Temperatures in Home Refrigerators and Mold Growth at Refrigeration Temperatures

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ABSTRACT

Air temperature in two home refrigerators ranged from 1.7 to 20.2°C during 4-day periods. Mean air temperatures at thermocouple locations varied from 3.9 to 11.9°C, and temperature changes resulting from opening refrigerators were usually 3°C or less, although increases as great as 18.5°C were recorded. Temperatures in the range at which refrigerators operated were evaluated for their effect on mold growth. Mycelia from a strain of aflatoxin-producing Aspergillus did not grow at 8°C during 504 h of incubation. Isolates of Penicillium obtained from refrigerated food had optimum growth rates of mycelia at 15°C or above but grew at 5°C. However, incubation at 5°C prevented germination of spores of all but one of the isolates that were tested.

During an investigation in this laboratory of molds in home-stored foods, some molds isolated from certain refrigerated foods produced toxic substances (6). A survey by Van Walbeek et al. (7) of temperatures in domestic refrigerators indicated that the minimum operating temperatures ranged from -0.5 to 10°C; however, no attempt was made to measure fluctuations in temperature that might occur during home use. Fluctuations in storage may enhance mold spoilage of certain foods (3) and would influence preservation of all refrigerated foods. A detailed study of activity rates for several species of bacteria indicated that more growth resulted when temperatures fluctuated to extremes equidistant from a given temperature than when incubation was constant at the temperature (4).

The present study was prompted by a concern for the presence, growth characteristics, and temperature response of toxigenic molds in refrigerated foods, and by a need to gain information about changes that occur in home refrigerators during use.

MATERIALS AND METHODS

Determining air temperatures in home refrigerators

Two families, each having two children between the ages of 5 and 14 years, were used for this study. The home refrigerator-freezers monitored were termed by the manufacturers to be frostless of frost-free. Refrigerator A had a capacity of 12.7 ft³, whereas that of refrigerator B was 14.0 ft³.

A Honeywell (Fort Washington, Pennsylvania) Type 15 Electronic Recorder, a continuous balance potentiometer with strip chart recorder equipped with copper-constantan thermocouples, was used to monitor temperatures in refrigerators during 4-day periods. Thermocouples were taped in place to prevent their movement. Air temperatures recorded at each thermocouple, resulting from opening and closing the door, were evaluated to determine the initial temperature before the door opened, maximum temperature attained, and the time required for temperature to return to the initial temperature (recovery time).

Mold strains

Isolates of Aspergillus, Penicillium, Mucor, and Rhizopus obtained from moldy food (6) were inoculated onto yeast extract-sucrose-gelatin medium, described below, and were evaluated for growth at 15 and 5°C. Of these, two isolates identified as belonging to the genus Penicillium and designated Penicillium sp. 0543 and Penicillium sp. 0546, were judged by dry weight and linear growth measurements of colonies to grow better than the other isolates at these temperatures, and were used in the growth experiments along with the aflatoxigenic Aspergillus parasiticus NRRL 2999 and nonaflatoxigenic Aspergillus flavus WB 1957. Spores used as inocula for growth experiments were obtained by flooding 10-day old cultures grown at 25°C on Mycological Agar (Difco) with sterile water and then gently rubbing the agar surface with an inoculating loop. The resultant spore suspension was filtered through a double layer of sterile cheesecloth into a sterile container, washed twice with distilled water to remove nutrients from the suspending medium, and held in distilled water at 5°C for 2 weeks before use. When used, the inoculum was again filtered through cheesecloth to remove chains of spores and provide a suspension of mostly single spores as judged by microscopic examination.

Medium

The medium for growth analyses contained 20 g of yeast extract, 200 g of sucrose, and 150 g of gelatin per liter of distilled water.

Inoculation and incubation of cultures

Spores were suspended in 0.5% molten agar at 45 to 50°C and inoculated at the center of medium in petri plates. One group of inoculated plates was immediately distributed at incubation temperatures of 25, 15, 8, and 5°C. During incubation, spores were examined with the aid of a microscope to detect outgrowth. A second group of inoculated plates was incubated at 21°C and spores were observed for germination and outgrowth. Mycelia appeared in all cultures within 30 to 54 h upon incubation at 21°C. After outgrowth of spores was observed by examination of the medium surface with a microscope, plates were distributed to incubators maintained at 15, 8, or 5°C.

