Public Health Significance of Molds and Mycotoxins in Fermented Dairy Products

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ABSTRACT
Mold growth on cheese and other fermented dairy products is a common and recurring problem. Potential mycotoxin contamination is serious since some molds can grow and produce mycotoxins at temperatures as low as -2 to 10°C. Work can be divided into: 1) incidence, types, and mycotoxin-producing potential of molds in fermented dairy products, 2) experimental mycotoxin production on cheese under conditions of storage and aging of cheese, 3) natural occurrence of mycotoxins in commercial samples of cheese, and 4) potential toxicity of Penicillium roqueforti and its significance in blue veined cheeses.

Molds most common on cheese and fermented dairy products are Penicillium species. Mycotoxins produced by these organisms are penicillic acid, patulin, ochratoxin A, and citrinin. Percentages of molds in cheese capable of producing some commonly studied mycotoxins ranged from 1.8% to 12.4%. Cheese is an excellent substrate for mold growth but a poor substrate for mycotoxin production. Several natural occurrences of mycotoxins in cheese include small and variable amounts of patulin, penicillic acid, sterigmatocystin (600 μg/kg), penitrem A, and mycophenolic acid. Penicillium roqueforti is capable of producing toxic alkaloids and other compounds. The significance of these substances for human health is unclear.

The decision to trim or to discard moldy cheese can be aided by considering the risk versus benefit based on storage history (temperature), extent of mold growth, appearance of mold (color), and size of cheese.

INTRODUCTION
Mold growth on cheese and, to less extent, other fermented dairy products stored at low temperatures is a common and recurring problem (19, 36). It is a problem for the cheese manufacturer during aging and for the retailer and consumer during refrigerated storage. Mold growth on cheese causes economic losses from discolorations, poor appearance, and off-flavors. Often severe trimming of the cheese is necessary. However, of even more serious concern is that some molds are capable of producing toxic metabolites known as mycotoxins. Some of these toxins, such as aflatoxins, are known carcinogens. In addition, certain molds can produce mycotoxins at temperatures as low as -2 to 10°C (6); many of these molds belong to the genus Penicillium and are capable of growth in household refrigerators and supermarket display cases (53). Some psychrotrophic molds may grow at temperatures below 0°C (38). Recently, Torrey and Marth (68) reported isolation of potentially toxic molds from home refrigerators and cheese and other foods stored in home refrigerators. Thus, mold growth on cheese and fermented dairy products may pose potential hazards to food safety and human health.

This report reviews research concerning molds and mycotoxins in cheese and fermented dairy products and assesses the significance to public health of these findings. The work can be divided into four areas: 1) incidence, types,
and mycotoxin-producing potential of molds in fermented dairy products; 2) experimental mycotoxin production on cheese under conditions that cheese is stored and aged; 3) natural occurrence of mycotoxins in commercial samples of cheese; 4) potential toxicity of *Penicillium roqueforti* and its significance in blue veined cheeses.

### INCIDENCE, TYPES, AND MYCOTOXIN–PRODUCING POTENTIAL OF MOLDS IN FERMENTED DAIRY PRODUCTS

The incidence, types, and mycotoxin-producing potential of molds associated with cheese and fermented dairy products have been studied. In one study, 82% of the molds on refrigerated Cheddar cheese belonged to the genus *Penicillium*, 7% were *Aspergillus* species, 1% were *Fusarium* species, 1% *Alternaria* species, and 9% were distributed over several other genera (8). Toxicological screening indicated that 19.8% of these isolates were toxic to chicken embryos, causing 50% mortality or more. The toxicity of only 7.2% of the isolates could be accounted for by known mycotoxins through thin layer chromatographic examination. Mycotoxins produced by these cultures included patulin, penicillic acid, ochratoxin A, and aflatoxins.

Of molds isolated from Swiss cheese, 87% were *Penicillium* species, most of which were capable of growing at 5°C (4). In fact, of the isolates that grew at 5°C, 93% were *Penicillium* species. Toxicological screening of the isolates showed that extracts of 34% of all isolates and 35% of the *Penicillium* isolates were toxic to chicken embryos. Known mycotoxins were penicillic acid, patulin, and aflatoxins. The isolates producing known mycotoxins accounted for only 5.5% of the toxic activity in chicken embryos.

Bullerman (5) obtained moldy cheese trimmings from cheese manufacturing plants and retail outlets and found that *Penicillium* species predominated and accounted for 93% of the isolates. *Cladosporium* (4.0%), *Fusarium* (.9%), and other genera (1.8%) accounted for the remaining isolates. *Aspergillus* species were not found. However, these isolates represented organisms that developed at low storage temperatures, and this may account for the absence of *Aspergillus*. Four of the *Penicillium* cultures produced penicillic acid, one produced patulin, and one produced ochratoxin A. This represents 1.8% of all the molds isolated. Isolates from this study were not tested for toxicity to chicken embryos.

