Behavior of Aflatoxin M₁ During Manufacture and Storage of Queso Blanco and Bakers’ Cheese

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ABSTRACT

Queso Blanco and bakers' cheese were prepared from milk naturally contaminated with aflatoxin M₁ (AFM₁), and then were stored at 4°C and at -23 ± 6°C for 2 months. AFM₁ was found in both curd and whey. There was a 2.84-fold increase of AFM₁ in curd of Queso Blanco cheese over the amount present in milk from which the cheese was made. The AFM₁ content of the cheese varied during refrigerated and frozen storage, but AFM₁ was present near initial levels at the end of storage. Bakers' cheese was prepared with and without added rennet. More AFM₁ was found in cheese and whey than in milk from which cheese was made. AFM₁ tended to be concentrated in curd. Cheeses made without rennet had greater enrichment (4.24-fold increase over that found in milk) than those made with rennet (2.97-fold increase over that found in milk). The AFM₁ content in both types of cheese was variable but toxin remained in cheese through 1 month of refrigerated and 2 months of frozen storage.

Aflatoxin M₁ (AFM₁) is a hepatocarcinogen found in milk of animals that have consumed feeds contaminated with aflatoxin B₁. The dairy industry and public health officials are concerned about AFM₁ in dairy products because AFM₁ has toxic and carcinogenic activity.

Recently the literature on AFM₁ and the dairy industry was reviewed (5). Since this review was written, the behavior of AFM₁ in several varieties of cheese was studied in our laboratory. Results of these studies are reported elsewhere (2,8,9,10). As was reported earlier, AFM₁ was concentrated in the curd rather than whey (2,8,9,10). The detectable content of AFM₁ in cheese is influenced by manufacturing procedures, conditions of cheese ripening and procedures used to extract AFM₁ from cheese. In some instances, there was an apparent increase of AFM₁ after cheesemaking over that which was in the milk used for cheese (2,9). This was related to variability in recovery of AFM₁ from cheese. AFM₁ was recovered from curd, whey, and wash water (where applicable).

Two noteworthy aspects of these studies were: (a) the variability in enrichment of AFM₁ in curd and (b) how the AFM₁ content of cheese changed (or did not) during ripening. Manufacturing procedures appeared to affect distribution of AFM₁ in cheese (2,8,9,10). For example, washing of curd reduced the AFM₁ content of brick and Limburger-like types of cheese. Wash water of these cheeses contained appreciable amounts of AFM₁ (8). Activity of lipases added in cheesemaking was thought to improve recovery of AFM₁ from Parmesan cheese (9). Changes in the AFM₁ content of cheeses aged for extended periods (up to 1 year) were correlated with proteolytic and lipolytic changes that occurred in cheese (8,9,10). Other studies of Brackett and Marth (7) showed that AFM₁ could bind to casein in model systems. Furthermore, when they treated naturally contaminated milk with Pronase®, more AFM₁ was recovered from treated than untreated milk (7).

During the manufacture of processed cheese spread, heating of the curd and use of emulsifying salts singly or together increased the apparent level of AFM₁ in the cheese spread(s). Mozzarella curd also had a large enrichment of AFM₁ over that found in milk. Brackett and Marth (9) thought heating and stretching of curd may have affected the amount of toxin recovered from this cheese.

Since manufacturing procedures used can affect partitioning and amount of AFM₁ in cheese, we believed it appropriate to examine the fate of AFM₁ in two additional cheeses, Queso Blanco and bakers’ cheese, which are made by procedures different from those used to make the cheeses that were studied earlier. Queso Blanco cheese was made by other investigators from milk artificially contaminated with AFM₁ rather than from naturally contaminated milk (18). Approximately half of the AFM₁ in the milk appeared in curd and the rest in the whey. However, these results may be different from what happens when cheese is made from naturally contaminated milk. AFM₁ in artificially contaminated milk does not always behave the same as does AFM₁ in naturally contaminated milk (12). Bakers’ cheese (and similarly produced cream and Neufchatel cheese) have not been studied before. Both Queso Blanco and bakers’ cheese are sometimes held frozen. AFM₁ in frozen milk either is thought to be partially lost or rendered more difficult to extract than is AFM₁ in unfrozen milk.
Could a similar phenomenon occur in frozen cheese? We sought the answer to this and other questions in these experiments.

