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Effect of Storage Atmosphere on Postharvest Growth of Mushrooms[1]

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Abstract. Controlled atmosphere (CA) storage prolonged the shelf life of mushrooms (*Agaricus bisporus*, [Lange] Sing.) if the O_2 concentration was 9% or the CO_2 concentration was 25 or 50%. Concentrations of 2 to 10% O_2 stimulated pileus expansion and stipe elongation with maximal stimulation of growth occurring at 5% O_2. Levels of CO_2 above 5% markedly inhibited growth, even after air was substituted for the CO_2 treatment. Five percent CO_2 stimulated stipe elongation and suppressed pileus expansion. Protein degradation, as indicated by protease activity and the level of α-amino N in the tissue, increased during postharvest maturation of mushrooms. As in starving bacteria, it is suggested that the main physiological function of proteolysis in the postharvest maturation of mushrooms is as a source of C and N.

Several techniques have been introduced which effectively delay the deterioration of harvested fruits and vegetables. Among these techniques controlled atmospheres (CA), involving a modification of storage atm by lowering the oxygen (O_2) and/or increasing the carbon dioxide (CO_2) with strict temp control, have shown the most promise. The retarding effect of low O_2 and increased CO_2 on mushroom respiration and deterioration was first recognized in the 1930's (10, 17). More recent work (21) indicated that low O_2, increased CO_2, and low temp, separately, prevented opening of mushroom caps. Subsequently it has been shown that gas permeable plastic films could increase the storage life of mushrooms (1, 20). Analysis of the internal atm indicated that those films which permitted an accumulation of about 10% CO_2 and a depletion of O_2 to about 2% yielded mushrooms of the highest quality and with the fewest caps open.

This study presents quantitative data on the effects of O_2 and CO_2 on the maturation (primarily growth of stem and cap) of harvested mushrooms. It has been reported that protein degradation is relatively slow in growing bacteria, but much faster when there is no source of C or N (15, 16, 22). Since maturation of the carpophore continues after harvest under conditions essentially analogous to starving bacteria, the changes in rates of proteolysis under CA are also presented in relation to postharvest maturation of mushrooms.

Materials and Methods

General. Freshly harvested mushrooms (tan strain) were purchased from Alpine Mushroom Culture, Morgan Hill, CA. The harvested mushrooms (first flush, medium size, U.S. No. 1) were placed at 0°C overnight and distributed to the various treatments the next morning.

The mushrooms were sorted by size and freedom from blemishes. Extremely small or large mushrooms were discarded and the mean pileus (cap) diameter of those selected was 3.6 cm (S.D. = ± 0.3 cm). To accurately measure postharvest stipe (stem) growth, the stipes were recut to a uniform length of 2.4 cm. Identically matched mushrooms were then distributed 1 to each sample until the sample size reached 15 mushrooms. Each sample was weighed, placed in a 10-L respiration jar and stored in the dark at 10°C (±0.5°), at which temp mushrooms mature at a rate neither too slow nor too fast for reasonable observation (19).

CA storage. To determine the effects of low O_2 or elevated CO_2 on maturation after harvest, mushrooms were stored under various atm with high humidity (ca. 90%). The treatments consisted of O_2 atm of 0, 2, 5, 10, 21 and 50%, or CO_2 atm of 0, 5, 25, and 50%.

The low O_2 and elevated CO_2 atm were prepared by diluting air with an appropriate amount of N_2 or CO_2. To maintain the O_2 level near 21% in the CO_2 series, an appropriate amount of O_2 was added to the higher CO_2 concns. The desired gas mixtures were established by use of flowmeters and metered to the samples at a rate such that respiration did not increase CO_2 by more than 0.3% in the effluent. The levels of O_2 and CO_2 in the various treatments were monitored daily using a Beckman G-2 O_2 analyzer and a Beckman infrared CO_2 analyzer.

The samples were exposed to CA for 3 or 7 days at which time they were sampled to measure growth, free α-amino N and protease activity. At these times duplicate lots were transferred to air at 10°C for 3 additional days and then sampled. CO_2 production by the low O_2 and air samples was measured at 12 hour intervals by the colorimetric method (11). Each time-treatment combination had 3 replicates.