In a survey of domestic and imported cheeses, Bullerman (7) reported that *Penicillium* species predominated, accounting for 80 to 86% of the isolates. The remaining genera included isolates of *Aspergillus* (2 to 5%), *Cladosporium* (2 to 5%), *Fusarium* (1%), and other genera (7 to 10%). In this study, potentially toxic species of *Penicillium* and *Aspergillus* were identified. The most common toxic species was *P. cyclopium*, which accounted for 1.4% of the isolates. The second most common toxic species was *P. viridicatum*, which accounted for 5.5% of the isolates from domestic cheeses. This organism was not recovered from imported cheeses. *Aspergillus flavus* accounted for 1.8% of all isolates, .08% of the isolates from domestic cheeses, and 2.3% of isolates from imported cheeses. Mycotoxins were produced by 12.4% of the isolates. The toxins included penicillic acid, patulin, ochratoxin A, citrinin, and aflatoxins.

Recently, Torrey and Marth (68) reported that molds isolated from refrigerated and nonrefrigerated foods stored in private homes were primarily *Penicillium* (49%) and *Aspergillus* (38%) species. Of these isolates, 10.7% were capable of producing known mycotoxins including aflatoxins, kojic acid, ochratoxin A, penicillic acid, and patulin. Aflatoxin-producing molds were more common in nonrefrigerated foods than in refrigerated foods. In this study, 30 samples of cheese and cottage cheese yielded 25 isolates. Of these isolates, 21 (84%) were *Penicillium* species, 2 (8%) were *Aspergillus* species, 1 (4%) was a *Mucor* species, and 1 (4%) was unclassified. The two *Aspergillus* isolates from the dairy products produced aflatoxin, and one of the *Penicillium* isolates produced ochratoxin A. Thus, 12% of the isolates from cheese were potential mycotoxin-producing molds. In addition, 3.4% of the isolates produced toxic effects in mice, including lethality.

Northolt et al. (42) reported that molds isolated from visibly molded Gouda, Edam, and processed cheeses obtained from shops, homes, and warehouses in the Netherlands were predominately *Penicillium* species (86.5%). Other molds were *Aspergillus* (7.6%) and several other...
The incidence, types, and mycotoxin producing potential of molds in cheese and fermented dairy products are summarized in Table 1. *Penicillium* species predominate the mycoflora of these products and include molds capable of producing known mycotoxins. Thus, a potential for contamination of these products with known mycotoxins exists. However, it may not be a serious public health hazard since only a small percentage (2 to 15%) of the molds produced known mycotoxins. Further, when molds develop on cheese during refrigerated storage, *Penicillium* species are almost exclusively present, and the percentage of known mycotoxin producing molds appears to be very low. Thus, under refrigerated storage, growth of nontoxic penicillia seems to predominate and reduces the chances of the cheese becoming toxic. It is likewise important that cheese be aged and stored at temperatures low enough (below 10°C) to prevent growth of *Aspergillus* species, particularly *A. flavus*, *A. parasiticus*, and *A. versicolor* to avoid the production of aflatoxins and sterigmatocystin in cheese.

The significance of the toxicity of the *Penicillium* isolates, as measured in biological assays (chick embryos, chicks, and mice),...
is difficult to interpret because total toxicity cannot be accounted for on the basis of known mycotoxins. It is not known if this toxicity was caused by other known mycotoxins, which have not been studied extensively and for which standards were not available, or if this represents toxicity caused by toxic metabolites not yet studied and characterized. Thus, it is not possible to speculate on the significance of this toxic activity relative to public health and food safety. *Penicillium* species are capable of producing a number of toxins, and this currently is being studied. Chemical and toxicological properties of this unexplained toxicity remain to be determined.

**EXPERIMENTAL MYCOTOXIN PRODUCTION ON CHEESE**

Several workers have studied formation of mycotoxins in artificially inoculated cheeses. Lie and Marth (33) showed that *A. flavus* and *A. parasiticus* will grow and produce substantial quantities of aflatoxins on Cheddar cheese at room temperature. Oldham et al. (43) working with *A. flavus* and Cheddar cheese obtained small amounts of aflatoxins at 25°C but none at 4.4 or 7.2°C. Kiermier and Groll (25) produced aflatoxin on Tilsit cheese but not on Camembert and Romadur cheeses. Other workers have reported aflatoxin production in Tilsit and Emmentaler cheeses (18, 55). Polzhofer (51) showed that processed cheese was a good substrate for aflatoxin production by *A. flavus* at 25°C and that increasing emulsifying salt from 3 to 8% and addition of 6% sodium chloride reduced aflatoxin production. Shih and Marth (62) reported production of aflatoxins on brick cheese at both 12.8 and 23.9°C by *A. parasiticus* after 1 wk of incubation and by *A. flavus* after 14 wk of incubation at 23.9°C. *A. flavus* failed to produce aflatoxin at 12.8°C. It is believed generally that *A. flavus* and *A. parasiticus* will not grow and produce aflatoxins at temperatures below 10 to 13°C (14, 22, 56, 57, 64). However, van Walbeek et al. (72) reported that a strain of *A. flavus* produced aflatoxin at 7.5 and 10°C in 4 wk. Differences in strains might account for aflatoxin production at temperatures approaching refrigerated storage, but, for the most part, aflatoxins are not a problem for foods under adequate refrigeration.

