Analyzing volatile compounds in dairy products

Michael H Tunick*

Abstract

Volatile compounds give the first indication of the flavor in a dairy product. Volatiles are isolated from the sample matrix and then analyzed by chromatography, sensory methods or an electronic nose. Isolation may be performed by solvent extraction or headspace analysis, and gas chromatography is often employed with various detectors to identify odorants. The human nose is also used as a detector, and electronic noses are being developed to qualitate and quantitate volatiles. A reliable technique for analyzing odorants in dairy products has not yet been invented.

HISTORY

The first aspect of flavor that a consumer encounters is aroma, which consists of volatile compounds. Scientists started to investigate volatiles in cheese and other dairy products in the 1890s and began to publish papers and books about the subject in the following decade. For many years, volatile compounds had to be identified by tedious wet chemical methods and many volatiles went undetected. For instance, a 1910 publication described the separation of volatile organic acids from cheese as follows: samples weighing 250–300 g were ground with sand, dilute H₂SO₄ was added, the mass was distilled with steam, a liter of distillate was neutralized with Ba(OH)₂ and redistilled, the solution was evaporated to 150–200 mL, 0.1N H₂SO₄ was added, the resulting BaSO₄ was filtered off, and the acids were separated by fractional distillation.¹ The largest molecule found by this method was hexanoic acid.

The study of volatile compounds in dairy products was limited until the emergence of chromatography in the mid 1940s. Gas chromatography (GC) was first described in 1952² and was coupled with mass spectrometry (MS) a few years later, allowing for easier identification of volatiles.³ A 1957 review of flavor chemistry of milk, butter and cheese showed that a relative handful of major compounds in these products was known; GC and MS were mentioned just once in the article.⁴ Progress in GC determination of volatiles in dairy products then accelerated,⁵ becoming the technique of choice in most applications. Researchers interested in aromas soon began to sniff the GC effluent to match odors with compounds, leading to development of gas chromatography with detection by olfactometry (GC-O). The first practical GC-O method was reported in 1971⁶ and has provided a new dimension in odor identification. The electronic nose, which has been the focus of much research over the past 15 years may ultimately prove to be a reliable technique for the determination of key odorants in dairy products.

ISOLATION OF VOLATILES

Volatile compounds have to be isolated from the sample before they can be analyzed. Isolation techniques may result in bias during an analysis since volatiles are released at different rates and thermally released or non-volatile artifacts may be introduced.⁷ The following sections describe the most common methods of separating volatile compounds from the sample matrix.

Solvent extraction

Procedures for isolating volatiles from dairy products include solvent extraction, vacuum distillation, simultaneous distillation with solvent extraction (SDE) and solvent-assisted flavor evaporation (SAFE). Advantages and drawbacks of these techniques are shown in Table 1.

In solvent extraction, samples are shaken in the solvent of choice and the liquid is evaporated or centrifuged. Lipids are also soluble in organic solvents and have to be separated from the aroma compounds.⁸,⁹ Vacuum distillation requires 4 h of distillation under high vacuum and mild heat, with condensation of the volatile compounds and subsequent solvent extraction.¹⁰ SDE was devised in 1964 and consists of boiling an aqueous solution or slurry of sample in one flask while boiling solvent in a second flask; the vapors are mixed, condensed and separated by density.¹¹ SAFE was first described in 1999 and involves distillation under high vacuum during solvent extraction.¹²

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Two other solvent extraction methods are steam distillation and supercritical fluid extraction (SFE). Steam distillation is not recommended for dairy products because artifacts are produced at the elevated temperature. SFE, which uses supercritical CO2 as the solvent, removes lipids as well as flavor compounds and is applicable to samples containing little fat, which would tend to have low concentrations of volatiles.

The most-cited papers describing solvent and distillation extraction of volatiles from representative dairy products are listed in Table 2. The SAFE papers are from the past decade, but three of the other publications date back to the 1960s and three more are at least 24 years old. Solvent extraction and vacuum distillation appear to have been supplanted by headspace methods.

### Headspace methods

The volatiles emanating from a sample in a sealed container may be collected from the headspace without solvent extraction. Sampling of the headspace may be performed by using inert gas to displace volatiles from the sample on to an adsorbent (dynamic or purge-trap methods) or in equilibrium (static methods). A comparison of the techniques is shown in Table 3.

In purge-trap, volatiles are stripped from the sample with a flowing inert gas, concentrated in a cold trap or adsorbed on an inert polymer, and then thermally desorbed or eluted with a solvent. In static sampling, 1 mL is withdrawn from the headspace and analyzed. Static headspace methods would appear to be the best way of analyzing volatiles coming from a sample, but their lack of sensitivity means they are not applicable to less volatile and less abundant compounds. These shortcomings are mostly overcome by solid-phase microextraction (SPME), which was first described in 1990 and greatly shortens the time required to analyze samples. SPME consists of a 1 cm fiber (a piece of fused silica coated with a liquid phase and attached to a plunger) that absorbs the volatiles and then desorbs them into a gas chromatograph injection port. The method was first used for cheese in 1996 and now appears to be the technique of choice for instrumental flavor analysis of dairy products. A newer variation of SPME is solid-phase aroma concentrate extraction, in which a high-capacity absorbent is coated on a 9 cm stainless steel rod. Static methods such as SPME are semi-quantitative and are better suited for qualitative purposes.