Measurements. Increases in pileus diameter and stipe length were measured using Vernier calipers. Pileus diameter was measured at the widest portion of the cap and stipe length was measured from the top of the cap to the stipe cut.

For total free α-amino N determination, a random sample of 80–100 g of mushroom tissue was boiled in 95% ethanol, cooled, and the mixture was homogenized in a Waring blender. The resulting homogenate was filtered and the filtrate brought to a known volume with 80% ethanol. The α-amino N was estimated by the method of Yemm and Cocking (24) using a Gilford spectrophotometer at 570 nm with an alanine standard.

Protease activity was determined in the CA stored mushrooms by

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preparing an acetone powder in a manner after Yamaguchi et al. (23). The amount of enzyme activity in the powder was determined using a method previously described (18). Enzyme protein was determined by the Folin phenol method (14).

Results

CA effect on cap growth. After 3 days storage at 10°C 5% O_2 significantly promoted pileus expansion when compared with the air control, while 0% O_2 or 5, 25 and 50% CO_2 significantly retarded pileus expansion (Fig. 1). When stored for 7 days, pileus expansion of mushrooms in O_2 concn higher than 0% was not significantly different from the control. The effects of the 0% O_2 and the high CO_2 treatments had apparently maximized by this time. The degree of inhibition by 5, 25 or 50% CO_2 seemed concn dependent, being 63, 82 and 100%, respectively. There was essentially no effect of 10% O_2 or 50% O_2 on growth during either the 3- or the 7-day storage period.

After storage for 3 days in CA and then 3 days in air, the 0% O_2 and 25 and 50% CO_2 treatments showed large increases in cap expansion relative to what they were prior to transfer to air (Fig. 2). However, the growth increase during the 3-day post-treatment interval in air was less than those stored in air, except for those mushrooms stored in 5% CO_2. During the total 6-day period mushrooms from these treatments, but not 5% CO_2, had grown significantly less than those stored only in air.

The effect of the CO_2 treatment in retarding subsequent cap growth in air was more evident when mushrooms were held for 7 days than when held for 3 days before transfer. The growth of mushrooms in air after 7 days of the CO_2 treatment was generally about 33% of the growth increase in air exhibited after 3 days of the CO_2 treatment.

CA effect on stipe growth. Storage in 0% O_2 significantly retarded stipe elongation after 3 or 7 storage days (Fig. 1). Levels of O_2 greater than 0%, but less than 21%, had no significant effect on promotion or retardation of stipe growth after 3 days. However, after 7 days of storage 5 and 10% O_2 significantly promoted stipe elongation. Whereas stimulation of cap expansion by low O_2 was limited primarily to 3 days storage, some degree of stimulation of stipe elongation was present over the entire 7 days.

Depending on concn, increased CO_2 resulted in stimulation or retardation of stipe elongation. A 21% stimulation of stipe growth was achieved with a 5% CO_2 treatment after 3 days which dropped to 13% after 7 days of storage. Both 25 and 50% CO_2 significantly retarded elongation during the entire 7 days.

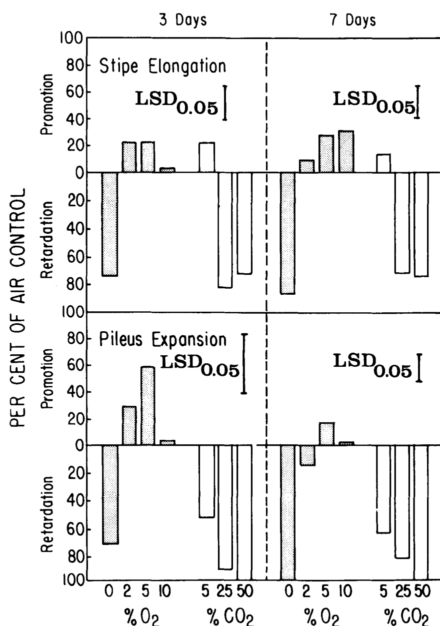

Fig. 1. Effects of decreased O_2 or increased CO_2 in the atm on growth of pileus and stipe of mushrooms stored for 3 or 7 days at 10°C. Retardation or promotion are expressed as percent of the air control.