Maintenance of low temperatures to prevent aflatoxin production in stored cheese is a key point; however, temperatures in refrigerators and refrigerated display cases may vary considerably. Van Walbeek et al. (72) reported in a survey of domestic refrigerators that the minimum temperatures ranged from 0 to 10°C. More recently, Torrey and Marth (69) reported that mean temperatures in two home refrigerators were 3.9 and 11.9°C and ranged from 1.7 to 20.2°C. In that study, an aflatoxigenic strain of *A. parasiticus* did not grow at 8°C over 21 days; however, *Penicillium* species grew at 5°C. Maxcy (37), however, reported that the mean temperature of water in household refrigerators was 3.6°C, considerably lower than reported by van Walbeek et al. (72) and Torrey and Marth (69). Maxcy (37) also showed that the range of temperatures was not as great as formerly reported. Since Maxcy (37) was measuring the temperature of water stored in refrigerators, and the other studies (69, 72) were measuring air temperature, differences would be expected. Recently, Kiermeier and Behringer (24) reported aflatoxin formation in moistened milk powder at temperatures between 1 and 5°C and at 10°C. However, Lieu and Bullerman (34) reported that *A. flavus* did not grow on any of several food substrates, including Mozzarella and Swiss cheeses at 5 or 12°C, but grew extensively and produced aflatoxins at 25°C (Table 2). However, less aflatoxin was produced on cheeses than on other substrates. While two of the papers just cited reported aflatoxin production below 10°C, the bulk of the published work indicates that aflatoxins only can be produced on cheese at temperatures above 11 to 13°C and probably would not be produced below these temperatures. Also, the amounts of aflatoxin produced would be expected to vary with type of cheese since reports indicate amounts ranging from 0 to low amounts in surface ripened cheeses to high amounts in other types of cheeses.

On Cheddar and brick cheeses at about 24 to 25°C (room temperature) aflatoxins may diffuse up to 1.3 cm from the point of growth in Cheddar cheese and 4 cm on prolonged incubation in brick cheese (33, 62). Thus, ordinary trimming of the moldy cheese, without removing a considerable amount of the cheese, would not remove the aflatoxins.

Because low temperatures prevent aflatoxin...
production maintenance of storage and aging temperatures below 10°C is extremely important. If cheese is kept at 5°C, aflatoxin-producing molds most likely will not be able to grow and produce aflatoxins. From 5 to 10°C, there may be some question as to whether certain toxigenic strains of *A. flavus* might be able to grow, but for the most part, this temperature range also should suppress growth of aflatoxicogenic molds and consequently prevent aflatoxin production. However, above 10°C *A. flavus* and *A. parasiticus* will grow, and aflatoxin production can occur.

A merchandising practice of displaying natural cheeses at room temperature is potentially dangerous. Even if the cheese is refrigerated during hours when the outlet is closed, the cheese may be subjected to room temperatures for 12 h or more each day. This could be followed by 10 to 12 h at refrigerated temperatures. This sort of temperature cycling may permit growth of aflatoxin producing molds and in fact, may favor higher aflatoxin production than occurs at continuous incubation at 25°C. Preliminary studies in our laboratory have shown with broth substrates that cycling temperatures of 25°C for 12 h followed by 5°C for 12 h for periods up to 6 wk can result in high growth and production of aflatoxins by *A. parasiticus* (47).

Cheese and fermented dairy products should be kept under refrigeration at all times, and the practice of displaying cheese at room temperature should be discouraged. Allowing cheese to come to room temperature for short times, such as for serving, does not pose a hazard.

There has been work on production of other mycotoxins in cheese, particularly those produced by *Penicillium* species. Stott and Bullerman (67) inoculated Cheddar cheese with a patulin-producing strain of *P. patulum*, and observed that the mold grew extensively at 5 and 25°C. Patulin production, however, was not observed at 5°C, and only small variable amounts of patulin were found at 25°C. In samples containing patulin, the toxin was localized in the mold mycelia and the first 3-mm layer of cheese. Lieu and Bullerman (34) observed similar results with organisms grown on Swiss and Mozzarella cheeses producing patulin and penicillic acid. The *Penicillium* species grew extensively on the cheese at 5, 12, and 25°C; however, no patulin or penicillic acid were detected in the cheese. Olivigni and Bullerman (45), working with an atypical isolate of *P. roqueforti* capable of producing both patulin and penicillic acid, obtained patulin and penicillic acid production at 5, 12, and 25°C on laboratory media, but toxins were not produced on Cheddar or Swiss cheeses (Table 3). Pohlmeier and Bullerman (49) reported that an ochratoxin-producing strain of *Penicillium* isolated from Cheddar cheese produced ochratoxin A on Cheddar cheese incubated at 25°C for 3 wk. Ochratoxin A was detected up to 7 mm from the point of mold growth. At 12 and 5°C, the mold grew extensively, but less of ochratoxin was produced. The amounts of ochratoxin in cheese ranged from a low of 4 μg/kg to a high of 600 μg/kg.

