Quantitation of Growth of Mold on Cheese

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ABSTRACT

Earlier work by others indicated that a mold colony grows radially at a constant rate on solid media. This concept was used in our study to develop a method for quantifying growth of mold on cheese. The ability of molds to grow on cheeses or pasteurized process cheese made with or without addition of sorbate was compared. Cheeses tested were mild Cheddar, aged Cheddar, aged-smoked Cheddar, brick and pasteurized process cheese. Pasteurized process cheeses were made from the natural cheeses by addition of water and a phosphate salt, then the mixture was heated. Some pasteurized process cheese from mild Cheddar was made to contain 0-500 ppm sorbic acid. Natural cheeses were sliced under aseptic conditions and were placed in sterile petri-plates. The hot and molten pasteurized process cheeses were poured into petri-plates. A spore suspension of Aspergillus parasiticus or Penicillium camemberti was inoculated onto the center of the cheese slice or pasteurized process cheese, and plates were covered and incubated at 22°C. The radius of mold colonies was measured at 24-h intervals. Data were analyzed by linear regression and lag period and rate of radial growth were calculated. Mold colonies grew radially at constant rates on cheeses and pasteurized process cheese. Lag in growth of each mold was longest on aged Cheddar cheese and pasteurized process cheese made from it, whereas it was shortest on mild Cheddar, brick and pasteurized process cheeses made therefrom. A. parasiticus grew faster on all cheeses and pasteurized process cheeses than did P. camemberti. Aged Cheddar cheese and pasteurized process cheese made from it effectively slowed the growth of both molds that were studied. Pasteurized process cheese containing sorbic acid inhibited growth of both molds. Generally, the higher the concentration of sorbic acid in the pasteurized process cheese, the slower was mold growth and the longer was the lag period.

Hard cheeses are usually ripened for several months at a suitable temperature and relative humidity. During the ripening period, molds may grow on cheese causing some economic losses. Also, since many molds produce toxins, growth of mold on cheese may be potentially hazardous to the health of consumers.

Several factors influence growth of mold on synthetic media (1). Growth of mold on cheese can be influenced by such factors as compositional, physical and microbial properties of cheese as well as the environmental parameters in the ripening rooms. A single factor may cause slight or major inhibition of mold growth on cheese. If the effects of these single factors are quantitated, manufacture or storage of cheese may accordingly be altered to maximize inhibition of growth of mold on cheese.

Currently, there is no objective method for quantitation of growth of mold on cheese. This work is an attempt to develop a method for measuring growth of mold on artificially contaminated cheese. The study is an application of a phenomenon that was observed earlier by others when molds were grown on solid synthetic media. When a small inoculum of mold spores is applied to the surface of a suitable solid medium, a circular colony is formed that grows radially at a constant rate (8). Potential uses for this method include (a) quantifying inhibition of mold caused by changes in manufacturing or processing of cheese or other solid foods, and (b) measuring the ability of a food to sustain growth of mold.

In this study, cheeses or pasteurized process cheeses were used as solid media to support surface growth of two different molds. One of the two molds used (Penicillium camemberti) can be found in the environments of some dairy factories (5), and is used in the manufacture of Camembert cheese. The other mold, Aspergillus parasiticus, is a potential contaminant in the cheese industry, and an earlier report (2) indicates this mold can grow and produce aflatoxin on cheese. The ability of these two molds to grow on different cheeses (as quantitated by our method) was compared. Inhibition of the molds on cheeses containing an antifungal agent (sorbic acid) was also studied.

MATERIALS AND METHODS

Cheeses

Brick and mild, aged and smoked-aged Cheddar cheeses were obtained from the dairy factory of the University of Wisconsin-Madison. Slices of cheese blocks were swabbed with pieces of cotton soaked with ethyl alcohol. Each block was then placed on a sterile piece of aluminum foil. The outer layers (several millimeters) of the cheese block were removed with a sterile knife. Cheese was then sliced aseptically with a sterile cheese slicer. Cheese slices (ca. 4-mm thick) were trimmed to fit into 10-cm (diameter) petri-plates.
**Pasteurized process cheese**

Pasteurized process cheeses were prepared from the natural cheeses as follows. Cheese was shredded and 132 g was weighed into a 500-ml beaker. Three g of dibasic sodium phosphate were dissolved in 15 ml of water, and this solution was added to the shredded cheese. The mixture was steamed (in a steamer) for 5 min. An electric egg-beater, equipped with sterile blades, operating at medium speed was used to mix the cheese for 1 min. Steaming and mixing were repeated. About 30 g of the molten mixture was poured into a sterile petri plate. Plates were covered and kept at 22°C until the mixture cooled and solidified. Occasionally, moisture condensed on the inside of the plate’s cover. These covers were replaced with new sterile covers.