Petri plates were placed in polyethylene bags containing moistened pieces of cheesecloth, and wetted strips of cheesecloth were hung inside incubators to maintain a moist atmosphere.

Determination of dry weight

Three plates containing a given mold culture were used to determine dry weight at each sampling period and were placed on a steam bath to liquify the gelatin. Mold growth floating on the surface of the medium was removed with a spatula and floated on water at 50°C to remove medium adhering to the colony. The washed colony was then dried at 60°C for 24 h and weighed.
RESULTS AND DISCUSSION

Monitoring refrigerator temperatures

Data obtained from Refrigerator A are in Table 1. Operating temperature, considered to be the initial air temperature before the refrigerator door was opened, varied from 1.7 to 14.0°C, and mean values for initial temperature at all locations ranged from 3.9 to 6.7°C. The maximal air temperature attained at each thermocouple location during door opening was from 15.4 to 20°C. Mean temperature attained on opening the door ranged from 7.8 to 19°C. Comparison of means for initial and maximal temperatures increases during door openings ranged from 1.1 to 9.0°C. Also, from comparison of mean air temperature values, the least temperature change on door opening occurred at the upper rear of the refrigerator cabinet, and the greatest change occurred at the bottom of the cabinet. Temperature increases at the top and bottom of the door were intermediate to extremes detected in the cabinet. Mean recovery time for temperatures at all locations ranged from 9.5 to 13.0 min.

A summary of air temperature changes detected in Refrigerator B is in Table 2. Initial or operating temperatures varied from 4.8 to 12.9°C, and mean values at all locations ranged from 5.8 to 11.9°C. The maximal temperature during door openings at each location was from 7.8 to 18.8°C, and mean temperatures attained on opening the door ranged from 6.4 to 12.5°C. Mean values for initial and maximal temperatures at the same location indicate that mean temperature increases during door openings ranged from 0.4 to 3.5°C. In contrast to temperatures observed in Refrigerator A, the greatest temperature change in response to door opening at any location in Refrigerator B occurred at the upper rear of the refrigerator cabinet. Mean recovery time for all locations was from 5.4 to 13.9 min.

No effort was made to standardize conditions in the two refrigerators, therefore detailed comparisons of data on air temperature have not been attempted. Instead, emphasis was on monitoring fluctuations in temperature that occurred during normal use.

Several factors that were not controlled probably influenced air temperature profiles. The temperature and surface area of cold refrigerated food, acting as a driving force to cool ambient air introduced during door openings, was not controlled. Location of food-stuffs near thermocouples also was not controlled, and it is possible that in some instances food almost touching thermocouples influenced the time required to return the air temperature to its initial value. Other uncontrolled factors considered to have influenced temperature rise and recovery time during door openings were length of time the door was open, disturbance of air flow patterns within the refrigerator caused by rearrangement or removal of food, and the manner in which the refrigerator door was opened and closed - fast, slow, or jerking motion.

Some comparisons of data in Table 1 and 2 are of interest. Door openings for Refrigerator A numbered 73 during the 4-day monitoring period in June, while

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**TABLE 1. Air temperature changes in refrigerator A during home refrigeration**

<table>
<thead>
<tr>
<th>Thermocouple location</th>
<th>Temperature, °C</th>
<th>Recovery time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Box</td>
<td>Initial&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Maximal&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Left side</td>
<td>6.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.8</td>
</tr>
<tr>
<td>Right side</td>
<td>6.7</td>
<td>7.8</td>
</tr>
<tr>
<td>Door</td>
<td>Top, butter keeper</td>
<td>6.7</td>
</tr>
<tr>
<td>Bottom</td>
<td>1.7-9.5</td>
<td>4.5-20.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Temperature immediately preceding door opening; also operating temperature for each location.
<sup>b</sup>Each set of numbers represents, reading down, the mean, median, and range.
<sup>c</sup>Highest temperature reached during door opening.
<sup>d</sup>Time required to return to initial temperature after door opening.