The studies with mycotoxigenic *Penicillium* species indicate that these organisms grow well on cheese at temperatures as low as 5°C. However, patulin and penicillic acid either do not occur or occur at such low levels that the toxins are detected only in the mycelia of the mold. Ochratoxin can be produced on cheese at low temperatures; however, the amount is much less on cheese than certain other substrates and also is less at lower temperatures of incubation. Thus, the importance of keeping cheese at low temperatures (approximately 5°C) is again evident.

In the work just reviewed on the production of aflatoxins, patulin, penicillic acid, and ochratoxin on cheese, toxins produced were generally much lower than on other food substrates such as cereal products. The lack of production of patulin and penicillic acid

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**TABLE 2. Aflatoxin production by *Aspergillus flavus* NRRL 5520 on several substrates incubated at 25°C for 14 days (34).**

<table>
<thead>
<tr>
<th>Substrates</th>
<th>B₁</th>
<th>B₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swiss cheese</td>
<td>1.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Mozzarella cheese</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Bacon</td>
<td>0.1</td>
<td>+</td>
</tr>
<tr>
<td>Bologna</td>
<td>14.7</td>
<td>1.6</td>
</tr>
<tr>
<td>Cooked corn meal</td>
<td>3.4</td>
<td>.1</td>
</tr>
</tbody>
</table>

a⁺ = Less than .05 μg/g.
bND = None detected.
and reduced production of aflatoxins and ochratoxin A on cheese may be because these substrates are low in carbohydrate and high in protein (11, 18, 34, 45, 62, 66). This is supported by other natural substrates high in protein, such as soybeans, peanuts, and cottonseed, likewise not supporting penicillic acid production (9). Also, soybeans are a relatively poor substrate for aflatoxin production compared to grains (15). Laboratory media lacking in carbohydrate but high in protein support extensive growth of molds producing patulin and penicillic acid but little or no production of either toxin (45, 66). Incubation temperature is not the determining factor controlling patulin or penicillic acid production by *Penicillium* species since these organisms produced toxins in suitable substrates, including potato dextrose broth, yeast-extract sucrose broth, shredded wheat, and cooked cornmeal at 5, 12, and 25°C (34, 45, 66). Low available water in the cheese also would affect adversely production of patulin, penicillic acid, and ochratoxin A in these substrates (39, 40, 41). However, this does not explain adequately the extensive growth without production of these toxins in broth substrates containing no carbohydrates but high amounts of protein (45, 53).

Pohlmeier and Bullerman (49) reported that broth substrates devoid of carbohydrate, but containing washed casein or washed casein plus calcium lactate, supported extensive growth of an ochratoxin producing *Penicillium* species but little or no ochratoxin production at temperatures of 5, 12, and 25°C. An aqueous extract of Cheddar cheese likewise supported extensive mold growth but less ochratoxin production than substrates containing a carbohydrate source. Only little ochratoxin was produced on cheese extract at 12°C, and no ochratoxin was found at 5°C on the cheese extract. Similarly, Engel (16) reported that lactate did not support aflatoxin production at 27°C by *A. flavus* and *A. parasiticus*. The apparent lack of patulin and penicillic acid productions and low production of aflatoxins and ochratoxin on cheese is due, in part at least, to the composition of the cheese substrate. Molds grew well on cheese, utilizing carbon from lactate and amino acids for energy, but they produced no toxins or only low amounts of certain toxins. Substrate components other than carbohydrate probably also exert an effect on toxin production; however, these have not been identified.

Another factor influencing mycotoxins in a food product is stability of the toxin in the food. Lieu and Bullerman (34) found that aflatoxins B1 and G1 were essentially stable in Swiss cheese for up to 1 wk at 5°C (Figure 1). Recoveries of aflatoxins ranged from 90 to 100%. However, patulin and penicillic acid appear to be less stable than aflatoxins in certain foods. Scott and Sommers (59) found that both patulin and penicillic acid were stable in grape and apple juice but not in orange juice and flour. They attributed the instability in orange juice and flour to thiol compounds. Pohland and Allen (48) also reported that patulin was stable in apple juice and dry corn

### TABLE 3. Production of penicillic acid and patulin (μg/g) on several food substrates after 6 wk at 5°C and 4 wk at 12°C (45).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>5°C</th>
<th>12°C</th>
<th>25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheddar cheese</td>
<td>ND^a</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Swiss cheese</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Summer sausage</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Corn tortillas</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Corn meal</td>
<td>14.2^b</td>
<td>1.8^b</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>43.2^b;24^c</td>
<td>21.2^b</td>
<td></td>
</tr>
<tr>
<td>Shredded wheat</td>
<td>105^b;120^c</td>
<td>36.0^b</td>
<td></td>
</tr>
</tbody>
</table>

^aND = none detected.