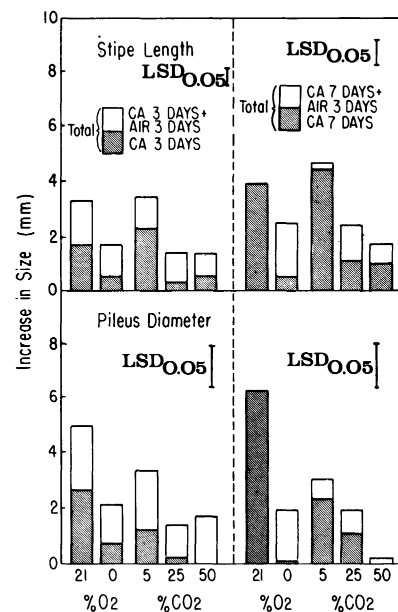

Fig. 2. Effects of previous holding in N_2 (0% O_2) or in elevated CO_2 on subsequent pileus expansion and stipe elongation in air at 10°C. Initial mean cap diameter and mean stipe length were 36 and 23 mm, respectively.

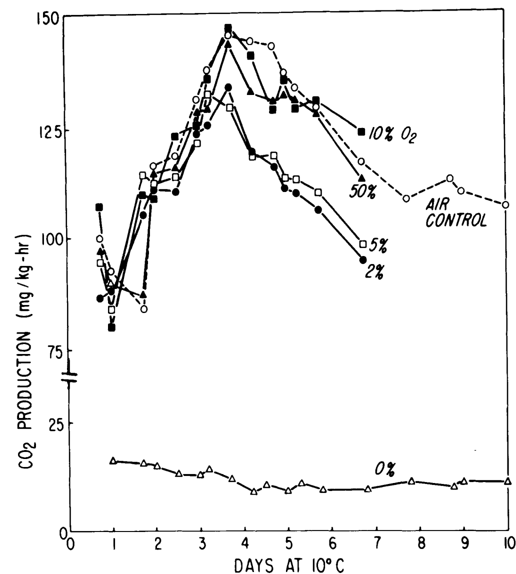

Fig. 3. Effects of O_2 levels on respiration rate of mushrooms held at 10°C.

Stipe elongation increased after transfer to air from 0% O_2 or 25 and 50% CO_2 (Fig. 2), but the total length of the stipe remained significantly shorter than that of the air control. The total increase in stipe length in mushrooms stored 3 days in CA then air was about 40% less than the increase exhibited by the air control.

CA effect on respiration. The rates of CO_2 production indicate that low O_2 has very little effect on the intensity of the respiratory peak (Fig. 3). However, 2 and 5% O_2 slightly reduced respiratory intensity and advanced the timing of the respiratory peak by about 12–24 hr. Storage in 0% O_2 reduced respiration to about one-tenth of that in air.

CA effect on protease activity and free α-amino N. When mushrooms were stored under either decreased or increased O_2 at 10°C, the increase in protease activity and decline in free α-amino N were similar to the pattern displayed by the air control (Figs. 4 and 5). Storage in 0% O_2, however, had a very dissimilar effect. A rise in enzyme activity did not occur during the storage period, while the

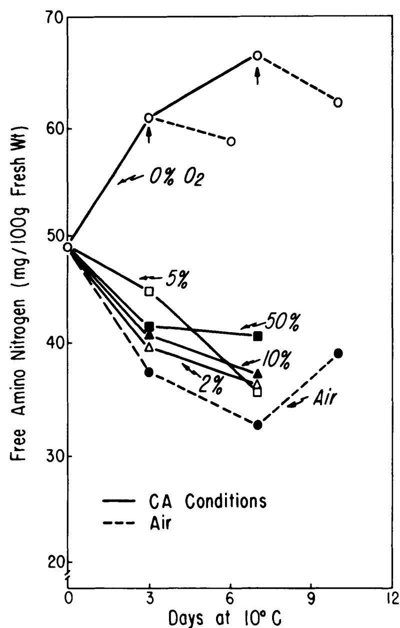

Fig. 4. Changes in levels of free α-amino N in mushrooms as affected by O_2 concn during storage at 10°C. Arrows (↑) indicate time at which samples were transferred from 0% O_2 to air for 3 additional days.

