Detection of Vegetable Oil Adulteration in Ice Cream

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ABSTRACT
This study was to demonstrate the application of various analytical methods to the detection, identification, and quantitation of vegetable oil adulteration of ice cream. Total fat content, sterols, long- and short-chain fatty acids, vitamin E, Reichert-Meissl values, and Polenske values were measured in ice cream. All methods except total fat determination were capable of detecting vegetable oil adulteration. Sterol determination was the most effective and versatile measurement because it provided information not only on the detection and extent of adulteration but also on the possible identity of the adulterant.

INTRODUCTION
Definitions and standards for ice cream are described in detail in the Code of Federal Regulations (6), which states that "ice cream contains not less than 10 percent milk fat . . . ." Definitions and standards for milk and cream are also stated in the regulations (7). Thus, a clear distinction is made between ice cream and products that simulate ice cream with various amounts of nonmilk fat, i.e., vegetable fats and oils and other animal fats. On occasion a few, generally small firms have placed into interstate commerce some ice cream substitutes or imitations that do not meet the specifications described in the regulations. No health or safety issues are involved but rather instances of adulteration and misbranding.

The adulteration of fats and oil, including milk fat, has been of concern to chemists for more than a century. Roos (16) in 1963 summarized and discussed the methods available for the detection of, and the problems associated with detection of, adulteration of milk fat by foreign fat(s). He indicated that no single general method available for detecting milk fat adulteration was applicable to all possible adulteration situations, but rather, a number of methods were available for the determination of various physical and chemical characteristics, e.g., fatty acids, sterols, tocopherols, triglycerides, color, and unsaponifiable composition. The information obtained from these determinations forms a basis for the detection of adulteration and possible identification and quantitation of the adulterant. Roos discussed classical methods, including the sterol acetate method (10) and microbiological method (11) for detecting phytosterol. Many of these methods are still in use (1, 3, 4, 15). He introduced the subject of gas chromatography (GC), but its applications were not explored in detail. The advances in GC since 1963 have made the determination of fatty acids (2, 13, 17, 18) and sterols (5, 8, 12, 17, 19) relatively rapid and simple compared to classical methods.

The purpose of this paper is to alert the scientific community that adulteration of ice cream is occurring and to demonstrate some of the available scientific approaches and their application to solve quality control and regulatory problems of adulteration. New methods are not described but rather some selected existing analytical methods (1, 13, 14, 17) used to detect, identify, and quantitate adulterants are evaluated so that they may be used by quality assurance and regulatory chemists. The Association of Official Analytical Chemists sterol acetate melting point method (3) and microcrystal test (4), which are effective for the identification but not the quantitation of vegetable fats in butter fat, were not evaluated. An absorbance method not described here has been used effectively to detect adulteration by the Minnesota Department of Agriculture (H.E.)

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2Analytical data used to develop graphic presentation are available from the authors on request.

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RESULTS AND DISCUSSION

Total Fat

The average fat (lipids) content of the samples is presented in Table 2. All samples met the basic minimum requirement of 10% fat content (6), as expected, because testing fat content is the simplest and most basic test that would be made, and the potential adulterator would hope that sample testing would stop at this point.

Sterols

The sterol pattern will be discussed before fatty acid determination because the former can be used to identify the source of fats and oils and is the basis for classifying the amount of vegetable fat found.

The butyrate derivatives are prepared from the sterols contained in an aliquot of the long-chain fatty acid (LCFA) methyl esters. The hydroxyl group at the 3-position of the A-ring of a sterol does not react during the preparation of the fatty acid esters. Also, the fatty acid esters are not affected by the butyrating reagent mixture. During GC of the sterol butyrates, the fatty acid methyl esters