^bPenicillic acid.

^cPatulin.

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but unstable in wet corn, Durum wheat, and sorghum. Hofmann et al. (21) and Ceigler et al. (11) investigated potential production of patulin and penicillic acid in meat and meat products. They concluded that both patulin and penicillic acid at the pH of meat and meat products would react with sulfhydryl compounds and amino acids normally occurring in meat and, therefore, would not be detected by chemical assay procedures. Stott and Bullerman (67) found that patulin became undetectable when added to Cheddar cheese and stored at 5 and 25°C for various times (Figure 2). Further, Lieu and Bullerman (34) found that patulin and penicillic acid became undetectable in bologna after 12 and 48 h, respectively, when stored at 5°C. But in Swiss cheese, a low patulin and penicillic acid persisted with 5 to 8% of the added toxins being detected after 1 wk at 5°C (Figures 3 and 4). In these same studies, patulin and penicillic acid were more stable in cooked corn meal with about 80% of the toxins recoverable after 1 wk at 5°C. Thus, patulin and penicillic acid are less stable in foods that are high in protein such as cheese and meats than they are in grain and fruit products.

The instability of patulin and penicillic acid in foods such as cheese and meats has been attributed to combination of the toxins with amino acids and compounds containing sulfhydryl groups (11, 21, 35, 48, 59). Binding of patulin and penicillic acid to sulfhydryl groups reduced biological activity of these toxins. Hofman et al. (21) found that patulin bound to glutathione was no longer toxic to chicken embryos, mice, and rabbit skin. The S-alkylated adducts formed by combination of penicillic acid with cysteine or glutathione were nontoxic to mice and quail (11). In the same study, by chicken embryo tests penicillic acid adduct of glutathione was about 40 to 50% as toxic as penicillic acid, and the penicillic acid adduct of cysteine was as toxic as penicillic acid itself. Ceigler et al. (10) showed that the adduct of patulin and cysteine was nontoxic to mice and chicken embryos but remained teratogenic to chicken embryos. Lieu and Bullerman (35) studied the toxicity of the adduct of combinations of patulin and penicillic acid with glutathione and cysteine and found they were
Figure 4. Comparative recovery of penicillic acid from Swiss cheese, bologna, and cooked corn meal after storage for various times at 5°C (34).

Noninhibitory to *Bacillus subtilis* at 50 μg of toxin equivalent. Patulin and penicillic acid adducts of cysteine at 50 and 150 μg of toxin equivalent were nontoxic to brine shrimp larvae, and the patulin-glutathione adduct and patulin-cysteine adduct both at 100 μg of patulin equivalent were not toxic to 4-day-old chicken embryos. However, the penicillic acid adduct of cysteine at 100 μg equivalent of penicillic acid possessed considerable toxicity to embryos, but the penicillic acid-glutathione adduct in the same amount was less toxic. Simulated peptic digestion of S-alkylated adducts of the two toxins did not result in regeneration of free toxins, and no teratogenic effects were observed.

Ochratoxin appears to be more stable in foods than patulin or penicillic acid but probably less stable than aflatoxins. Harwig et al. (20) studied the effect of canning dried, white beans contaminated with ochratoxin on stability of ochratoxin A. Thermal processing at 121°C for 1 to 4 h reduced the ochratoxin content by 11% and 34%, respectively. Soaking and blanching resulted in losses of 21% and 10% of ochratoxin, respectively. In another study, autoclaving of oatmeal and rice cereals for 3 h reduced the amount of added ochratoxin by 60% (70). Simulated coffee roasting conditions, where temperatures of 93°C for 5 min were reached, destroyed ochratoxin (32). The instability of ochratoxin was mainly from the effects of heating. For the most part, cheese would not be subjected to these types of cooking temperatures. However, in the manufacture of process cheeses, a heating step is involved (65°C for 30 s); whether this might result in partial destruction of ochratoxin is not known. Pohlmeier and Bullerman (49) found that when ochratoxin was added to whole wheat bread, summer sausage, and Cheddar cheese, the percent recovery declined with storage time. However, up to 49% of the amount of ochratoxin added to Cheddar cheese was recovered after 48 h of storage at 25°C.

It appears that of the mycotoxins of concern in cheese and fermented dairy products, aflatoxin would be expected to be the most stable. Ochratoxin A also would be expected to be stable to a degree, but probably less than aflatoxin, whereas the stability of patulin and penicillic acid would be least in high protein foods such as cheese. More studies on the stability of these and other mycotoxins in foods are needed fully to assess the long stability of all mycotoxins in foods in general and cheese and fermented dairy products in particular.

