Instrumental Milk Fat Determination. I. Effects of Potassium Dichromate Concentration and Sample Storage Time on Milko-Tester Results

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Abstract
At 0.08 to 0.80% levels of $K_2Cr_2O_7$ the milk fat content determined with a Milko-Tester Automatic was not measurably affected up to 3 days storage at 21 to 27 C. Official methods recommend 0.21%. A depression of less than 0.05% in the fat percentage is detectable by the Milko-Tester Automatic after three days of sample storage. Deviations of more than 0.05% between duplicates and fat percentage depressions of more than 0.1% occurred with and after the fifth day. Storage results at Days 7 and 12 were still more unsatisfactory. A reinvestigation of milk sample preservatives is recommended to establish criteria of use and quality for users and manufacturers.

Introduction
One of the questions often raised with regard to milk sample preservation is whether the amount of preservative added affects the results of milk fat determinations.

Common preservatives are mercuric chloride ($HgCl_2$), potassium dichromate ($K_2Cr_2O_7$) and formalin (36 to 40% aqueous solution of formaldehyde, HCHO). Use recommendations vary with states, countries and manufacturers. For the official determination of fat in milk in the United States, samples should be preserved with "tablets containing $HgCl_2$, $K_2Cr_2O_7$, or other suitable preservative, at least 0.5 g active ingredient per tablet for each 8 fluid ounce of milk, but total weight of such tablet of not more than 1 g, or 35% solution of HCHO, 0.1 ml (2 drops) per fluid ounce" (2).

In the United States, $HgCl_2$ is the preservative generally used in milk plants. It is poisonous and corrosive, is an effective preservative, and usually is available in two tablet sizes of about 0.5 and 1 g. About 46.7% of a tablet is $HgCl_2$, the remainder is inert filling and binding material such as sodium chloride, acacia and mineral oil. Because of its mercury content and eventual disposal with sewage, the dairy industry may experience a shift away from this preservative because of increasingly adverse publicity that may be generated by mercury found in our environment. During the preparation of this manuscript the Pesticides Regulation Division, Agricultural Research Service, U.S. Department of Agriculture, was reviewing all pesticide uses of mercury (Federal Register, December 3, 1970, p. 18409). This review may or may not affect the dairy industry or require a revision of milk sample preservation recommendations.

Manus and Bendixen (3, 4) stated that of several milk sample preservatives, $HgCl_2$ reduced Babcock fat readings of composite and single milk samples more than did $K_2Cr_2O_7$.

Armandola (1) investigated the suitability of five different milk sample preservatives and concluded that $HgCl_2$ (0.1%) and $K_2Cr_2O_7$ (0.1%) were the best.

Potassium dichromate is commonly used by Dairy Herd Improvement Associations. Because of its relative noncorrosiveness it was selected for Milko-Tester use. It is also the accepted preservative for milk samples in Germany where recommended amounts are 0.1 to 0.2%. Several manufacturers produce $K_2Cr_2O_7$ tablets. In this study we used tablets distributed by NASCO (Fort Atkinson, Wisconsin). The average weight of a tablet was 100 mg, containing 41 mg of the active ingredient. Consequently, 1 tablet in 100 ml milk amounts to about 0.04% of $K_2Cr_2O_7$.

This study ascertained the effect of concentration of $K_2Cr_2O_7$ (and accompanying substances) on the fat content of milk samples and also the effect of length of preservation time. Effective preservation time refers to bacteriostatic and bactericidal effects and to all other physicochemical phenomena that disrupt milk and thereby interfere with accepted ways of taking sample aliquots, such as mixing and subsequent pipetting.
Experimental

Raw mixed herd milk with 3.59% fat was used. All fat percentages were obtained only with a Milko-Tester Automatic (MTA). Reproducibility of Milko-Testers is extremely good. No deviations of more than 0.02% in the fat content of the 3.59% milk were found in multiple serial analyses.

Milk agitated gently and constantly was dispensed from a can in 50-ml amounts into 6-oz. Whirl-Pak plastic bags (NASCO, Fort Atkinson, Wisconsin), containing from 1 through 10 K₂Cr₂O₇ tablets. Thus, on closing the bags and dissolving the tablets by gentle shaking, concentrations of 0.08, 0.16, 0.24, etc., up to 0.80%, were obtained. Normally observed use is generally near 0.04%. All samples were prepared in duplicate. They were kept in an open rack at 21 to 27 C. At Days 3, 5, 7 and 12 samples were analyzed. The milk in each bag was mixed to ensure incorporation of the cream layer; further mixing was with the instrument stirrer. Each bag was sampled only once to avoid the errors usually caused by excessive and repetitive handling and stirring.

Results and Discussion

The results are in Table 1. Addition of K₂Cr₂O₇ tablets slightly depressed fat percentages at the third day of sample storage, but they were not depressed more by increased preservative. Thomasow and Mrowetz (5) reported a slight increase in the fat content of milk preserved with 0.1 and 0.2% K₂Cr₂O₇, and also with 0.4 and 0.8%. The deviation is in the third significant figure and would most likely not be detected with the Gerber or Babcock method. The Röse-Gottlieb (Mojonnier) method, however, indicated the expected decrease in fat percentages with increasing dilutions of the samples with added potassium dichromate. They (5) also indicated no significant change in the fat of milk samples with different fat percentages (1.35 to 7.82%) with 0.3% K₂Cr₂O₇.