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**TABLE 2. Air temperature changes in refrigerator B during home refrigeration**

<table>
<thead>
<tr>
<th>Thermocouple location</th>
<th>Temperature, °C</th>
<th>Recovery time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Box</td>
<td>Initial&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Maximal&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Upper back near light panel</td>
<td>7.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.6</td>
</tr>
<tr>
<td>Left front</td>
<td>6.4</td>
<td>9.0</td>
</tr>
<tr>
<td>Near door latch</td>
<td>6.6</td>
<td>8.6</td>
</tr>
<tr>
<td>Right front</td>
<td>6.7</td>
<td>7.8</td>
</tr>
<tr>
<td>Bottom (under vegetable crisper)</td>
<td>5.6-7.3</td>
<td>7.3-12.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>See Table 1 for explanation of these footnotes.
Refrigerator B was opened 39 times during a December period of identical length. Ranges for mean initial temperatures and mean recovery times at all thermocouples in both refrigerators for the 4-day test period (3.9 to 11.9 C, 5.4 to 13.9 min) indicate that both were operated at temperatures recommended for home refrigeration (5) and were efficient in cooling air that entered during opening. However, butter and cheese storage areas in both refrigerators maintained temperatures (mean values of 6.7 and 11.9 C) in excess of the 2.2 to 4.4-C range preferred for storage of these dairy products (2). Dairy products including cheese were frequently found among moldy samples of refrigerated foods in a survey of food stored in the home (6).

**Growth studies**

Initial plans were to study how air temperature in refrigerators fluctuated and then apply results of that study to an analysis of the effect of temperature fluctuations on growth of molds isolated from home-stored foods. However, air temperature profiles of the two refrigerators examined (Table 1 and 2) indicated that, although occasional increases in air temperature of 18.5 C occurred and recovery times as long as 71 min were detected, most temperature fluctuations were less than 3 C and lasted 12 min or less. We, therefore, limited our experiments to temperature influence on mold growth at constant temperatures, and determined how growth from mycelia and from spores of selected cultures responded to those temperatures. Comparison of rates of growth by different isolates seemed to be done best by use of dry weight measurements, and this required a solid substrate that could be removed easily before mycelial growth was weighed. A medium made solid with gelatin suited this purpose since all incubations were below the temperature at which gelatin is a liquid. The average of triplicate dry weight values were plotted and lines were visually fitted to the points. Growth rates for mycelium at 5 and 8 C appeared to decrease before dry weight values reached 100 mg, and linear regions were difficult to assign with certainty to most of the curves.

Mycelial growth of aflatoxin-producing *A. parasiticus* NRRL 2999 was appreciably reduced at 15 C compared with growth at 25 C (Fig. 1). Mycelium developing at 15 C remained a creamy white, and sporulation was not detected. No lag was detected for mycelial growth at 25 or 15 C. No growth of mycelium occurred at 5 and 8 C. Spores germinated at 15 C after a lag period that exceeded 100 h. Spores incubated at 5 and 8 C did not germinate during the 504-h (21 days) incubation.

Spores and mycelia of *A. flavus* WB 1957 incubated at 5 and 8 C failed to germinate or grow. Growth of spores or mycelia at other temperatures was not attempted with this strain.

Van Walbeek et al. (7) examined six aflatoxigenic strains of *A. flavus* for growth and toxin production in broth and agar cultures at 7.5 and 10.0 C with or without preincubation at room temperature. Spores germinated and aflatoxin was detected in one instance after incubation for 1 week. Two cultures grew at 7.5 and 10 C with no preincubation at room temperature, but failed to produce aflatoxin during incubation for 4 weeks. The authors suggested that absence of spores from cultures growing at 7.5 and 10.0 C presented an additional health hazard since the green color associated with *A. flavus* growth was not available to aid in detection. Lack of growth at 5 and 8 C by strains of *A. flavus* and *A. parasiticus* reported in our study contrasts with results of van Walbeek et al. (7), who used strains of the same species. Differences in the media employed and/or variation in temperature response of the strains may be responsible for this lack of growth.