**NATURAL OCCURRENCE OF MYCOTOXINS IN CHEESE**

There are few reports concerning the natural occurrence of mycotoxins in cheese as the result of mold growth on the cheese. Bullerman (4) found trace amounts of penicillic acid in Swiss cheese that developed mold during storage at 5°C for 6 wk under experimental conditions. The cheese had not been inoculated artificially, and the mold that developed was a part of the natural contamination of the cheese. Penicillic acid was in 4 of 33 samples, and its identity was verified by derivatization and TLC, UV, and IR absorption spectra. After verification of the toxin identity, too little of it remained for biological testing. However, the amount was estimated to be 0.5 μg/g of cheese. Bullerman (5) also reported that in moldy cheese trimmings, ochratoxin was detected in one sample, and aflatoxin B1 appeared to be in another sample. There was not a large enough sample to verify the toxin identities or quantitate the amounts.

Recently, the natural occurrence of the mycotoxin, penitrem A, in refrigerated cream cheese was reported (54). The occurrence came to light after the cream cheese became moldy in a home refrigerator, was discarded, and partially consumed by two dogs from the same neighborhood (3). Both dogs developed
symptoms of severe muscle tremors and generalized seizures. Examination of the moldy cheese resulted in isolation of *Penicillium crustosum* and penitrem A (3). Penitrem A is a mycotoxin that is produced by several species of *Penicillium* of the section Asymmetrica, subsection Fasciculata, which causes severe trembling and muscle spasms in animals (12). The effects of the toxin can be fatal to mice. The amount of penitrem A in the naturally molded sample was not determined, but approximately 0.15 g of the original sample of cream cheese caused neurologic symptoms when given orally to each of three mice; two of the mice died within 2.5 h (3). When 30 g of the moldy cream cheese was fed to a 10 kg male Beagle dog, panting, generalized muscle tremors, and limb rigidity developed within 2 h after ingestion (3). Laboratory studies involving the artificial inoculation of the mold isolate into nonmoldy cream cheese followed by incubation at 27°C for 2 wk, then at 4°C for 1 wk, resulted in the development of penitrem A in the cheese (54).

Northolt et al. (42) reported the occurrence of sterigmatocystin in cheese ripening in warehouses in the Netherlands, and molded with *Aspergillus versicolor*. Sterigmatocystin was found in 9 of 39 samples in the moldy surface layer of the cheese in concentrations ranging from 5 to 600 μg/kg. LaFont et al. (27) also reported patulin, penicillic acid, mycophenolic acid, and sterigmatocystin in cheeses in France.

Several other reports exist on the natural occurrence of aflatoxins, ochratoxin, and sterigmatocystin in cheese (63). In addition, other mycotoxins that have received less study, including mycophenolic acid, cyclopiazonic acid, β-nitropropionic acid, roquefortines A and B, and isoferugemilacalin have been reported. These have all been associated with cheeses produced in Europe. For the most part, mycotoxins in naturally contaminated cheeses have been little and difficult to detect and quantitate. However, the demonstration of toxicity with the cream cheese molded with *P. crustosum* and containing penitrem A indicates that possible human toxicity also could result from consumption of similarly molded products. Further study is needed.

Another way for aflatoxin to become a contaminant of cheese and fermented dairy products would be through the use of milk from dairy animals that have consumed feed contaminated with aflatoxin. Aflatoxin contamination of milk has resulted in contamination of cheese or other fermented milk products. When this occurs, usually only aflatoxin M1 is detected since the animal converts aflatoxin B1 to M1 before secretion into the milk. Kiermeier et al. (26) found aflatoxin in 79 of 419 samples (19%) of milk shipped to dairy plants in Germany. Polzhofer (50), working in Germany, tested milk, milk powder, yogurt, fresh cheese, Camembert cheese, hard cheese, and processed cheese and detected aflatoxin M1 in some samples of all of the products tested. Maximum amounts ranged from 0.3 μg/kg in milk to 2.0 μg/kg in milk powder. Corbion and Fremy (13) examined 100 samples of commercial cheese from 40 factories in France and found aflatoxin M1 in trace amounts in only one sample. Recently, milk and cheese produced in the southwestern US was found contaminated with aflatoxin as a result of aflatoxin contamination of dairy feeds (52). While contaminated milk may be a vehicle for transfer of aflatoxins into cheese and other fermented dairy products, there is no evidence that any of the other mycotoxins can be transmitted in this way.