After more than five days of storage the results were substantially depressed. This is probably due to changes in physical structure of the aging milk samples. Moreover, agreement of duplicates became worse with storage beyond the fifth day. Again, preservative concentration had no influence. At Day 3 all 10 sample duplicates agreed closely, differing not at all or by less than 0.05%. Such deviations can usually not be detected with the Babcock method. At Day 5, 2 of 10 duplicates differed by more than 0.05%, whereas at Days 7 and 12 there were 6 out of 10 and 9 out of the 10 samples, respectively, that differed by more than 0.05% between duplicates.

The initial bacterial count of a milk sample and the species of microorganisms also determine the length of effective preservation. Refrigeration of samples does extend this time. Samples preserved with K₂Cr₂O₇ will eventually sour and coagulate. This is accompanied by milk changing from yellow to grey-green, an indication of a reduction, most likely by reducing bacteria or by acid conditions, of the orange hexavalent chromium anion in dichromate (Cr₂O₇²⁻) to the trivalent chromium ion which

Table 1. Milk fat percentages (average of two) of samples containing various amounts of potassium dichromate and analyzed with a Milko-Tester Automatic after 3, 5, 7, and 12 days of storage. Initial fat in milk was 3.59%.

<table>
<thead>
<tr>
<th>Number of preservative tablets/50 ml milk</th>
<th>Preservative (%)</th>
<th>Estimated fat content (%)</th>
<th>3 Days</th>
<th>5 Days</th>
<th>7 Days</th>
<th>12 Days</th>
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<tbody>
<tr>
<td>1</td>
<td>0.08</td>
<td>3.56</td>
<td>3.46</td>
<td>3.44</td>
<td>3.20</td>
<td></td>
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<tr>
<td>2</td>
<td>0.16</td>
<td>3.54</td>
<td>3.52</td>
<td>3.45</td>
<td>3.27</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.24</td>
<td>3.56</td>
<td>3.33</td>
<td>3.33</td>
<td>3.10</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.32</td>
<td>3.54</td>
<td>3.48</td>
<td>3.46</td>
<td>3.26</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.40</td>
<td>3.57</td>
<td>3.48</td>
<td>3.47</td>
<td>3.26</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.48</td>
<td>3.58</td>
<td>3.44</td>
<td>3.35</td>
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<tr>
<td>7</td>
<td>0.56</td>
<td>3.57</td>
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<td>3.42</td>
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<td>3.54</td>
<td>3.48</td>
<td>3.36</td>
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<td>9</td>
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<td>3.58</td>
<td>3.46</td>
<td>3.38</td>
<td>3.24</td>
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<tr>
<td>10</td>
<td>0.80</td>
<td>3.56</td>
<td>3.46</td>
<td>3.42</td>
<td>3.16</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>3.56</td>
<td>3.46</td>
<td>3.41</td>
<td>3.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
may appear as green Cr\(^{3+}\) or Cr(OH)\(_4\)\(^{-}\) in solution.

Based on these results, the following observations and recommendations can be made:

1. Potassium dichromate at 0.08 to 0.80% may be used in milk samples stored at 21 to 27 C without changes in fat percentage, if fat is determined within three days.

2. After five or more days of milk sample storage at 21 to 27 C the fat content will be at least 0.1% lower.

3. To conform with AOAC (2) recommendations, and to obtain 0.5 g active ingredient per tablet for each 8 fluid ounces milk (0.21%), it would be necessary to use 12 to 13 NASCO tablets, each containing 41 mg K\(_2\)Cr\(_2\)O\(_7\) for 8 ounces of milk.

4. A reinvestigation of milk sample preservatives should be initiated to bring actual practice and official recommendation together, to encourage standardization and more encompassing labeling for preservative tablets, and to propose other effective preservatives.

5. Coagulated milk samples and those in which the original yellowish dichromate has changed cannot be accurately analyzed for fat by the Milko-Tester.

Extrapolation of these results to other conditions should not be made. Investigations as to the effects of other preservatives, composite samples, and sample care have begun or are expected to be carried out later.

Acknowledgments

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References


Simplified Procedure for Determining Fat and Total Solids by Mojonnier Method

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Abstract

Following extraction of milk fat with the Mojonnier method, defatted solids may be determined by drying the residue which remain in the extraction flask after the solvents are removed. Different techniques may be used to determine solids and results agree with official methods.

Introduction

Solids-not-fat may be easily determined from the residue which remains in the Mojonnier extraction flask after removing the solvents. Fat may be determined following the traditional procedure. Solids-not-fat may be determined by evaporating the water, ammonia and ether on a steam bath or with another known method for estimation of solids. This technique is useful because it allows two determinations on the same sample. Evaporation of the defatted solids suspension does not present the problems of normal determinations where fat sometimes burns.

Experimental Procedure

It is important that reagents for the Mojonnier method are used in the right amounts to obtain complete fat extraction. The appearance of a gelatinous emulsion in the flask after ether is added indicates insufficient alcohol, then