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Description</th>
<th>Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vanilla</td>
<td>11.8</td>
</tr>
<tr>
<td>2</td>
<td>Butter pecan</td>
<td>14.0</td>
</tr>
<tr>
<td>3</td>
<td>Vanilla</td>
<td>10.9</td>
</tr>
<tr>
<td>4</td>
<td>Vanilla</td>
<td>13.8</td>
</tr>
<tr>
<td>5</td>
<td>Vanilla</td>
<td>10.5</td>
</tr>
<tr>
<td>6</td>
<td>Neapolitan vanilla, chocolate, strawberry flavored</td>
<td>10.9</td>
</tr>
<tr>
<td>7</td>
<td>Vanilla</td>
<td>10.5</td>
</tr>
<tr>
<td>8</td>
<td>Vanilla</td>
<td>10.4</td>
</tr>
<tr>
<td>9</td>
<td>Vanilla, chocolate, strawberry flavored</td>
<td>10.6</td>
</tr>
<tr>
<td>10</td>
<td>Vanilla</td>
<td>10.7</td>
</tr>
<tr>
<td>11</td>
<td>Coffee</td>
<td>10.4</td>
</tr>
<tr>
<td>12</td>
<td>Neapolitan vanilla, chocolate, strawberry flavored</td>
<td>10.3</td>
</tr>
</tbody>
</table>

1 Average of four determinations.

MATERIALS AND METHODS

Methods

Seven methods were used to measure characteristics of ice creams to provide information on type(s) of fat present. The determinations, methods, and references are summarized in Table 1. The GC conditions for determining the short-chain fatty acid (SCFA) butyl esters (13) were slightly revised as follows: 8 ft x 3 mm i.d. Pyrex column packed with 10% Silar 10C coated on 100/120 mesh Gas-Chrom Q; 270°C injector and detector temperature; 50 ml/min carrier gas flow; column temperature programmed at 70°C for 4 min, then 5°C/min to 130°C, followed by 10°C/min to 245°C. All other methods were used as published. If ice creams contained nuts, they were removed before sample analysis. Each sample was analyzed four times with each method.

Samples

Twelve samples were examined. Four samples were authentic ice creams. The other eight samples were collected from the northeastern United States during 1980 and early 1981 because of suspected adulteration. All samples were labeled as ice cream and gave no indication of possible substitution of vegetable oil for part or all the required milk fat. The samples are briefly described in Table 2.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat (lipids)</td>
<td>Gravimetry</td>
<td>(17)</td>
</tr>
<tr>
<td>Long-chain fatty acids</td>
<td>GC</td>
<td>(17)</td>
</tr>
<tr>
<td>Short-chain fatty acids</td>
<td>GC</td>
<td>(13)</td>
</tr>
<tr>
<td>Sterols</td>
<td>GC</td>
<td>(17)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Colorimetry</td>
<td>(14)</td>
</tr>
<tr>
<td>Reichert-Meissl value</td>
<td>Titration</td>
<td>(1)</td>
</tr>
<tr>
<td>Polenske value</td>
<td>Titration</td>
<td>(1)</td>
</tr>
</tbody>
</table>

1 Gas chromatography.
The concentration of the individual sterols in each sample was quantitatively determined using the external standard method. The sterol patterns shown by samples 5 and 6 were similar to those for authentic ice creams (Figure 1B). Samples 11 and 12 had sterol patterns similar to that of soybean oil (Figure 1D), indicating essentially a 100% substitution of soybean oil for milk fat in these samples. Samples 7 to 10 had sterol patterns intermediate between 1B and 1C, indicating an approximately 50% substitution of soybean oil for milk fat. The percent of dilution (substitution) of soybean oil for milk fat in these ice cream samples was calculated according to the following equations:

\[
\text{Percent of milk fat} = \left( \frac{\text{cholesterol content of sample}}{\text{mean cholesterol content of authentic ice cream}} \right) \times 100
\]

\[
\text{Percent of dilution} = 100 - \text{percent of milk fat}
\]

The data for each sterol concentration were plotted against percent of dilution and are shown in Figures 2 and 3. Each of the individual sterols gave a linear response for concentration vs. percent of dilution. In addition to cholesterol, \( \beta \)-sitosterol is the most suitable plant sterol to use to calculate the amount of adulteration because the slope of its curve is greater elute immediately after the solvent front and before the sterol peaks (17, 19) (Figure 1).

