ABSTRACT

The objective of this study was to ascertain whether the sensitivity of a test for vegetable fat adulteration of milk fat might be improved by considering the seasonal variation of the criteria for identifying suspect samples. The test is based on the ratio of the concentrations of butyric acid to oleic acid (C4: C18:1). Samples of milk fat that exhibit a ratio value greater than 2 standard deviations from the mean can be considered abnormal and possibly adulterated with vegetable fat. For the 6 mo of November through April, when the value of this ratio is typically high, the criteria for identifying suspect samples can be modified, that is, the minimum value of the ratio for pure milk fat can be increased. The minimum amount of vegetable fat in a sample of milk fat that can be detected using this method is reduced for 10.5% to approximately 8.2%.

The second objective of this study was to determine the suitability of the test for detecting vegetable fat adulteration of milk fat from sources other than block Mozzarella, i.e., from ice cream and Mozzarella cheese from frozen pizzas.

INTRODUCTION

A screening test has been proposed for detecting vegetable fat adulteration of milk fat in Mozzarella cheese (3). The test is based on the relative concentration of butyric and oleic acids in a sample of fat extracted from cheese. Because of the differences in the concentrations of the fatty acids in milk fat and in vegetable fat, the addition of vegetable fat to milk fat will alter the relative concentrations of butyric and oleic acids in milk fat and will lower the value of the ratio of the concentrations of butyric to oleic acid. The maximum sensitivity of the test is limited by the normal variability of the value of the ratio, which is due, to a large extent, to variability in the concentration of oleic acid in milk fat. This variability can be attributed to factors such as season, stage of lactation, type of feed, and breed of cow (1, 4, 5, 6, 7).

Whatever the cause, the variability of the concentrations of some of the fatty acids, and therefore the value of the ratio, manifests itself in a seasonal pattern. If the pattern can be predicted, the sensitivity of the test might be improved by introducing the approximate time or season the product was manufactured as a variable when assessing the results of the test. If the seasonal variation in the ratio is ignored, the effective maximum sensitivity of the test is about 10.5% added vegetable fat. The value of the ratio must be depressed to at least 0.133 (−2 SD from mean) before a sample can be considered suspect with reasonable confidence (>95%). If the seasonal variation is considered, the sensitivity of the test might be improved and the incidence of false positive results might also be reduced.

The objectives of this study are first, to determine how the inclusion of seasonal variations in the concentrations of butyric and oleic acids affect the sensitivity of the test, and second, to determine if the test can be applied to other selected dairy products. The proposed test has been previously evaluated with bulk Mozzarella cheese and butter (3). The present study will evaluate the test’s suitability for ice cream and Mozzarella cheese obtained from frozen pizzas.
TABLE 1. Seasonal variation of butyric acid, oleic acid, and ratio of butyric acid to oleic acid in 104 samples of Mozzarella Cheese.

<table>
<thead>
<tr>
<th>Month</th>
<th>Butyric CV (%)</th>
<th>Oleic CV (%)</th>
<th>Ratio CV (%)</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>Jan</td>
<td>3.97</td>
<td>24.53</td>
<td>1617</td>
<td>12</td>
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<tr>
<td>Feb</td>
<td>3.99</td>
<td>24.41</td>
<td>1657</td>
<td>11</td>
</tr>
<tr>
<td>Mar</td>
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<td>24.60</td>
<td>1729</td>
<td>10</td>
</tr>
<tr>
<td>Apr</td>
<td>4.14</td>
<td>24.15</td>
<td>1701</td>
<td>9</td>
</tr>
<tr>
<td>May</td>
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<td>25.99</td>
<td>1584</td>
<td>7</td>
</tr>
<tr>
<td>June</td>
<td>4.15</td>
<td>27.75</td>
<td>1502</td>
<td>7</td>
</tr>
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<td>28.86</td>
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<td>5</td>
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</tr>
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<td>Sep</td>
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<td>1494</td>
<td>12</td>
</tr>
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<tr>
<td>Dec</td>
<td>4.11</td>
<td>25.70</td>
<td>1613</td>
<td>11</td>
</tr>
<tr>
<td>Mean</td>
<td>4.11</td>
<td>25.98</td>
<td>1593</td>
<td>104</td>
</tr>
</tbody>
</table>

Figure 1. Monthly variation in the concentration of oleic acid in Mozzarella cheese fat and in milk fat as observed by the authors (---) and as published by Gray (—) and Lund (----). Gray and Lund data (4, 7) are for milk fat.

