Effect of Controlled Atmosphere on Growth of Mold on Synthetic Media and Fruit

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Growth of seven spoilage molds on agar plates at several temperatures in both controlled atmosphere (CA) and in air was studied. Each mold responded somewhat differently to CA at each temperature; however, there were some general tendencies. The lag phase was generally increased by CA and, in some cases, was substantially extended when incubation was just above the minimum growth temperature. The mycelial structure of molds seems to be different when grown in CA than when grown in air. With only two exceptions of 24 holding conditions, the maximum amount of mycelia was always less in CA than in air. Spore development varied with each mold at each temperature; generally, it was considerably less in CA than in air. CA storage of cherries above 34 F (1 C) did not retard mold infection to any extent; at 34 F, mold growth was inhibited and storage life was extended several days as compared to air storage. CA storage of strawberries at 34 F resulted in a mold-free product after 7 days of incubation, whereas the air-stored berries were slightly infected. However, when mishandled berries showing some mold growth were stored at 34 F, CA did not stop further mold growth.

Controlled atmosphere (CA) is defined as an environment with elevated carbon dioxide and decreased oxygen levels as compared with air. It has been used in conjunction with refrigeration to extend the shelf life of certain fresh food products (6). CA gas composition may be adjusted by several means. The earliest method took advantage of the natural respiration of the commodity in storage (3). However, several weeks were generally required to develop the desired atmosphere. Faster methods of obtaining the desired CA composition involved the use of dry ice or compressed CO₂. In recent years, CA generators (Whirlpool Corp., St. Joseph, Mich.) have been developed which burn natural gas and instantly deliver a CA of the desired composition.

The extension of storage life of food held in CA is partially attributed to inhibition of the microbial flora (2). There have been a number of studies on growth of bacteria and fungi in CA (6), but few studies have been made on mold growth in CA developed by generators (1, 5; C. W. Hastings, M.S. Thesis, Univ. of Illinois, Urbana, 1967). The purpose of this research was to compare the growth of selected spoilage molds in CA with that in air on a synthetic medium and on cherries and strawberries at several temperatures.

MATERIALS AND METHODS

Pure culture study. Mold cultures were inoculated on the surface of poured agar plates. The molds were: Penicillium expansum NRRL 976, Aspergillus niger NRRL 337, Macer hiemalis NRRL 1419, Fusarium bulbigenum NRRL 1985, Cladosporium herbarum NRRL 2175, Rhyzopus oryzae NRRL 1472, and Alternaria sp. NRRL 1284.

The synthetic agar medium was of the following composition: dextrose (anhydrous), 4.0 g; peptone (Difco), 1.0 g; agar (Difco), 1.5 g; water, 100 ml.

Agar plates were inoculated by dispersing mold from a stock culture in 5 ml of sterile 0.01% Triton X-100 solution. Then 0.1 ml of inoculum was spread over the poured agar surface with a bent glass rod. Each inoculated plate was placed, uncovered, within an individual plastic box to prevent cross-contamination. The plastic box, 4.25 inches (10.8 cm) by 4.25 inches by 1.5 inches (3.8 cm), was covered and sealed except for inlet and outlet ports (19/64 inches in diameter) for gas flow at opposite ends. The boxes were placed in refrigerated incubators and connected to a gas manifold by vinyl tubing. The gas, either CA or air, was pumped in order through a cotton sterilizing filter, a copper tube for cooling to incubation temperature, and a water trap to remove condensed water from the saturated (100% relative humidity) atmosphere. Air had been humidified before the pump, and CA was obtained with a high relative humidity. The gas was then forced through another cotton filter before passing through the manifold to each plastic box. Outlet gas was led outside the incubator via another manifold.

The CA gas of 10.5% CO₂ and 2.0% O₂ was developed by a generator (Tectrol Division, Whirlpool Corp., St. Joseph, Mich.).