**POTENTIAL TOXICITY OF PENICILLIUM ROQUEFORTI**

*Penicillium roqueforti* has produced several toxic compounds. It has been implicated as the principal organism in toxicity of cattle feed in Wisconsin that was associated with abortion and placental retention (65). Earlier *P. roqueforti* was associated with toxic cattle feed in Japan (71). A number of toxic compounds have been isolated from *P. roqueforti*. These toxins have been referred to as PR toxin, a sesquiterpenoid compound, and a series of alkaloids known as roquefortines A, B, and C, and festuclavine. The PR toxin has an oral LD₅₀ in weanling rats of 115 mg/kg, and oral doses above 130 to 160 mg/kg of body weight were fatal to 60 g rats within 36 h (75). Toxicities of the alkaloid compounds are low (58). Roquefortine is a neurotoxin that reportedly causes convulsive seizures in mice (60). Other toxic responses in mice include liver damage and hemorrhage in the digestive tract (23). Roquefortine has been recovered from Blue cheese.
from seven countries by Scott and Kennedy (60). The toxin was associated with the mold mycelia rather than the nonmoldy areas of the cheese. Subsequently, Scott et al. (61) showed that four strains of \textit{P. roqueforti} isolated from Blue cheese produced both roquefortine and PR toxin in semisynthetic media. Wei and Liu (74) obtained PR toxin from four type cultures of \textit{P. roqueforti} by semisynthetic media. However, Still et al. (65) were unable to show mammalian toxicity in a commercial strain of \textit{P. roqueforti} used in Blue cheese production. Egyptian workers have reported finding a toxic lipid fraction in Roquefort cheese that caused severe injury to the liver and other organs of rats during a 7 mo feeding trial (1, 2). Olivigni and Bullerman (46) reported that an atypical wild strain of \textit{P. roqueforti} isolated from Cheddar cheese produced patulin and penicillic acid simultaneously. However, three commercial strains of \textit{P. roqueforti} and seven isolates from domestic and imported blue veined cheeses did not produce either patulin or penicillic acid. Leistner and Pitt (31) reported isolating strains of \textit{P. roqueforti} from fermented sausages that produced patulin, and in one case patulin plus citrinin. Lafont et al. (29) reported that of 50 strains of \textit{Penicillium} used in the cheese industry in Europe, about half produced no toxic effects in chicken embryos, cell cultures, or mice. The rest gave variable results in the three systems. Of these, two caused rapid death in mice, and 15 gave some type of positive response in at least two of the test systems. The authors concluded that the toxic effects could not be related to a single toxin and that further studies were needed to determine whether this toxicity (produced in synthetic media) had any significance to public health. Further studies by Lafont et al. (28) showed that of 16 strains of \textit{P. roqueforti}, all produced mycophenolic acid in vitro in amounts of .8 to 4 mg/g of dry culture. Best production occurred at 15°C in 10 days of incubation. Wilson (76) lists mycophenolic acid as an antibiotic substance that appears to have a low oral toxicity (LD\textsubscript{50} 2500 mg/kg in rats).

The significance of the various toxins produced by \textit{P. roqueforti} to public health is not clear. Patulin, penicillic acid, and citrinin have been observed only in wild type isolates of the organism and not in commercial strains. As such, these wild isolates represent no greater significance than any other toxinogenic isolates of other species. The significance of PR toxin, mycophenolic acid, the roquefortines, and related alkaloids to human health is likewise unclear, particularly in view of the limited toxicological information available on these compounds, some of which indicate that roquefortine A and mycophenolic acid are only weakly toxic. Likewise, the significance of toxicity of \textit{P. roqueforti} cultures grown on synthetic substrates to cell cultures, embryos, and mice is unclear. This, combined with the fact that blue veined cheeses have been consumed for centuries without apparent ill effect, suggests that the hazard to human health may be minimal or nonexistent. The true significance of the \textit{P. roqueforti} toxins will not be known until further studied.

**SUMMARY AND CONCLUSION**

Incidence of molds in cheese and fermented dairy products indicates that those most commonly found (predominant flora) belong to the genus \textit{Penicillium}. From 82 to 87% of all molds isolated from various cheeses in several studies were \textit{Penicillium} species. In one study involving moldy cheese trimming, the percentages of \textit{Penicillium} isolates was even higher, 93%. Thus, the mycotoxin-producing potential of the molds developing on properly refrigerated cheese and fermented dairy products would be those toxins most likely to be produced by \textit{Penicillium} species. Indeed, the most commonly detected mycotoxins in extracts of cultures isolated from cheese trimmings were penicillic acid, patulin, and ochratoxin A. Citrinin, another \textit{Penicillium} toxin, also was found but in much lower incidence. Members of the genus \textit{Aspergillus} were recovered in low incidences ranging from .5 to 8.0% in several surveys and were not recovered at all from moldy cheese trimmings. A small percentage of the \textit{Aspergillus} species was capable of producing aflatoxins. In most of the studies, this ranged from .3 to 1.8% of all isolates. In one study, the incidence was as high as 8.0%, but this was based on a small number of samples. The incidence of aflatoxin-producing molds was higher in imported cheese than in cheese produced in the US. Overall, percentages of molds in cheese capable of producing some commonly studied mycotoxins were low, ranging from 1.8% in one study to 12.4% in another. The highest incidence was in
the study that included imported cheeses. The lowest incidence of potentially toxic molds (1.8%) was in moldy cheese trimmings. These molds represent strains that grew during storage at low temperatures. Thus, low temperatures help prevent growth of the potentially toxic molds.

In general, cheese is an excellent substrate for mold growth but is a poor substrate for mycotoxin production. Patulin and penicillic acid were not produced to any extent on cheese. Ochratoxin was produced in small amounts but not to the extent that it was produced on other substrates. Likewise, aflatoxins were produced only if the storage temperature was above 10°C. Aging and storing cheese at low temperatures, preferably 5 to 7°C, most likely will prevent development of aflatoxins and along with substrate effects will minimize other mycotoxins produced by Penicillium species.

