Potential Aflatoxin Hazards to Human Health from Direct Mold Growth on Teleme Cheese

GREGORY K. ZERFIRIDIS
Laboratory of Dairy Technology
Faculty of Agriculture
University of Thessaloniki
Greece

ABSTRACT

Ninety-four commercial Teleme cheese samples were examined for aflatoxins produced by direct mold growth.

The mycoflora on the cheese and in the atmosphere of Teleme cheese plants also were monitored. *Penicillium* and *Aspergillus* genera were tested for aflatoxin production after growth on Teleme cheese at 25°C. In all cases, over 78% of the molds were *Penicillium* species. *Aspergillus* made up 3.8 to 3.9% and 0 to 7.3% of the mold on cheese and in the plant atmosphere, respectively. None of the commercial samples contained aflatoxins and none of the 448 *Penicillium* isolates was an aflatoxin producer. Of 22 *Aspergillus* species, one was capable of producing aflatoxins after direct growth on cheese. Because the physicochemical characteristics of Teleme cheese (high moisture, low pH, and medium salt concentration) favor mold growth, care should be taken to avoid contamination of the cheese by aspergilli.

INTRODUCTION

Teleme is a white soft cheese kept in brine and consumed worldwide. Mold growth on this cheese is a common problem during ageing, in retail markets, and in home refrigerators. Mold imparts off flavors to the cheese and is undesirable for aesthetic reasons. It also may be undesirable because of the potential production of mycotoxins (8).

The natural occurrence of aflatoxins in cheese has been investigated (4). There are reports on the experimental production of mycotoxins in cheese and on the potential mycotoxin production of molds isolated from a limited number of cheese varieties (2, 3, 5, 17). Few reports refer to sources of mold contamination of cheese (16, 25). However, molds isolated from cheese and other foods were screened for their ability to produce mycotoxins by growing them on artificial media (2, 3, 5, 21, 25, 26). Our interest was to study the ability of isolated molds to produce mycotoxins on natural substrates such as cheese used for human consumption (10, 25).

Because aflatoxins are mycotoxins hazardous to human health, an investigation was initiated to study: 1) the presence of aflatoxins in commercial Teleme cheese, 2) the mycoflora of Teleme cheese, 3) mycoflora of the atmosphere in Teleme cheese making plants, and 4) the aflatoxin-producing potential of certain mold isolates grown on Teleme cheese.

MATERIALS AND METHODS

Sources of Cheese and Origin of Mold Isolates

Mold isolates were obtained from Teleme cheese and the atmosphere of Teleme cheese making plants. Seventy-five domestic and 19 imported cheese samples, visually without molds, were randomly obtained throughout the year from market places and cheese making plants of northern Greece. The cheese sample was a whole block approximately 11 × 11 × 7 cm, the surface of which was measured and then aseptically scraped about 2 to 3 mm deep (3). Scrapings were collected in a sterile screw cap jar and immediately examined for molds. The remaining scrapings were retained for chemical analyses. During the same period, the atmosphere of the cheese making plants, as a source of mold contamination of the cheese, was examined twice in each of four Teleme plants in the same area.
Mold Counts and Determination

Molds in cheese samples were enumerated according to the American Public Health Association (1) using potato dextrose agar (Oxoid) at pH 3.5. Plates were incubated in duplicate and counts reported as number of mold colonies per square centimeter (3). To estimate mold contamination of cheese from the atmosphere of Teleme cheese making plants, two plates containing potato dextrose agar at pH 3.5 were placed at each of the following areas of the plant: receiving and pasteurization, manufacturing, draining and salting, preripening, and packaging. Plates were opened for 10 min (16) to expose them to the atmosphere; then they were covered and transferred to the laboratory. Counts are reported as number of mold colonies per plate after incubation at 25°C for 5 d. Mold colonies on the plates from cheese and atmosphere were separated into groups of the same genus, and representative colonies from each group were transferred to potato dextrose agar slants. The number of picks per group was relative to the number of colonies in the group. The agar slant was incubated at 25°C for 5 d and the mold culture was then kept at 5°C until used. All isolates were classified for genus using the keys of Smith (24), Raper and Thom (19), and Raper and Fennell (18).