MATERIALS AND METHODS

Preparation of naturally contaminated milk
Naturally contaminated milk was obtained from two Holstein cows that received dietary aflatoxin B<sub>1</sub> as described elsewhere (6). Milk was collected twice daily and held at 7°C until it was processed. Skim milk for bakers’ cheese was obtained by forewarming raw milk to 40°C and then separating the milk with a hand-operated separator (Sübitas, Istanbul, Turkey). Skim milk was pasteurized at 64°C for 30 min before it was used to make bakers’ cheese.

Manufacture of Queso Blanco and bakers’ cheese
Queso Blanco cheese was prepared using a method similar to that described by Kosikowski (13). Whole milk was heated for 30 min at 83°C. To insure efficient stirring in the cheese vat, at least 100 lb of milk had to be used. Trial 1 used 111 lb and trial 2 used 135 lb of milk. Cheese curd was formed by addition of 1.25 ml of glacial acetic acid per lb of milk. The concentrated acid was diluted 1:10 with tap water before it was added to milk in three portions. Milk and acid were stirred for 5 min after which curd was allowed to settle for 15 min. Then whey was drained from the vat; the curds were weighed and salted (5 lb per 100 lb of curd). Queso Blanco curd is the acid-precipitated material before it is pressed into cheese. Curds were placed (1.5-lb portion) into 2-lb cheese hoops lined with cheese cloth and the cheese was pressed overnight. Cheeses were waxed and stored at 4°C.

Bakers’ cheese was prepared using the method described by Kosikowski (13). Ten lb of pasteurized skim milk were inoculated with 1% lactic culture (Hansen’s culture 70, Chr. Hansen Laboratories, Milwaukee, WI). Two trials were with calf-rennet (Dairyland Foods, Waukesha, WI) added to milk and two trials did not use rennet. The inoculated milk was incubated overnight at 22°C. Curd was dipped the next morning when the pH reached 4.3–4.4. Whey was drained by placing curd in a pail lined with cheesecloth which was placed in a refrigerator at 4°C. Drained curd was packaged in paperboard containers and then stored in a refrigerator at 4°C or in a freezer at -23 ± 6°C.

Determination of moisture, milkfat and salt
The moisture content of cheese curd and finished cheese was determined by the atmospheric oven method (14). Milkfat contents of Queso Blanco cheese curd and finished cheese and of bakers’ cheese were measured by the Babcock procedure for cheese (14). However, bakers’ cheese (9-g portion) was digested in a beaker and then was transferred to Babcock cock bottles used for skim milk (1-g bottles) to obtain readable columns of milkfat. Values from the column were multiplied by 2 to obtain the percent milkfat. Salt content of Queso Blanco curd and finished cheese was measured using the Quantab strip method (11). Extracts of cheese and curd were made with 140 rather than 90 ml of hot water to achieve salt concentrations within the accuracy of the test strip.

Determination of aflatoxin M<sub>1</sub>
Aflatoxin contents of milk, cheese, curd and whey were extracted and then analyzed using the method of Stubblefield (17). Suppliers and grades of reagents used are given elsewhere (21). Thin layer chromatography plates (Redi Plates®, Fisher Scientific, Pittsburgh, PA) with milk and whey samples were developed in one direction with ether-methanol-water (95 + 4 + 1, v/v/v), whereas plates with cheese samples were developed first in ether-methanol-water (95 + 4 + 1, v/v/v), dried and rotated 90° and then redeveloped in chloroform-acetone-isopropanol (87 + 10 + 3, v/v/v). Fluorescence of AFM<sub>1</sub> spots was measured with a fluorometer (G. K. Turner Associates, Palo Alto, CA) connected to a recorder (Linear Instruments, Irvine, CA).

