Identification and Quantitative Determination of 2-Furfurol in Sterilized Concentrated Milk

Abstract

A large peak appearing in the gas chromatograms of flavor concentrates obtained by vacuum steam distillation of stale sterilized concentrated milk (SCM) was identified as 2-furfurol by gas chromatography combined with mass spectrometry. Quantitative gas chromatography demonstrated that the amount of 2-furfurol in SCM increased rapidly through 26 weeks storage at 27 °C. The amount of 2-furfurol in samples stored for 26 weeks exceeded the flavor threshold (10-12.5 ppm) for this compound in milk. This suggests that 2-furfurol formation contributes to the flavor deterioration of SCM.

The unknown component was collected from three successive chromatographic runs in 1.8-mm by 100-mm glass capillary tubes cooled with dry ice. The collected fraction was subsequently analyzed by capillary column GLC in conjunction with mass spectrometry (MS).

An F & M Model 810 gas chromatograph equipped with a 0.254-mm-id by 91.44-m capillary column coated with butanediol succinate (BDS) was utilized for separation of components of the trapped fraction. Total effluent from the capillary GLC column was directed to an Atlas CH-4 mass spectrometer equipped with a double ion source. Operating parameters for GLC-MS analysis were as follows:

**GLC**
- Column: BDS
- Column temperature: 150 °C
- Carrier gas flow: one ml/min
- Injector temperature: 250 °C

**MS**
- Attenuation: ×10 initially
- Electron voltage: 20 eV and 70 eV
- Filament current: 20 eV source—45 μA
- Acquiring voltage: 70 eV source—12 μA
- Accelerating voltage: 3,000 V
- Multiplier voltage: 1.6 KV
- Analyzer pressure: 1.5 × 10⁻⁶ Torr
- Scanning speed: 5 sec from m/e 25 to m/e 250

The mass spectrum of the unknown component was as follows: m/e 98 (molecular ion, 100%), 39 (98%), 41 (97%), 42 (77%), 53 (77%), 54 (62%), 29 (64%), 97 (53%), 27 (47%), 69 (43%), 70 (37%), 31 (35%), 51 (30%). This is in excellent agreement with the published mass spectrum of 2-furfurol (1).

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References
The retention time of 2-furfurol was identical to that of the unknown component on both the Carbowax packed and BDS capillary columns. The unknown component was concluded to be 2-furfurol. Patton and Josephson (6) first isolated and characterized 2-furfurol in heated skim milk in 1949.

The size of the 2-furfurol peak in chromatograms of flavor concentrates from stale SCM prompted an interest in the concentration of 2-furfurol in fresh and stored SCM samples. Quantitative information was obtained using the gas entrainment, on-column trapping GLC technique of Morgan and Day (3). Ten-milliliter samples of SCM diluted to whole milk concentration with distilled water were employed. Samples were submerged in a 90°C water bath and purged with nitrogen 10 ml/min for 10 minutes. A 0.32-em-od by 3.66-m column packed with 2.5% Carbowax 20 M on 80-100 mesh Chromosorb G was employed for separation of components. A standard curve of recorder response (peak height) vs. 2-furfurol concentration was prepared by analyzing samples of fresh whole milk containing varying concentrations of added 2-furfurol. Five parts per million of n-octanol was used as an internal standard in all samples to correct for variations in recorder response.

Recorder response was found to be linear over the range 0-100 ppm of 2-furfurol in milk. The concentration of 2-furfurol in SCM was thus determined by comparing corrected recorder response for the 2-furfurol peak with the standard curve. The concentrations of 2-furfurol found in fresh SCM and SCM stored for various periods of time are shown in Table 1.