Several natural occurrences of mycotoxins have been reported. These included patulin, penicillic acid, sterigmatocystin, penetrem A, and mycophenolic acid. Except for sterigmatocystin, the amounts were low and variable. The significance of patulin, penicillic acid, and mycophenolic acid in cheese in small amounts is probably not great from a public health standpoint because of to their low oral toxicity. Sterigmatocystin is of more concern because of its carcinogenicity and that it occurred in amounts up to 600 μg/kg. However, low temperatures (5°C) should prevent the growth of A. versicolor and production of sterigmatocystin. The importance of maintaining adequate temperature control again is emphasized. Penetrem A in naturally moldy cream cheese is also of concern because of the animal toxicity associated with consumption of the cheese. However, the cheese was heavily molded and not fit for consumption. This is attested by the fact that the cheese had been discarded and under normal circumstances would not have been consumed by humans.

What then is the significance of molds on cheese and fermented dairy products? Also, what should be the recommendation to consumers who encounter mold on cheese either in home refrigerators or retail outlets? First of all, it is not accurate to say that the molds that develop on cheese are harmless. It can be said that most of the molds that develop on cheese are probably not toxic since the incidence of toxic molds on cheese is low and there appears to be a tendency for nontoxic molds to overgrow the toxic types. Also, it can be said that cheese is a poor substrate for mycotoxin production, particularly when properly stored at low temperatures of 5 to 7°C. At these temperatures, the molds that develop will be Penicillium species, which do not produce aflatoxins or sterigmatocystin. Thus, storage at 5 to 7°C should prevent aflatoxin and sterigmatocystin production. Storage at these temperatures will shift the mold flora such that fewer toxic types will develop, and those that might develop would be likely to be producers of patulin and penicillic acid, mycophenolic acid, penetrem A, and possibly ochratoxin. These toxins are much less stable in cheese and less toxic than aflatoxins. At low temperatures, it is likely that these toxins probably would not be produced to any great extent with the possible exception of penetrem A. Thus, patulin, penicillic acid, and mycophenolic acid may be considered to be less of a hazard than aflatoxins. In the experimental studies in which these toxins were produced on cheese and in the natural occurrence of penetrem A on cheese, mold growth associated with it was extensive. Thus, the toxins have been recovered only from severely molded cheese.

The decision to trim or to discard moldy cheese cannot be answered simply (17). Because of the value of cheese as a food and source of protein, it is undesirable to discard or waste cheese where it is not necessary, especially if the potential hazard may be minimal and can be minimized even further or eliminated by adequate trimming. However, no one would want to be exposed or see others exposed to potential health hazards of mycotoxins.Trimming is not advisable if the cheese has been abused by storage at high temperatures and there is a possibility of aflatoxin contamination. A reasonable approach is to base recommendations on information available in the scientific literature from research on this subject.

Some suggested guidelines that consumers can use to assess the risk/benefit ratio of a particular situation have been made by a number of workers (30, 44, 73). These can be summarized in the following points:

1. Has the cheese been kept under refrig-
eration (5 to 7°C)? And did the mold develop under refrigerated conditions?

2. Does the mold exhibit any color? Blue to gray, bluish gray, or bluish colored molds are likely to be *Penicillium* molds, whereas olive green, dark forest green, or dull green colors may be indicative of *A. flavus*, *A. parasiticus*, or *A. versicolor*, potential aflatoxin or sterigmatocystin producers. White mold may not have formed spores yet, and it may be impossible to tell if the mold is a potential aflatoxin or sterigmatocystin producer.

3. How extensive is the mold growth, and what is the size of the cheese? If the growth is extensive, and the size of the cheese is relatively small, the risks probably outweigh the benefits and it is probably most prudent to discard. However, if the piece is large and there is little mold growth, such as one or two small colonies, trimming with removal of about 1.3 cm or more of cheese around and beneath the mold is warranted. The benefits would outweigh the risks, especially if the cheese has been kept refrigerated.

4. This approach would be best used on solid cheeses and not on soft, semi-solid cheeses, such as Cream cheese or Brie, nor on semi-liquid fermented dairy products such as sour cream or yogurt. With these products, diffusion of mold metabolites would occur more extensively, and simply skimming the mold would not necessarily remove all of the mold metabolites. Even if mycotoxins were not present, other mold metabolites may impart undesirable flavors to the product.

5. Finally, mold growth on cheese can be minimized by good sanitation in production and handling through the entire food chain, from producer to consumer, and further minimized by exclusion of air from the cheese through vacuum packaging or other means whenever possible. It is important to keep cheese and fermented dairy products under refrigeration at all times. Other than for serving purposes, cheese should not be kept at room temperature long. The practice of displaying cheese at room temperatures in merchandising outlets should be avoided unless mold growth can be prevented by means other than temperature control.

**REFERENCES**


SYMPOSIUM: MICROBIAL METABOLITES OF IMPORTANCE IN DAIRY PRODUCTS


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