Mold growth measurements. The time required for
appearance of definite growth was noted. This growth was considered to be aerial mycelia rather than thin, thread-like mycelia on the surface. Storage was terminated when there seemed to be no apparent increase or change in the mold.

The number of spores on the plate at termination was determined by plate count. The entire mycelial mat, including the agar, was blended with 100 ml of 0.01% Triton X-100 solution in a 300-ml cup (Omni-Mixer, Sorvall, Inc., Norwalk, Conn.). Dilutions were pour-plated on the synthetic medium described above and incubated at 75 to 80°F (24 to 27°C). The counts were expressed as the number of spores per plate.

This expression assumed that outgrowth of mycelial fragments would not make a significant increase in the number of colonies. For those molds (Mucor, Rhizopus, Penicillium and Aspergillus) whose spores could be counted directly with an improved Neubauer AO Spencer Bright Line counting chamber, this was shown to be true at the 95% confidence level.

The dry weight of the mycelia was determined from the homogenate used to make the spore count. All of the homogenate, except for the 0.5% that had been removed for plating, was melted in boiling water and vacuum-filtered through a tared Whatman no. 1 filter paper on a Buchner funnel. After rinsing with hot water, the paper and mycelia were air-dried overnight at 150°F (66°C) before weighing.

Fruit storage. Bing cherries of good quality were purchased from local stores. The cherries were placed in sealed boxes (same as used for the plates) and continuously flushed with CA or air. The boxes were held at 60, 45, and 34°F (16, 7, and 1°C). They were examined for degree of infection and breakdown. Strawberries were shipped directly from California. The berries were ripe and quite soft upon arrival, with many exhibiting gray mold growth. The poorest berries were discarded. As one treatment, the strawberries were placed in plastic boxes for storage at 60, 40 (4°C), and 34°F and continuously flushed with CA or air. As a second treatment, the berries were held overnight at warm room temperature so that the mold growth was barely evident before the experiment was started. Then the experiment was performed as the first treatment, except that storage temperatures were 40 and 34°F.

Replication. In every experiment, there were two plastic boxes of each inoculated plate or fruit at each condition. All synthetic media experiments were duplicated and some were replicated as many as four times. The fruit experiments represent one trial.

RESULTS AND DISCUSSION

Pure culture study. The time at which definite growth occurred and the extent of final growth as indicated by spore count per plate and total mycelial weight are given for each mold at several temperatures in Table 1.

| Mold | Temp (F) | Time for appearance of growth (days) | Log of spore count on plate at termination of growth | Dry weight of mycelia at termination of growth (g) |
|------|----------|--------------------------------------|------------------------------------------------------|--------------------------------------------------|
| Penicillium expansum | 70 | 1 | 9.6 | 9.6 | 0.078 | 0.078 |
| | 60 | 2.5 | 3 | 9.6 | 9.6 | 0.078 | 0.078 |
| | 45 | 7 | 9 | 9.4 | 9.3 | 0.075 | 0.075 |
| | 34 | 13 | 26 | 8.6 | 7.3 | 0.030 | 0.020 |
| Aspergillus niger | 70 | 1.5 | 4 | 9.0 | 9.1 | 0.20 | 0.23 |
| | 60 | 3.5 | 6 | 8.1 | 5.9 | 0.10 | 0.05 |
| | 55 | 7.5 | 10 | 5.0 | 4.9 | 0.02 | 0.01 |
| Mucor hiemalis | 70 | 1 | 1 | 8.3 | 8.8 | 0.16 | 0.11 |
| | 60 | 3.6 | 4 | 8.3 | 8.4 | 0.11 | 0.06 |
| | 45 | 6 | 8 | 7.2 | 6.5 | 0.14 | 0.08 |
| | 34 | 19 | 21 | 4.3 | 5.5 | 0.02 | 0.04 |
| Rhizopus oryzae | 70 | 1 | 1 | 7.7 | 4.5 | 0.14 | 0.12 |
| | 60 | 3 | 4 | 7.7 | 4.3 | 0.08 | 0.07 |
| | 55 | 4.5 | 6 | 3.0 | 3.8 | 0.08 | 0.06 |
| Cladosporium herbarum | 60 | 1.5 | 2 | 7.6 | 6.5 | 0.26 | 0.08 |
| | 50 | 6 | 14 | 7.2 | 5.6 | 0.20 | 0.026 |
| | 45 | 9 | >15 | 7.1 | 5.4 | 0.11 | 0.01 |
| Alternaria sp. | 70 | 2 | 2.3 | 7.3 | 7.3 | 0.098 | 0.097 |
| | 60 | 3.5 | 5.5 | 7.1 | 5.8 | 0.075 | 0.02 |
| | 45 | 7.5 | 11.5 | 6.8 | 5.5 | 0.045 | 0.005 |
| | 34 | 26 | >50 | 6.5 | 6.5 | 0.015 |
| Fusarium bulbigenum | 60 | 3.5 | 4 | 8.1 | 7.8 | 0.073 | 0.05 |
| | 50 | 6.5 | 9 | 7.7 | 7.7 | 0.059 | 0.038 |
| | 45 | 9.5 | 14 | 7.7 | 7.7 | 0.050 | 0.035 |