RESULTS AND DISCUSSION

The moisture content of Queso Blanco cheese (Table 1) was similar to values reported elsewhere (13). This cheese has no Federal standard of identity. Cheesemakers producing this cheese can use milk with various amounts of milkfat and also can add various amounts of salt to the cheese. Our cheese had more salt and less milkfat than Kosikowski (13) suggested for Queso Blanco cheese made from whole milk.

The AFM<sub>1</sub> contents of milk, whey, curd and cheese are given in Table 2. There was a 2.84-fold enrichment of AFM<sub>1</sub> in cheese over that found in milk from which the cheese was made. AFM<sub>1</sub> is associated with casein (7), and so this enrichment during cheesemaking was expected. This enrichment was less than that found for most other cheeses (8,9). Brick cheese, where cheese curd is washed with water before pressing, also had a low enrichment of AFM<sub>1</sub> in the finished cheese (10). Acid precipitation may have formed small curd particles, some of which can be lost in the whey fraction. Loss of fine curd was thought to be a factor in the low yield of brick cheese and in the loss of AFM<sub>1</sub> when that cheese was made (10).

The variability of the AFM<sub>1</sub> content in Queso Blanco cheese during storage makes it difficult to determine the distribution of AFM<sub>1</sub> during cheesemaking. The AFM<sub>1</sub> concentrations of milk, whey, curd and cheese at each time period are given in Table 2.
TABLE 3. Concentration of aflatoxin M<sub>1</sub> in cheese, curd, whey and milk from which Queso Blanco cheese was made.<sup>a,b</sup>

<table>
<thead>
<tr>
<th>Trial</th>
<th>Milk</th>
<th>Curd</th>
<th>Whey</th>
<th>Cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
</tr>
<tr>
<td>1</td>
<td>27.9</td>
<td>3.5</td>
<td>98</td>
<td>15.77</td>
</tr>
<tr>
<td>2</td>
<td>31.6</td>
<td>2.8</td>
<td>87</td>
<td>15.77</td>
</tr>
</tbody>
</table>

<sup>a</sup>The amounts of milk, curd and whey based on fraction of total curd needed to prepare cheese. Total AFM<sub>1</sub> represent μg of aflatoxin M<sub>1</sub> present in that amount of product.

<sup>b</sup>Safety restrictions prohibited collection of whey; total amounts of whey were estimated by subtracting mass of curd from mass of milk.

<sup>c</sup>No datum.

TABLE 4. Composition of bakers' cheese.<sup>a</sup>

<table>
<thead>
<tr>
<th>Trial</th>
<th>Fat (%)</th>
<th>Moisture (%)</th>
<th>Rennet used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.27</td>
<td>87.3</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>0.19</td>
<td>88.1</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>0.46</td>
<td>83.6</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>0.43</td>
<td>84.3</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<sup>a</sup>Average of triplicate analyses.

During storage, the AFM<sub>1</sub> content of cheese varied. However, AFM<sub>1</sub> remained in Queso Blanco cheese through 2 months of refrigerated or frozen storage (Table 2).

Bakers' cheese does not have a Federal standard of identity. Our cheese (Table 4) had more moisture than the values suggested by Kosikowski (13) for this cheese. Rennet is used in the manufacture of this cheese to aid in the expulsion of whey. Rennet was used to produce the cheese in trials 3 and 4. Cheese from these trials had less moisture than did cheese made without rennet.

As with Queso Blanco cheese, there was more AFM<sub>1</sub> recovered from bakers' cheese and whey than was found in milk from which the cheese was made (Table 5). This apparent increase has been observed for other types of cheese, and may have resulted because of analytical variability (16,17) or improved recovery of AFM<sub>1</sub> after cheesemaking. Apparent increases of AFM<sub>1</sub> content have been noted in some cultured dairy products (20,21).

AFM<sub>1</sub> remained in bakers' cheese through 1 month of refrigerated storage and 2 months of frozen storage. Use of rennet did not appear to appreciably alter the distribution or concentration of AFM<sub>1</sub> in cheese or whey. AFM<sub>1</sub> in both types of bakers' cheese behaved similarly.