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MATERIALS AND METHODS

Samples (n = 104) of bulk whole milk Mozarella cheese, representative of brands manufactured in the northeastern US, were purchased from retail outlets over an 18-mo period and were analyzed for fatty acid (FA) composition by GLC (2, 3). The method for quantifying the fatty acids in the samples consisted of three steps. First, the fat was extracted from the cheese by a modified Mojonnier method. Second, the fat was saponified using 6 ml of an isopropyl alcohol-KOH solution (25 mg KOH/ml) added to .5 g milk fat and held at 100°C for 20 min. In the third step, the FA were quantified by GLC. Fifty milligrams of the dried mixture of FA-potassium salts are added to 4 ml of 80% (vol/vol) ethanol containing .25 mg/ml of C7 and 1.0 mg/ml of C17 as internal standards. A 1-μl aliquot is then injected into a gas chromatograph, Hewlett-Packard (Avon- dale, PA) model 5790. The operating conditions are as follows: 1.83-m glass column, 2-mm i.d., packed with 5% DEGS-PS, 100/120 Supelcoport (Supelco, Bellefont, PA); injection port temperature 250°C; detector temperature 250°C; helium carrier at 60 ml/min; and oven temperature 100 to 200°C with an 8°C/min increase following a 2-min delay. In addition, the chromatograph was modified with a trap to saturate the carrier gas with formic acid. The value of the ratio of butyric acid to oleic acid, C4: C18:1, was calculated for each of the samples. The mean and standard deviations for each month of sampling were determined. In addition, 41 samples of butter, 35 samples of frozen pizza, and 12 samples of ice cream were tested.

An additional step in sample preparation of cheese from frozen pizzas was necessary. The cheese was removed from the surface of the pizza while it was still frozen and it was then washed in distilled water to remove traces of...
RESULTS AND DISCUSSION

Seasonal Variation of Ratio

The mean observed value of the ratio $C_4: C_{18:1}$ for the entire population of tested Mozzarella cheese samples was .1593 with a standard deviation of .0130 (CV = 8.16%). Because the cheeses were commercial brands purchased at retail outlets, exact dates of manufacture were unobtainable. For the purposes of this study, 8 wk prior to the expiration date on the package was used as the estimated date of manufacture of the bulk Mozzarella cheese (K. M. Nilson, Dairy Products Technology Center, California Polytechnic University, personal communication).

The seasonal trends of butyric acid, oleic acid, and of the ratio are shown in Table 1. Analysis of variance indicated that there were significant differences in the monthly values of the ratio ($P<.01$). The observed monthly trend for oleic acid was similar to that observed in other parts of the world by Gray (4) in New Zealand and by Lund (7) in Denmark (Figure 1). Because there is a very strong correlation (> .99) between the concentration of oleic acid and the value of the ratio, it is expected that the ratio also would exhibit a similar trend in these parts of the world.

For the 6 mo of November through April (Figure 2), when the average value ratio is high, the minimum value of the ratio that can be considered normal can be increased to allow for the detection of smaller concentrations of added vegetable fat. For those months, the average value is .1652 with a SD of .0108 (CV = 6.48%). For the remaining 6 mo, the average value is .1513 with a SD of .0114 (CV = 7.53%). The differences between the grouped monthly values were significant ($P<.01$). Under
these conditions, normal value cutoff could be raised from about .133 to about .144 for products manufactured between November and April. A sample of milk fat with a ratio value at the high end of normal, approximately .185, would require an adulteration at a level of about 8.2% vegetable fat to achieve this degree of depression of the ratio. Using the .133 value, the level of adulteration would need to be about 10.5% to be detected. This is an increase in sensitivity of about 20% for milk fat samples at the high end of normal.

Other Products

There were 104 samples of bulk Mozzarella cheese, 41 samples of butter, 35 samples of cheese pizza, and 12 samples of ice cream tested by the method. There were no significant differences (P<.05) in the value of the ratio among any of the products. This suggests that the test can be applied to all these products successfully.

Samples of frozen pizza labeled as containing imitation cheese as well as real cheese were also analyzed. The value of the ratio from these samples averaged .0150 and ranged from .02 to .005. By extrapolating from the response curve of the addition of vegetable fat to milk fat (3) (Figure 3), we can assume that these values would correspond to approximately 70 to 90% vegetable fat.

Time Requirements

A technician experienced in preparing the sample and the operation of a gas chromatograph can analyze a single sample in about 135 min, and if multiple samples are tested simultaneously and an automated GLC injection and integration system is used, 12 samples can be analyzed in about 11 h.

CONCLUSIONS

The overall sensitivity of the test for vegetable fat adulteration can be increased to approximately 8.2% vegetable fat in milk fat (from 10.5%) by considering the general time of manufacture of a sample of Mozzarella cheese when interpreting the test results. In addition to bulk Mozzarella cheese and butter, the test is also appropriate for use with ice cream and Mozzarella cheese from frozen pizzas.

ACKNOWLEDGMENTS

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REFERENCES