Information regarding the flavor threshold of 2-furfurol in milk was not available. Therefore, the threshold was determined by presenting a series of samples of fresh whole milk containing varying amounts of added redistilled 2-furfurol to a trained flavor panel of ten judges. Positive and negative reference samples also were presented. The series of samples was presented to the panel on two different occasions, and panel members were asked to indicate if they could detect 2-furfurol in the samples. A plot of concentration of 2-furfurol vs. per cent positive response was prepared. The average flavor threshold was considered to be the concentration at which the plot crossed the 50% positive response line (6).

The average flavor threshold for 2-furfurol in milk was determined to be 10-12.5 ppm. Comparing this value with the concentrations of 2-furfurol found in stored SCM samples, it is apparent that 2-furfurol exceeds its threshold concentration after 26 weeks of storage at 27°C. Considering the possibility of additive interaction between 2-furfurol and other flavor compounds, levels less than 10-12.5 ppm might also be significant. The data suggest that 2-furfurol becomes increasingly important to the flavor of SCM during storage.

Considering the relatively high levels of 2-furfurol found, it is interesting to note that this compound is not among the list of compounds identified in stored milk products (5). When the techniques used in previous investigations are considered, the reason becomes apparent. The majority of the workers were looking specifically for carbonyl compounds using carbonyl derivative reagents, or were using headspace techniques. Both of these techniques would preclude the identification of 2-furfurol. Two exceptions are the investigations of Muck et al. (4) and Arnold et al. (2), who used solvent partitioning and solvent extraction techniques in their isolation schemes. In both cases, hydrocarbon solvents were used. It appears that 2-furfurol, being completely miscible with water, was not partitioned into the extraction solvent. Hence, 2-furfurol was not isolated and identified until flavor concentrates were recovered directly from SCM by vacuum steam distillation in this investigation.

Acknowledgments

The assistance of Dr. L. M. Libbey in obtaining mass spectra is greatly appreciated.

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References


J. DAIRY SCIENCE Vol. 51, No. 2

TABLE 1

Average concentration of 2-furfurol in stored sterilized concentrated milk, expressed as parts per million on whole milk basis

<table>
<thead>
<tr>
<th>Commercial sample</th>
<th>Weeks storage at 27°C</th>
<th>ppm 2-Furfurol</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>0</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>25.3</td>
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<tr>
<td>B-1</td>
<td>0</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>15.3</td>
</tr>
<tr>
<td>B-3</td>
<td>130</td>
<td>57.0</td>
</tr>
</tbody>
</table>

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Quality of Commercial Buttermilks 1

Abstract

Buttermilks from ten regional dairies were judged for flavor and analyzed for acetaldehyde, diacetyl, volatile acidity, and microbial quality. Large variations in flavor score and in concentration of acetaldehyde, diacetyl, and volatile acidity were observed. In none of the samples was the diacetyl:acetaldehyde ratio near the 4:1 value reported to be necessary for a good flavor balance. In contrast to the objective chemical data, the microbial quality of these buttermilks was good.

In recent years, much progress has been made in the chemical definition of the characteristic flavor of cultured buttermilk. It is now accepted that lactic acid, diacetyl, acetaldehyde, dimethyl sulfide, and acetic acid are the most important flavor compounds in buttermilk (4), and the quantitative relationships of these compounds are important in defining buttermilk flavor. Nevertheless, the development of sufficient amounts of desirable culture flavor and aroma is still one of the major problems confronting the cultured dairy products industry.

A number of methods for providing enhanced culture flavor have been proposed. These include the use of modified cultures, starter distillates, and direct addition of diacetyl to the product. These methods improve flavor, but the attainment of uniform, desirable culture flavor is not easily achieved (4). Recently, Lindsay et al. (4) described the formulation of butter culture flavor concentrates. Their results indicated that a large preference panel showed equal preference for high quality naturally cultured products and artificially flavored products. The present study was made to show the variation in commercially available buttermilk and provide additional support for the use of synthetic flavor concentrates in the manufacture of this product.

Experimental Procedure

Buttermilk samples from ten different re-

1 Supported in part by funds granted by the American Dairy Association.

J. Dairy Science Vol. 51, No. 2