Screening Mold Isolates for Aflatoxin Production

Isolates of aspergilli and pencilli were screened for aflatoxin production by growing them on Teleme cheese. Aspergillus flavus NRRL 6554, a known aflatoxin producer, also was grown on Teleme cheese for comparison. This mold was obtained from the US Department of Agriculture, Northern Regional Research Center, Peoria, IL.

A 2-ml aqueous suspension of conidia from each mold culture was prepared according to Shih and Marth (23) and kept at 5°C up to 1 mo until used for inoculations. Manufactured Teleme cheese blocks, 7 x 7 x 7 cm, had a pH of 4.6 to 4.7, moisture content 55%, and salt concentration from 5 to 6%. The cheese block was inoculated on the upper surface by the conidial preparation representing one mold culture and was placed in a plastic screw cap container disinfected with hypochlorites. A small amount of water was added to the bottom of the container, after which it was incubated at 25°C until the inoculated surface was either covered by the mycelium after about 10 d or the mold seemed to stop growing on the cheese after 15 to 20 d. The container was aseptically opened periodically for observation and for entry of air. The mycelium and the top 1-cm layer of the cheese were cut off, mixed, and examined for aflatoxins.

Chemical Analyses

The pH was measured electrometrically, moisture by oven drying to constant weight, and salt by the International Dairy Federation (11) method. Salt in the moisture was estimated as the ratio of [salt %/(moisture % + salt %)] × 100.

Aflatoxins were determined by the method of Shih and Marth (22). Standard silica gel plates 20 x 20 cm (Merck No. 5715) were used for thin-layer chromatography after activation for 2 h at 110°C. The reflectance of the extracts from all isolates and from the standard aflatoxin producers were compared with those of reference aflatoxins B₁, B₂, G₁, and G₂ (Aldrich, Europe, B-2340 Beerse, Belgium. Code No. 85636-3) at 366 nm using an ultraviolet chamber.

RESULTS

Physicochemical Characteristics of the Cheese

Moisture content of Teleme cheese ranged from 52 to 55% (legal maximum 56%); pH was generally below 4.8, and only 9 domestic samples were between 4.9 to 5.1; salt in the moisture was less than 5% for 48 of the domestic and 3 of the imported samples, whereas the rest were as high as 6.8%.

Mold Counts on Cheese and in the Atmosphere of Cheese Plants

Among the domestic cheese, 20, 23, and 38 samples had mold counts of <1, 1 to 10, and >10/cm² (range 12 to 199), respectively. Of the imported cheese 3, 6, and 10 samples had mold counts <1, 1 to 10, and >10/cm² (range 39 to 208), respectively.

Mold counts of the atmosphere of cheese plants are shown in Table 1. The number of colonies per plate decreased in the plant in the order: receiving and pasteurization, manu-
TABLE 1. Mold counts per petri dish in the atmosphere of Teleme cheese making plants.

<table>
<thead>
<tr>
<th>Area of plant</th>
<th>Plant</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receiving and pasteurization</td>
<td></td>
<td>5</td>
<td>38</td>
<td>40</td>
<td>21</td>
</tr>
<tr>
<td>Manufacturing</td>
<td></td>
<td>7</td>
<td>27</td>
<td>42</td>
<td>5</td>
</tr>
<tr>
<td>Draining and salting</td>
<td></td>
<td>2</td>
<td>15</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td>Preripening</td>
<td></td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Packaging</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

1 Means of four Petri dishes.

Although the physicochemical characteristics of the samples were favorable for mold growth, counts were low compared with other types of cheese (3, 6, 12). This may be due to early enclosure of Teleme cheese into containers filled with brine, sealed, and kept at low temperatures as compared with other cheese varieties, which may be exposed to the atmosphere for long periods, and therefore, may develop mold before cleaning and packaging. Jantea et al. (12) reported similar results.