Growth curves representing the temperature response of two isolates of *Penicillium* appear in Fig. 2 and 3. The isolates grew at refrigeration temperatures in preliminary studies described in Materials and Methods. Visual examination of slopes of growth curves for *Penicillium* sp. 0546 (Fig. 2) indicates that the greatest growth rate occurred when mycelia from germinated spores were incubated at 25 C. Comparison of mycelial growth curves fro 5, 8 and 15 C in the region from about 10 to 100 mg of dry weight indicates that growth rates for these regions are similar to each other. The difference between curves appears to reflect a lag time after

![Graph showing growth of mycelium and spores at different temperatures](http://meridian.allenpress.com/jfp/article-pdf/40/6/393/1649817/0362-028x-40_6_393.pdf)
incubation of mycelia was begun. The lag increased with decreasing temperature of incubation.

Spores of isolate 0546 held at 15 C had germinated by 150 h, but almost 300 h were required before spores incubated at 8 C germinated. Spores at 5 C did not germinate within 504 h (21 days).

Growth of strain 0543 at 5, 8, and 15 C is described in Fig. 3. The lag preceding the maximum growth rate is much less evident at 5 and 8 C for strain 0543 than that described for 0546 in Fig. 2. Time required for spore germination at 8 C was about twice that needed for germination at 15 C. Spores of strain 0543 did germinate at 5 C.

Since an increased lag period characterizes growth as the temperature is decreased, it is necessary to consider this lag in growth as well as the slope of the growth curves when temperature sensitivity of different strains is being compared.

Growth curves of 0543, 0546, and NRRL 2999 in Fig. 4 take into account lag periods that precede apparent linear portions of the mycelial growth curves (Fig. 1, 2, and 3). Values plotted are the reciprocal times required to attain an arbitrarily selected dry weight. Parallel lines representing different weights of the same strain are an indication that growth curves maintain a constant relationship with change in dry weight. The nonlinear character of plotted values (Fig. 4) for times required to attain 10 mg may be the result of weighing errors.

**Figure 2.** Growth of mycelium and growth from ungerminated spores of Penicillus sp. 0546 on yeast extract-sucrose-gelatin medium at 5 (unlabeled curve), 8, 15, and 25 C. Spores incubated at 8 C had not germinated when examined at 255 h. Spores incubated at 5 C did not germinate during 504 h of incubation.

**Figure 3.** Growth of mycelium and growth from ungerminated spores of Penicillus sp. 0543 on yeast extract-sucrose-gelatin medium at 5, 8, and 15 C. Spores incubated at 5 C had not germinated when examined at 309 h.

*A. parasiticus* NRRL 2999 had a growth rate greater than that of 0546 at 25 C, as indicated by its larger value for reciprocal time. At 5, 8, and 15 C strain 0543 had the greatest rates of growth.

Strain NRRL 2999 is the most temperature-sensitive over the range investigated since no growth was detected at 8 C, and the slope determined by values for growth at 15 and 25 C is the greatest.

Temperature sensitivity of isolate 0546 is slightly more than that of isolate 0543 as determined from the greater slope (-0.29) for isolate 0546 compared to a slope of -0.32 for isolate 0543 (calculated for 100 mg dry weight values).

**Figure 4.** Growth rates of mold mycelia computed from time required to attain 10, 50, and 100 mg dry weight when grown at constant temperatures from 5 to 25 C.
Deverall (1) has suggested that psychrophilic molds should have a growth optimum near or below 10°C. Growth rates for the isolates of *Penicillium* used in this study continued to increase with increasing temperature (Fig. 4), over the range of 5 to 15°C for isolate 0543 and of 5 to 25°C for isolate 0546. Although they were isolated from refrigerated food and can grow at 5°C, they are not considered psychrophiles according to Deverall's definition.

Profiles for air temperature in home refrigerators and growth studies of molds presented in this report indicate that even when refrigerators are operated according to manufacturer's recommendations, growth of molds may occur. Although isolates of *Aspergillus* that we studied did not grow at 8°C, results of studies by Van Walbeek et al. (7) indicate that some toxic strains of *A. flavus* can grow at that temperature.

The recommended temperature for refrigerated storage of some foods is a compromise between maintenance of quality and safety. In light of the isolation of toxigenic molds from refrigerated foods (6) and the general concern about molds in foods, refrigerator manufacturers may need to reexamine the design of cabinets so that temperature can be monitored, and to assure that critical items be stored at the recommended temperature. In the final analysis, consumer knowledge of safe food handling and storage practices, and awareness of potential health hazards presented by molds in food may be the most important defense against these hazards.

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**REFERENCES**