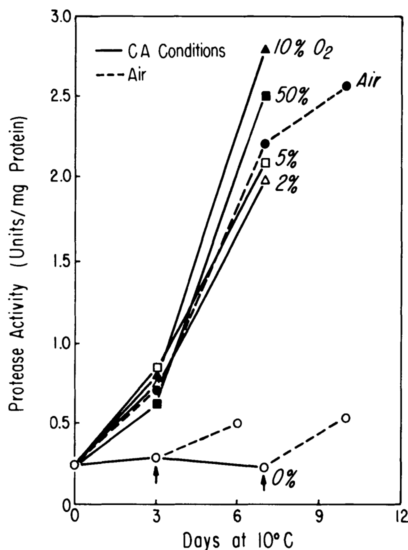

Fig. 5. Changes in protease activity in mushrooms as affected by O_2 concn during storage at 10°C. Arrows (↑) indicate time at which samples were transferred from 0% O_2 to air for 3 additional days.

level of α-amino N increased by nearly 50%. The data also show that upon transfer to air from 0% O_2 metabolism of protein and utilization of α-amino N occurred, although at a slower rate than if the mushrooms had been placed directly in air.

In contrast to the response shown at levels of O_2 above zero, storage of mushrooms in CO_2 above 5% generally resulted in an accumulation of α-amino N (Fig. 6) and a reduction in protease activity (Fig. 7). Storage in 5% CO_2 produced a noticeably different pattern of change in protease activity and α-amino N content when compared with the other CO_2 treatments. Protease activity in the 5% CO_2 treatment was almost twice that of the air control after 3 days but changed only slightly during the 4 subsequent days of storage. The α-amino N levels in mushrooms stored in 5% CO_2 remained constant during storage and changed little even upon transfer to air (Fig. 6). Storage in 50% CO_2 completely inhibited the rise in enzyme activity and caused about a 35% increase in α-amino N content which tended to level off after 3 days. Similarly, 25% CO_2 caused an increase in α-amino N of about the same magnitude. However, the 25% level was less effective than 50% CO_2 in retarding the increase in enzyme activity, although still highly effective when compared to the control (Fig. 7).

As demonstrated before with mushrooms stored in 0% O_2, upon removal of the CO_2 atm increased metabolism of protein and utilization of α-amino N occurred. It should be noted that there was a further accumulation of α-amino N in mushrooms stored 3 days in air after 7 days of 50% CO_2 (Fig. 6).

Discussion

Effects of O_2. Preliminary observations at 5°C had indicated that cap opening of mushrooms in storage seemed to be promoted by O_2 tensions lower than that in air. The stimulatory effect appeared to be maximal in mushrooms stored 4 days in 4% O_2. However, very low concentrations of O_2 (<1%) significantly retarded cap opening. The

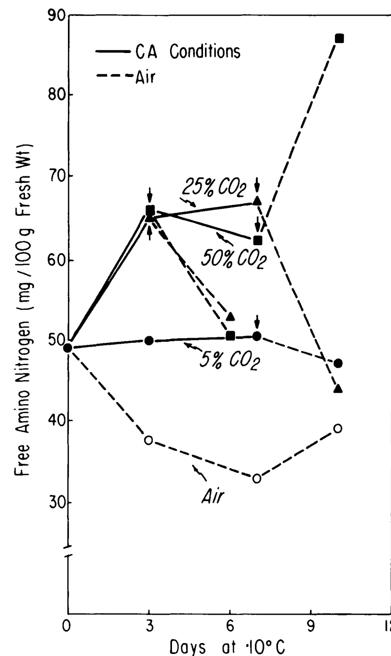

Fig. 6. Changes in levels of free α-amino N in mushrooms as affected by increased CO_2 during storage at 10°C. Arrows (↑) indicate time at which samples were transferred from CO_2 to air for 3 additional days.