Mold counts in the atmosphere of cheese plants were higher than those reported by Moreaux (16) in France obviously due to differences in climate, layout of the plants, their location, and the hygiene practiced in the plants. The surface of Teleme cheese is mainly contaminated by molds after it is formed into blocks in the manufacturing section. Thereafter, care should be taken to protect walls, roofs, and equipment from mold development to

TABLE 2. Genera of molds isolated from market Teleme cheese samples.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Domestic</th>
<th>Imported</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(no. isolates/% total isolates)</td>
<td></td>
</tr>
<tr>
<td>Penicillium</td>
<td>135/84.9</td>
<td>41/78.9</td>
</tr>
<tr>
<td>Mucor</td>
<td>8/5.0</td>
<td>5/9.6</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>6/3.8</td>
<td>2/3.9</td>
</tr>
<tr>
<td>Cladosporium</td>
<td>5/3.1</td>
<td>2/3.8</td>
</tr>
<tr>
<td>Fusarium</td>
<td>2/1.3</td>
<td>1/1.9</td>
</tr>
<tr>
<td>Other genera</td>
<td>3/1.9</td>
<td>1/1.9</td>
</tr>
<tr>
<td>Total</td>
<td>159/100.0</td>
<td>52/100.0</td>
</tr>
</tbody>
</table>

1 From Denmark and Holland.
TABLE 3. Atmospheric contamination of Teleme cheese by molds in the various areas of the plant during cheese making. Number of isolates is from mold colonies in 16 petri dishes.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Receiving and pasteurization</th>
<th>Manufacturing</th>
<th>Draining and salting</th>
<th>Preripening</th>
<th>Packaging</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(no. isolates/% total isolates)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillium</td>
<td>118/78.2</td>
<td>67/80.7</td>
<td>45/90.0</td>
<td>25/92.6</td>
<td>17/100</td>
</tr>
<tr>
<td>Mucor</td>
<td>12/7.9</td>
<td>8/9.7</td>
<td>2/4.0</td>
<td>1/3.7</td>
<td></td>
</tr>
<tr>
<td>Aspergillus</td>
<td>11/7.3</td>
<td>2/2.4</td>
<td>1/2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cladosporium</td>
<td>. . .</td>
<td>3/3.6</td>
<td>1/2.0</td>
<td>1/3.7</td>
<td></td>
</tr>
<tr>
<td>Fusarium</td>
<td>3/2.0</td>
<td>1/1.2</td>
<td>1/2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alternaria</td>
<td>4/2.6</td>
<td>. . .</td>
<td>. . .</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other genera</td>
<td>3/2.0</td>
<td>2/2.4</td>
<td>. . .</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>151/100.0</td>
<td>83/100.0</td>
<td>50/100.0</td>
<td>27/100.00</td>
<td>17/100</td>
</tr>
</tbody>
</table>

ensure that atmospheric contamination is minimal.

Incidence and types of molds encountered on Teleme cheese followed the same trend as those reviewed by Bullerman (4) for other kinds of cheese. The incidence of Penicillium and Aspergillus species in the atmosphere of the first sections of the plant was similar to that of the market cheese. This might lead to the assumption that cheese is mainly contaminated in the plant before packaging.

The production of aflatoxins by Penicillium species has been reported (8, 9, 12, 20, 27), but it is generally accepted that aflatoxins are produced only by the Aspergillus species. This is also supported by the results of this study.

The commercial samples of Teleme cheese had no aflatoxins and results were similar for other cheese varieties (4, 5, 7, 13, 14). This study indicates though that a small proportion of the Aspergillus isolates were able to produce aflatoxins in Teleme cheese after seeding the cheese surface with mold conidia. Thus, the potential hazard for the natural occurrence of aflatoxins in Teleme cheese is real, even though it would require massive contamination of the cheese by aspergilli and favorable conditions for growth.

ACKNOWLEDGMENT

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REFERENCES

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