**Sorbate treatments**

Mild Cheddar cheese was processed as indicated earlier except that potassium sorbate, and sodium phosphate were dissolved in 15 ml of water. Sufficient sorbate was added so cheese contained 0, 50, 100, 200, 300, 400 or 500 ppm expressed as sorbic acid.

**Molds**

*A. parasiticus* NRRL 2999 was obtained from the Northern Regional Research Laboratory, Peoria, IL and *P. camemberti* from K. B. Raper, Department of Bacteriology, University of Wisconsin-Madison. Freeze-dried spores of each mold were suspended in 10 ml of a sterile solution of 0.1% peptone. One ml of the spore suspension was spread on a slant of Mycological Agar, in a 200-ml prescription bottle. Slants were incubated at 22°C until spores were formed. Spores were harvested with 10 ml of sterile 0.1% peptone solution. The suspension contained $1 \times 10^3$ to $1 \times 10^4$ spores of either mold/ml.

**Growth of mold on cheese**

One $\mu$l of the spore suspension was inoculated onto the cheese at the center of the plates. Plates were partially sealed with an adhesive tape, incubated at 22°C and checked every 24 h for presence of measurable mold colonies. When growth was observed, two perpendicular diameters of the mold colony were measured (while the plate was covered) and the average radius of the mold colony was calculated. Average radius of the mold colony was measured every 24 h thereafter for up to 15 d. Growth of molds on Mycological Agar was also tested. Two trials were made of each experiment.

**Statistical analysis**

Data on growth of mold were analyzed using Minitab statistical software on a Compaq microcomputer. The radius (mm) of the mold colony was regressed against the period of incubation (h) using linear regression analysis. Lag period (Lag, h) and the rate of radial growth ($K_r$, mm/h) were calculated from the regression equation as follows. Regression equation: $Y = a + bt$ where $Y =$ the radius of the colony (mm), $t =$ period of incubation (h) and $a$ and $b$ are the estimated parameters of the equation. Lag period: $Lag = -a/b$ (h). Rate of radial growth: $K_r = b$ (mm/h).

**RESULTS AND DISCUSSION**

Shortly after the mold initiates growth on the surface of a solid synthetic medium, the mold colony grows radially at a constant rate (4,8). Brancato and Goulding (1) found that after a fixed period of incubation, the diameter of the mold colony depended on the type of mold and several environmental factors. The diameter that a mold colony attains after a fixed period of incubation depends on the lag period and rate of growth. In this study, we attempted to apply the former principles to molds growing on cheese. In a preliminary experiment, growth of *A. parasiticus* on aged Cheddar cheese was compared with that on Mycological Agar. The radius of the mold colony (mm) was regressed against the period of incubation (h). Results (Fig. 1) indicate that growth of *A. parasiticus* on aged Cheddar cheese was fitted closely to a straight line (coefficient of determination, $R^2$, was 99.7% for cheese and 100% for Mycological Agar). The regression line, fitted to the data, was extrapolated and the point of intersection with the x-axis ($Y = 0$) was calculated. The value at that point was considered the lag period (h). From data in Fig. 1, one can conclude that growth of mold on aged Cheddar cheese was delayed considerably (lag = 126 h) as compared with Mycological Agar (lag = 6.6 h). Furthermore, the mold grew slower on aged Cheddar cheese ($K_r = .123$ mm/h) than on Mycological Agar ($K_r = .157$ mm/h).

A comparison between lag periods exhibited by *A. parasiticus* and *P. camemberti* when each mold grew on different natural cheeses and on pasteurized process cheese made therefrom is illustrated in Fig. 2 and 3. In general, growth of both molds was delayed least (lag periods were the shortest) on mild Cheddar, whereas growth was delayed most on aged Cheddar. Smoked-aged Cheddar fell in between these two extremes. In most instances, lag periods of molds tested in this study were longer on natural cheeses than on corresponding pasteurized process cheeses. Lag periods were considerably shorter for both molds when they grew on Mycological cheese.

**Figure 1. Radial growth of colonies of A. parasiticus on aged Cheddar cheese and Mycological Agar.**

![Figure 1. Radial growth of colonies of A. parasiticus on aged Cheddar cheese and Mycological Agar.](http://meridian.allenpress.com/jfp/article-pdf/50/4/337/1651366/0362-028x-50_4_337.pdf)
Quantitating Mold Growth

Figure 2. Lag in growth of colonies of A. parasiticus and P. camemberti on different cheeses and Mycological Agar.