As was true for other varieties of cheese (8-10), use of contaminated milk will produce Queso Blanco or bakers' cheese that is contaminated with AFM<sub>1</sub>. This contamination will remain in frozen and refrigerated cheese.

Results of this research and that carried out in this laboratory but described elsewhere (3,4,8,9,10,21-25) indicate that AFM<sub>1</sub> is a relatively stable compound in dairy products.

TABLE 5. Distribution of aflatoxin in finished cheese, whey and milk from which bakers' cheese was made.<sup>a</sup>

<table>
<thead>
<tr>
<th>Trial</th>
<th>Milk</th>
<th>Cheese</th>
<th>Whey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg</td>
<td>kg</td>
<td>kg</td>
</tr>
<tr>
<td></td>
<td>AFM&lt;sub&gt;1&lt;/sub&gt; (μg/kg)</td>
<td>AFM&lt;sub&gt;1&lt;/sub&gt; (μg/kg)</td>
<td>AFM&lt;sub&gt;1&lt;/sub&gt; (μg/kg)</td>
</tr>
<tr>
<td>1</td>
<td>3.3</td>
<td>1.42</td>
<td>2.55</td>
</tr>
<tr>
<td>2</td>
<td>2.6</td>
<td>1.51</td>
<td>2.17</td>
</tr>
<tr>
<td>3</td>
<td>3.3</td>
<td>1.22</td>
<td>3.09</td>
</tr>
<tr>
<td>4</td>
<td>3.4</td>
<td>1.20</td>
<td>2.97</td>
</tr>
</tbody>
</table>

<sup>a</sup>4.5 kg of milk used to prepare cheese.

<sup>b</sup>Average of duplicate samples.

<sup>c</sup>No value.
ucts. Common manufacturing practices do not cause a loss of AFM1; AFM1 was stable in milk that was pasteurized (25) or given other heat treatments (22). AFM1 also was stable during the manufacture and subsequent storage of non-fat dried milk and dried buttermilk (23), ice cream and sherbet (24), and butter (23). AFM1 persisted during the manufacture and ripening of natural and processed cheeses (2,8,9,10) as well as cultured dairy products (21). Studies showed that AFM1 is not affected in the pH range encountered in most dairy products (22).

Bentonite, potassium sulfite, lactoperoxidase and hydrogen peroxide can remove or inactivate AFM1 in milk (3,4). In the future it may be practical to use one of these methods to remove AFM1 from milk. However, at this time the best way to deal with the problem is to prevent contamination of milk with AFM1 by insuring that dairy cows consume feed free of aflatoxin B1 or that the feed contains aflatoxin B1 at a concentration below that needed to cause occurrence of AFM1 in milk.

### TABLE 6. Aflatoxin M1 content of milk, whey and bakers’ cheese during manufacture and storage.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Aflatoxin M1 (µg/kg)</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Trial 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td></td>
<td>3.3</td>
<td>2.6</td>
<td>3.3</td>
<td>3.4</td>
</tr>
<tr>
<td>Whey</td>
<td></td>
<td></td>
<td>6.5</td>
<td>6.8</td>
<td>3.7</td>
</tr>
<tr>
<td>Initial cheese</td>
<td></td>
<td>13</td>
<td>11</td>
<td>9.0</td>
<td>11</td>
</tr>
<tr>
<td>Refrigerated cheese:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td></td>
<td>6.2</td>
<td>7.4</td>
<td>2.1</td>
<td>8.3</td>
</tr>
<tr>
<td>2 weeks</td>
<td></td>
<td>9.1</td>
<td>6.1</td>
<td>7.9</td>
<td>9.3</td>
</tr>
<tr>
<td>1 month</td>
<td></td>
<td>7.7</td>
<td>6.4</td>
<td>6.6</td>
<td>9.3</td>
</tr>
<tr>
<td>Frozen cheese:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month</td>
<td></td>
<td>6.0</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2 months</td>
<td></td>
<td>4.7</td>
<td>9.8</td>
<td>4.9</td>
<td>9.6</td>
</tr>
</tbody>
</table>

*Average of duplicate samples.
*No value.

### ACKNOWLEDGMENTS

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