nearly equal in air and CA over the temperature range of 70 (21°C) to 45°F (Fig. 2). However, as storage temperatures approached 34°F, the number of spores that developed in CA was about one-twentieth that found in air. Mycelial development (Table 1) showed little difference between air and CA over the temperature range of 70 to 45°F. However, growth at 34°F in CA was two-thirds that in air, and the submerged mycelia at 34°F in CA had an intense orange coloration.

A. niger. CA holding of A. niger caused a delay in initial appearance of growth by 2.5 days over the temperature range of 70 to 55°F (13°C; Table 1). The mold did not grow in either air or CA at 50°F (10°C). The spore count at termination of growth in air at 70°F was nearly 1,000 times more than in CA, but at 55°F the spore counts were equal. Mycelial weights in air and CA were equal at 70°F, but at 60 and 55°F growth in air was twice as great as in CA.
Fig. 1. *Penicillium expansum*: number of days required for the appearance of mycelia on agar plates incubated in air and controlled atmosphere at various temperatures.

Fig. 2. *Penicillium expansum*: effect of incubation temperature on number of spores on agar plates at termination of growth in air and controlled atmosphere.

**M. hiemalis.** The time for initial appearance of growth at 70 °F was only 1 day in both CA and air (Table 1). The times were also equal at 60 °F. However, at 45 and 34 °F, it took about 1 to 2 days longer in CA. Spore counts were similar in air and CA from 70 to 45 °F, but at 34 °F there appeared to be a greater count in the CA sample. Mycelial weight was greater from 70 to 45 °F in air than in CA (Table 1). However, at 34 °F there was better growth in CA than in air. Microscopic examination showed that the aerial mycelia in CA were deformed, very thick, and highly branched as compared with those in air storage.

*R. oryzae.* The increase in time for initial appearance of growth resulting from CA holding was about 1 day in the temperature range 70 to 55 °F (Table 1). The mold did not grow at 50 °F. At storage temperatures of 70 and 60 °F, there were about 1,000 times more spores produced in air than in CA. At 55 °F, which was near the minimum growth temperature, there was a greater development of spores in CA than air. The dry mycelial weight was always a little greater in air than in CA at the several storage temperatures (Table 1). Microscopic examination showed that the mycelia produced in CA were thicker and rougher, more deformed, and more highly branched than those in air. Many "immature" sporangia were noted in the CA samples.

*C. herbarum.* This mold showed high sensitivity to CA storage. Growth in air gave a dense mycelial mat with typical dark green color. Growth in CA was poor with only slight green color. Initial appearance of growth was delayed in CA, and the delay was increased with decrease in storage temperature (Table 1). At 45 °F, the lag in CA was at least 6 days, and, in several replications, the mold did not grow at all at this condition. At termination of holding at 50 and 45 °F, there were about 50 times more spores and 10 times more mycelia in air than in CA.