Figure 3. Lag in growth of colonies of A. parasiticus and P. camemberti on Mycological Agar and on pasteurized process cheeses made from mild Cheddar, aged Cheddar, aged-smoked Cheddar and brick cheeses.

Figure 4. Rate of radial growth of colonies of A. parasiticus and P. camemberti on different cheeses and Mycological Agar.

Agar than on natural cheeses or pasteurized process cheeses. No general conclusion can be drawn about which mold exhibited the longer delay in growth on cheeses, since the two molds behaved differently on different cheeses. For example, growth of P. camemberti was delayed longer than that of A. parasiticus when each was grown on aged Cheddar or the corresponding pasteurized process cheese. The opposite was observed when each mold grew on mild Cheddar or the corresponding pasteurized process cheese.

Figures 4 and 5 show that A. parasiticus grew faster than P. camemberti on all cheeses; pasteurized process cheeses and Mycological Agar. Both molds grew faster on brick and mild Cheddar than on aged Cheddar cheeses. In fact, both molds grew as fast or faster on mild Cheddar and brick cheeses than they did on Mycological Agar. This suggests that the ability of cheeses to delay initiation of mold growth is more significant than their ability to suppress growth of an established mold.

Differences between cheeses investigated in this study are related to variability in degree of ripening (e.g. mild vs. aged Cheddar), certain processing treatments (e.g. aged vs. aged-smoked Cheddar) and composition (e.g. brick vs. Cheddar cheeses). Ripening reduces the availability of moisture (6) and causes free fatty acids to be formed in cheese (3,7), which may have been responsible for the marked inhibition of mold growth on aged cheeses that was observed in this study. Surface ripening and the high moisture content of brick cheese may have provided an adequate environment for rapid initiation and growth of molds on that cheese.
During incubation of natural cheeses in plates, contaminating molds occasionally grew and measuring the diameter of a mold colony was difficult. Such plates were discarded; thus it was always necessary to start with more replicate plates than were actually needed.

Sorbic acid in pasteurized process cheese extended lag periods of both molds investigated in this study (Fig. 6). With P. camemberti, the greater the concentration of sorbic acid in pasteurized process cheese, the longer was the delay of mold growth. Concentrations of sorbic acid, up to 200 ppm, did not result in a noticeable delay in growth of A. parasiticus. Higher concentrations of sorbic acid, however, effectively extended the lag period of that mold. Sorbic acid slowed the growth of mold on pasteurized process cheese (Fig. 7). Generally, the higher the concentration of sorbic acid, the slower the growth of mold.

Although neither mold was inhibited appreciably by the concentrations of sorbic acid used in this study, the situation in a cheese factory could be quite different. Sorbate is usually applied to the surface of cheese only.

Figure 5. Rate of radial growth of colonies of A. parasiticus and P. camemberti on Mycological Agar and on pasteurized process cheeses made from mild Cheddar, aged Cheddar, aged-smoked Cheddar and brick cheeses.

Figure 6. Lag in growth of colonies of A. parasiticus and P. camemberti on pasteurized process cheese made from mild Cheddar cheese, and containing different levels of sorbic acid.

Figure 7. Rate of radial growth of colonies of A. parasiticus and P. camemberti on pasteurized process cheese made from mild Cheddar cheese and containing different levels of sorbic acid.
means cheese containing 500 ppm of sorbic acid should have a much higher concentration of sorbic acid at the surface. Furthermore, it is permissible in the U.S. to add up to 3000 ppm of sorbic acid in natural and 2000 ppm in pasteurized process cheese. Considering these factors, and assuming that our results can be extrapolated for higher concentrations of that additive, it is likely that treating cheese with sorbic acid in a dairy factory inhibits molds to much higher degrees than occurred in this study.

In conclusion, results of this study illustrate a method to quantify growth of molds on cheese. Using this method, we demonstrated that cheeses differ in their ability to support initiation and subsequent growth of molds. Ability of cheeses to delay initiation of growth of molds is an important factor in contributing to the general control of molds on cheese. Pasteurized process cheeses containing up to 500 ppm of sorbic acid were moderately inhibitory to both molds that we studied. Use of sorbic acid as practiced in dairy factories is likely to result in much greater inhibition of mold than was noted in these experiments.

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REFERENCES