dirt. For each temperature, 24 mushrooms were used for moisture loss and color change studies, and 16 (160 g) lots were prepared for microbial count. Mushrooms were divided into four subgroups, and each subgroup was placed in each storage chamber. Plastic weighing boats were used for handling the mushrooms to avoid damage. Each experiment was performed for 8 days. The individual mushrooms were weighed daily, and the color measurement and microbial test were performed every other day. Carbon dioxide was added to the air stream and the concentration of carbon dioxide was kept at 1%.

Moisture loss. The mushroom samples were weighed to the nearest 0.001 g using Mettler PE 360 balance (Mettler Instruments Co., Hightstown, U.S.A.). To minimize moisture loss during the weighing procedure, all the samples were kept in a desiccator containing distilled water while samples were weighed.

Microbial count. One 150-g mushroom lot from each chamber was removed and weighed directly into a sterilized Waring blender jar. An equal amount of sterile 0.1% peptone water was added and blended for 2 min into a homogeneous slurry. Each 20 ml of the homogenate was transferred to 80 ml of 0.1% sterile peptone water in a dilution bottle, resulting in a ten to one dilution. Further decimal dilutions were prepared using 11 ml transfers, and 1 ml of each dilution was plated onto petridishes in duplicate.

Media. For total aerobic count, Plate Count Agar (Difco, Detroit, U.S.A.) was used. For yeast and mold count, Potato Dextrose Agar (Difco, Detroit, U.S.A.) with chloramphenicol and chlorotetracycline (Sigma Chemical Co., St. Louis, U.S.A.) at 100 ppm concentration was used.

Incubation and count. For total aerobic counts, the plates were incubated for two days at 35°C before counting. For yeast and mold count, the plates were incubated for five days at 25°C and then counted.

Color measurement. A Gardner Digital Colorimeter (model XL-10, Gardner Laboratory Inc., MD, U.S.A.) was used for measuring the whiteness of the mushrooms. The 'L' value of the cap was measured.

Data analysis. The mean differences of weight and color changes of the samples that occurred during storage were examined using Duncan's multiple-range test. The tests for independent regressions for homogeneity were performed to compare the color change and weight loss rates at each RH and temperature.[8] The activation energy of the color change of the samples were obtained from the linear regression equations using regression programs (Minitab, Minitab Co., University Park, U.S.A.).

Results and Discussion

Weight loss. Table 1 shows the rates of weight loss for mushrooms at each RH during storage. At the same temperature, the weight loss increased as the RH was lowered. As the temperature increased, the weight loss of mushrooms at the same RH level also increased. At each temperature, the weight loss rates at different RH levels were all significantly ($p<0.05$) different except between 94 and 91% RH levels at 10 and 5°C. When the weight loss was compared at the same temperature, the largest discrepancy was observed between 91 and 99% at 15°C, and the differences were reduced as the temperature was lowered. The mushrooms stored at 15°C with 91% RH showed the highest weight loss rate (28.60±1.38 mg/cm^2/day), while those stored at 5°C with 99% RH had the lowest weight loss rate (1.34±0.06 mg/cm^2/day). The mushrooms stored at 15°C with 91% RH retained 44.4% of the initial weight, while those stored at 5°C with 99% RH had 96.5% of the initial weight during the 8-day test period. The high net weight loss

Table 1. The rate of weight loss for mushroom at each RH level during storage.

Temperature (°C)	Relative Humidity (%)			
	99[1]	97	94	91
15	4.50±0.10[2] xa	15.30±1.16 xb	21.80±1.96 xc	28.60±1.38 xd
10	2.65±0.11 ya	9.61±0.35 yb	17.10±0.74 xc	18.00±0.45 yc
5	1.34±0.06 za	6.02±0.09 zb	11.00±0.44 yc	12.30±0.36 zc

Values are means±SD for 6 samples.

[1]These values represent the expected humidities and may differ from the tested humidities.

[2]Each value represents the rate of weight loss (mg/cm^2/day).

[3]At each temperature, the rates with the same letter (a-d) are not significantly different ($p>0.05$). At each RH level, the rates with the same letter (x-z) are not significantly different ($p>0.05$).

Table 2. The rate of whiteness ('L' value) for mushrooms at each RH level during storage.