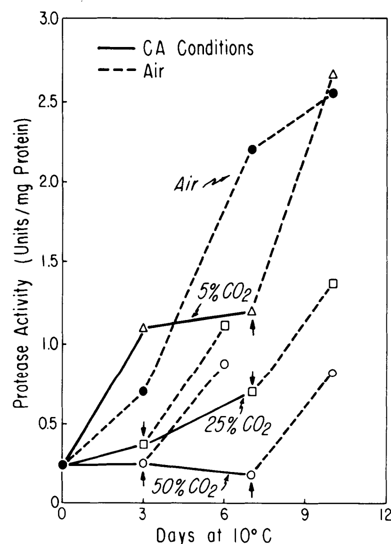

Fig. 7. Changes in protease activity in mushrooms stored in different CO_2 concn at 10°C. Arrows (↑) indicate time at which samples were transferred from CO_2 to air for 3 additional days.

quantitative results presented here, though at a higher temp, tend to substantiate these earlier visual observations.

Mushrooms show a maximal stimulation of growth in 5% O_2 and the growth stimulation is likely due to increased cell elongation, since cell division occurs very early in the development of the carpophore (2). To our knowledge this is the first report of stimulation of growth in fungi by low O_2 concn. The growth stimulation does not appear to be linked to an enhancement of energy production needed for growth since a stimulation of respiration did not occur in 2% or 5% O_2. However, a temporal shift in the respiratory peak suggests that postharvest maturation and development of the mushroom was hastened.

Our results with low O_2 are strikingly parallel to those reported for potato (8, 9) in which 5% and 10% O_2 stimulated sprout growth in storage. The degree of stimulation in potato, however, was dependent upon the time of treatment during the rest period.

Stimulation of stipe growth by low O_2 is not as great as that of the cap and appears to be spread over a longer period of time (Fig. 1). Since the lamellae are the center of growth control in mushrooms (12, 13) and abundant lamellae occur near the cap margin (5, 12), the nearness of these lamellae to the apparent site of active cap growth might explain the greater relative response of this tissue to the low O_2 stimulus. Alternatively, it could be that there is a difference in gas diffusion into the 2 meristematic areas involved. The meristematic area of the cap (e.g. the cap margin) could come under the influence of the low O_2 stimulus sooner than the meristematic area of the stem which is located well within the mushroom carpophore.

Effects of CO_2. Growth of fungi can be retarded by increasing the concn of CO_2 in the atm or culture media (3, 4). That CO_2 can strongly inhibit mushroom growth is apparent. However, 5% CO_2 while inhibiting cap expansion stimulated stipe elongation. Therefore, the response of mushrooms to CO_2 appears to involve a dual effect: on one hand 5% CO_2 stimulated stipe growth and suppressed cap expansion while concn much higher than 5% almost completely suppressed stipe and cap growth. Burton (6, 7, 8) also observed stimulation of sprout growth in potato tubers at concn below 7–8% CO_2, and suppression of growth at higher concn.

An increase in CO_2 concn above 0.03% may be beneficial even though extremely high concn can become injurious. This was most strikingly observed with mushrooms stored in 25 and 50% CO_2. Maturation was dramatically suppressed, yet slight injury in the form of surface pitting was evident in some mushrooms stored 7 days in the highest CO_2 atm. However, the physiological changes brought about as an indirect effect of the treatment with CO_2 appear to be of a temporary nature and do not seem to be extremely detrimental to the mushrooms.

Proteolytic activity. Mushrooms assimilate almost all low molecular wt compounds containing C and N from the substrate on which they are growing. Upon harvest the supply of C and N is cut off and the carpophores must now adapt to circumstances similar to those of bacteria under nongrowing conditions. It has been proposed that increased proteolysis may provide starving *E. coli* cells with a source of essential amino acids for the synthesis of enzymes needed for proper cell maintenance (15, 16). This also appears to apply for mushrooms during storage (19). Storage in 0% O_2 or increased CO_2 at 10°C caused an accumulation of α-amino N and a retardation of the rise in protease activity. Moreover, when mushrooms were transferred to air after 7 days storage in 50% CO_2 there was an additional accumulation of α-amino N with a corresponding increase in protease activity. In these cases growth was also markedly suppressed. However, in treatments where α-amino N decreased with time expansion of the cap and elongation of the stipe usually occurred. The apparent relationship of proteolytic activity to growth suggests that its main physiological function is to supply metabolites which function directly in the postharvest maturation of mushrooms.

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