*Alternaria sp.* CA holding caused a delay in the initial appearance of growth, and the delay increased with decrease in storage temperature. After 50 days of incubation, the mold had not grown in CA storage at 34 °F. At 70 °F, there was little difference in spore count between air and CA storage. At temperatures below 70 °F, there was about 10 times more spore development in air than in CA. Mycelial weight development at 70 °F was nearly equal for air and CA storage. At 45 °F, mycelial development in air was about 10 times greater than in CA, and, at 34 °F, the mold did not grow in CA (Table 1).

*F. bulbigenum.* CA holding caused a delay in appearance of growth by several days compared with that caused by air storage. Spore counts were equal for air and CA samples at 60, 50, and 45 °F (Table 1). The mold did not grow at 34 °F in either air or CA, even with extended incubation time. Mycelial weight determination showed that slightly greater development occurred in air than in CA.
**Fruit storage.** Cherries held in air at 60 F showed mold within 3 days, but the CA samples were still mold-free; after 5 days, several molds as well as yeasts were growing on the CA-stored cherries. After 12 days at 45 F, the air-stored cherries had a musty off-odor and several cherries were slightly infected with *Penicillium* and yeast; however, in CA there was little infection and the cherries were of firmer body, better odor, and brighter color. After 3 weeks at 34 F, storage in air resulted in some mold development but in CA there was none. Although CA cherries showed some bacterial and yeast growth, they had better color and odor than the air-stored cherries.

In treatment 1, strawberries at 60 F were highly infected within 1.5 days in either air or CA storage. At 40 F, the air-stored berries were visually infected by gray mold within 4 days and the CA berries within 5 days. After 7 days of storage at 34 F, berries in air were infected to a slight degree, whereas the CA-stored berries were free from mold, of brighter red color, and had greener caps.

In treatment 2, the objective was to observe the degree to which CA could stop or inhibit growth after it had been initiated. At 40 F, mold continued to grow well for about 1.5 days and then the growth rate decreased in both air and CA. After 7 days at 40 F, the berries in both atmospheres were spoiled but the CA samples showed less mold growth. After 7 days at 34 F in both air and CA, mold growth was greatly reduced as compared with storage at 40 F but neither sample was acceptable.

This study shows that the effect of CA on growth and development of seven spoilage molds is quite variable and, for the most part, is temperature-related. Of most interest in this work is the interaction effect of CA and storage temperature. The effectiveness of the CA was usually increased as storage temperature was reduced. Thus, for control of mold growth, it is evident that CA should generally be used with the lowest storage temperature that would be acceptable for the specific product.

**LITERATURE CITED**

1. Epstein, E., M. P. Steinberg, A. I. Nelson, and L. S. Wei. 1970. Aflatoxin production as affected by environmental conditions. J. Food Sci. 35:389–391.
2. Frazier, W. C. 1958. Food microbiology. McGraw-Hill Book Co., New York.
3. Kidd, F., and C. West. 1920. Department of Scientific and Industrial Research, Food Investigation Board, Fruit and Vegetable Committee (Great Britain). Food Invest. Board Rep. 1919, p. 17–24.
4. Kidd, F., and C. West. 1921. Department of Scientific and Industrial Research, Food Investigation Board, Fruit and Vegetable Committee (Great Britain). Food Invest. Board Rep. 1920, p. 16–25.
5. Littlefield, N. A., B. N. Wankier, D. K. Salunkhe, and J. N. McGill. 1966. Fungistatic effects of controlled atmosphere. Appl. Microbiol. 14:579–581.
6. Smith, H. W. 1963. The use of carbon dioxide in the transport and storage of fruits and vegetables. Advan. Food Res. 12:95.