Temperature (°C)	Relative Humidity (%)			
	99[1]	97	94	91
15	-3.10±0.59[2]	-3.90±0.24	-3.27±0.13	-3.47±0.03
	xab[3]	xa	xb	xb
10	-1.77±0.19	-1.34±0.20	-1.67±0.17	-1.66±0.10
	ya	ya	ya	ya
5	-1.04±0.12	-1.17±0.27	-1.82±0.19	-1.17±0.08
	za	yab	yb	za

Values are means±SD for 6 samples.
[1]These values represent the expected humidities and may differ from the tested humidities.
[2]Each value represents the rate of loss of whiteness ('L' value/day).
[3]At each temperature, the rates with the same letter (a-d) are not significantly different ($p>0.05$). At each RH level, the rates with the same letter (x-z) are not significantly different ($p>0.05$).

at 15°C with 91% RH indicates the importance of maintaining high humidity in mushroom storage. Both the storage temperature and RH levels in this experiment had significant effects on the weight loss of the tested mushrooms.

Loss of whiteness. The rate of loss of whiteness (the 'L' value) for mushroom at each test condition during storage is presented in Table 2, along with t-test results for the homogeneity of the independent regressions. The rate of loss of whiteness of the stored mushrooms showed highly correlated linear relationship with storage time at each temperature. At 15°C, the rate of loss of whiteness was significantly ($p<0.05$) higher than those at other temperatures. There was no significant ($p>0.05$) difference between the rates of loss of whiteness for the mushrooms stored at 10 and 5°C. At the same RH level, the rate of loss of whiteness became higher as the temperature increased. The rate of loss of whiteness of the mushrooms were not significantly affected by different RH levels at the same temperature. However, they became higher as the temperature increased. The reaction constant (K) were 0.016/day, 0.021/day and 0.048/day at the temperature of 5, 10, and 15°C, respectively. These data show that the reaction is apparent first-order in loss of whiteness of mushrooms. The activation energy (Ea) for the loss of whiteness of mushrooms, calculated from the slope of the Arrhenius plot (reaction constant vs absolute temperature), was 80.02 kJ/mol. The obtained Ea fell within the range of Ea values (40-120 kJ/mol) for color, flavor, and texture changes suggested by Saguy and Karel.[9]

Microbial activity. Neither bacteria nor fungi caused visible damages to the samples during storage. This may be in part due to the low initial population of the microbes in the samples. At the same temperature, different RH levels had no significant effect on microbial population, therefore, the data were grouped for each test temperature and further analyzed. The microbial counts for mushrooms are shown in Table 3. Bacterial counts at 15 and 10°C and yeast and mold counts at 15°C showed increasing trends. However, bacterial counts at 5°C and yeast and mold counts at 10 and 5°C did not increase. While the bacterial counts at 5°C was a minimum at day 4, the yeast and mold counts showed a maximum at day 4. This might be due to the competitive relationship between the two groups, which may result in a negative association where the better adapted microbial species predominate or eliminate the other species.[10] It has been reported that the major bacteria in the fresh mushrooms were fluorescent pseudomonads (54%) followed by flavobacteria (10%).[11] These bacteria have optimum growth temperature above 15°C. Thus, at 5°C, the growth of these bacteria might be slow, while that of fungi was not affected as much due to their relatively high tolerance to lower temperature. In mushrooms, the bacterial population was much higher than the yeast and mold populations throughout the storage period.

Pseudomonas species, the major bacteria found in mushrooms, do not grow below the water activity (Aw) of 0.95. Fresh fruits and vegetables generally have Aw of above 0.98. The RH in this study was maintained above 90%. It is expected, since severe dehydration did not occur, the micro-environment within all samples remained above the Aw of 0.95 throughout the experiment. This may be why there was no noticeable difference among microbial counts at different RH levels.

Table 3. Microbial counts for mushrooms during storage.

Temperature (°C)	Day		
	0	4	8
Bacterial count			
5	6.68±0.00[1]	4.94±1.20	7.56±0.23
	xyb[2]	xa	xb
10	6.50±0.00	7.94±0.30	7.97±0.10
	xa	yb	xyb
15	6.76±0.00	7.42±0.22	8.41±0.59
	ya	xya	yb
Yeast and Mold count			
5	2.30±0.00	2.78±0.40	2.30±0.16
	ya	xyb	xa
10	2.20±0.00	1.95±0.37	2.14±0.23
	xa	xa	xa
15	2.27±0.00	3.28±0.24	4.09±0.67
	xya	yb	yc

[1]Each value[(log CFU/g)±SD] represents the common logarithm of the average of duplicates at four different RHs.
[2]At each temperature, the counts with the same letter (a-c) are not significantly different ($p>0.05$). At each RH level, the counts with the same letter (x, y) are not significantly different ($p>0.05$).

In conclusion, weight losses of mushrooms were affected by both temperature and RH levels, while whiteness loss and microbial growth were affected only by temperature in this experiment. The results indicate that the use of clean mushrooms with low initial microbial counts and environment with high RH without condensation can be effective in keeping the quality and reducing the spoilage of stored mushrooms under refrigerated condition.

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