Milk fat contains mainly cholesterol and no significant amounts of plant sterols (9). Vegetable oils contain little or no native cholesterol in contrast to animal fats (12, 21). Sterol patterns vary with vegetable oil type, i.e., the ratio of the three major plant sterols, \( \beta \)-sitosterol, stigmasterol, and campesterol, will be different for different oils. Also, in some types of oil such as rapeseed, brassicasterol, a sterol unique to that oil, will be detected and can be used to estimate the amount of rapeseed oil present. Thus, the determination of sterol pattern is very useful not only for detection but also for identification of a vegetable oil.
Long-Chain Fatty Acids

Most food laboratories routinely perform LCFA determinations. If the vegetable oil and the degree of hydrogenation have been carefully selected, it can be difficult to find significant differences in the LCFA pattern of the adulterated sample vs. a sample containing milk fat. However, relationships between acids often may be useful, e.g., oleic acid is usually more prevalent in vegetable oils than in animal fats and the converse is generally the case for palmitic acid, although concentration differences are not as pronounced (18, 20). These general relationships were observed for the products examined and are shown in Figure 4. Changes in oleic acid or palmitic acid concentration, or the first linear regression equation, or the ratio of oleic acid to palmitic, or the graphed results may be used to estimate the degree of adulteration.

Short-Chain Fatty Acids

Short-chain fatty acids are somewhat more difficult to determine than LCFA because of volatility and water solubility problems. However, the formation of the butyl derivative overcomes both problems (13). Although each acid from a carbon chain length of one through ten forms individual straight weight line plots, butyric acid was selected because it is present in
ADULTERATION DETECTION IN ICE CREAM

Figure 6. Relationship between vitamin E content and percent dilution by vegetable oil in ice cream fat.

highest amount in milk fat. Although the butyric acid content in these authentic ice creams showed greater variability than normal, a negative linear relationship between the amount of butyric acid and the amount of vegetable oil present was obtained (Figure 5). The amount of adulteration can easily be determined graphically, using the calculated first linear regression equation or using proportion type calculation.

Conclusions

All methods used except total fat determination were effective for the detection of vegetable oil adulteration of ice cream. The sterol determination is the most versatile based on the information it provides the analyst, i.e., detection of adulteration, extent of adulteration, and an excellent possibility of identifying the vegetable oil used as the adulterant. The detection and quantitation of other animal fats in milk fat is generally more difficult because there is no distinct difference in sterol patterns among animal fats. However, based on the data obtained from the determination discussed in this report, identification and possible quantitation of related oils with intermediate chain fatty acids. The R-M and P plots (16) can be used to detect vegetable oil adulteration of ice cream (Figure 7). The R-M can be used alone since the differences in R-M for vegetable oil vs. milk fat are more pronounced than the P. The P alone is not of much use because the slope of the plot between 100% vegetable oil and 100% milk fat is of low magnitude. The more practical use of the R-M and P is to establish the ratio between them as related to the amount of adulteration. Graphic plots from experimentation or the first linear regression equation can be used to read the percent dilution (adulteration).

Vitamin E

Vitamin E is present in milk fat as well as in vegetable oil but is considerably higher in the latter (14). Figure 6 shows a linear relationship between tocopherol content and percent dilution. The vitamin E content of samples can be compared with those of genuine ice cream and vegetable oil, and the degree of adulteration can be calculated. The data used to produce Figure 6 indicate that adulteration of milk fat with vegetable oils may be detected based on vitamin E measurements.

Reichert-Meissl and Polenske

The Reichert-Meissl value (R-M) (1) is a measure of the amount of soluble volatile fatty acids and is a classical way to detect adulteration in milk fat. The Polenske value (P) (1) is a measure of the amount of insoluble volatile fatty acids and is useful to detect coconut and

Figure 7. Relationship between Reichert-Meissl (R-M) and Polenske (P) values and percent dilution by vegetable oil in ice cream fat.
animal fat adulteration in milk fat can also be achieved. Use of a sufficient number of methods has demonstrated that the regulatory analyst can develop an analytical interpretation based on several chemically unrelated methods to form a solid case for legal purposes.

REFERENCES