Stir bar sorptive extraction was introduced in 1999 as a solvent-free method of concentrating volatiles. A stir bar coated with polydimethylsiloxane is suspended in the headspace or used to stir a liquid sample. After desorption, the bar has to be rinsed in distilled water to remove non-volatiles, thus adding a handling step. The bar is dipped on a tissue to dry and the compounds are desorbed thermally or by a solvent. The differences in partition coefficients of the volatiles may affect the efficiency of the extraction, however.

Table 4 lists the most-cited papers describing the extraction by headspace methods of volatile compounds from representative dairy products. All but one of these papers was published in the last 18 years – a sharp contrast from those in Table 2.

### CHROMATOGRAPHY

GC is the chromatographic method of choice for analyzing the volatile compounds extracted from a sample. GC-O, which is partly a sensory method, is described in the next section.

GC uses a capillary column to separate compounds by polarity and volatility. Two columns with different polarities may be used in tandem in two-dimensional gas chromatography (GC × GC), which is sometimes employed for complex mixtures. The most common GC detectors for analysis of volatile compounds in dairy products are MS, the less expensive flame ionization detection (FID) and olfactometry (Table 5). Sulfur chemiluminescence detectors have been used for S-containing volatiles, and nitrogen phosphorus detectors have been applied to analysis of amines, pyridines and other N-containing volatiles.

FID is common in analytical chemistry of organic compounds and produces consistent results. Chromatographic peak retention
indices are compared with those of standard mixtures, but a compound will be labeled as unknown if the analyst cannot match its peak with a standard. MS instruments come with a library of reference spectra that give possible matches for each compound detected, but the set-up is quite expensive.

**SENSORY DETECTION**

The most sensitive detector of odor compounds in food is often the nose. GC is capable of detecting $10^9$ molecules of an odorant in a milliliter of air, but the human nose is $10-100$ times more sensitive than this. GC-O was created to take advantage of our olfactory system, combining an analyst’s opinion of an odor (its identification and intensity) and the retention index of the compound (the ratio of the retention time of the compound and that of a standard).

Several sensory-directed methods for evaluating aromas have been designed for GC-O. The first was odor activity value (OAV), devised for bread aromas in 1963, applied to GC-O of dairy products in the 1980s, and consisting of the ratio of odor concentration to its threshold concentration. The latter is defined as the lowest concentration detectable by humans, meaning that a barely detectable compound would have an OAV of $1$. OAV values were not found to be adequate indicators of intensity, prompting the creation of other techniques. In aroma extract dilution analysis (AEDA), developed in 1987, and combined hedonic aroma response measurements (CharmAnalysis or CHARM), devised in 1984, serial dilutions of volatile fractions are made until no odor is perceived in the GC effluent. The highest dilution at which an aroma is smelled is the flavor dilution (FD) factor. If the FD is 8, for example, the odor disappears with an eightfold dilution. CHARM takes duration of aroma into account by measuring the areas under the GC peaks. In the Osm technique, first used in 1990 for odor analysis of hops and wine, odor intensity is measured on a 16-point scale.

The major disadvantage of using human detectors is reproducibility: one person’s idea of an odorant may be different from another’s, sensitivity may vary from one session to the next and a strong smell may affect the ability to detect a subsequent odor. Training, aroma lexicons and practice help the situation, but no two people have exactly the same olfactory sensors. A panel of trained analysts can be used to improve precision, but the human factor is always a concern.

**ELECTRONIC NOSES**

An ideal instrument for identifying and quantitating volatile compounds would be a device that does not rely on opinion, while giving reliable and repeatable results. The electronic nose (e-nose) is an attempt at fulfilling these requirements. Introduced in 1982, the e-nose consists of systems for sample delivery, detection and computation. An aliquot of the headspace above a sample may be injected into the device, or the headspace itself may be directed into the instrument by a carrier gas. Sensors inside the e-nose react with the volatile compounds and send signals to a computer, which matches the compounds to ones in its library and calculates their concentrations. Hand-held units contain sensor arrays made of such materials as metal oxides, conducting organic polymers or quartz crystals; the first two respond to changes in conductivity and the third is affected by alterations in resonance frequency. An external MS detector is sometimes used but is not portable. Other sensors are constantly being developed.

E-noses began to be applied to dairy flavor analysis in the late 1990s. These devices should be tailored for the specific application to achieve the best results and are still not as sensitive as the human nose. Moreover, the e-nose has to be ‘trained’ to detect compounds of interest. The sensitivity characteristics of artificial sensors and biological receptors are different enough to prevents good correlations between e-noses and humans.

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**Table 3.** Comparison of headspace methods for extracting volatiles from dairy products

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purge-trap</td>
<td>Artifacts minimized, uses small sample</td>
<td>Some aromas may be absorbed more than others</td>
</tr>
<tr>
<td>Static</td>
<td>Easy to perform, representative of aromas present</td>
<td>Only the most abundant and volatile compounds are detected</td>
</tr>
<tr>
<td>Solid-phase microextraction</td>
<td>Easy to perform, sensitive, uses small sample</td>
<td>Inconsistencies when compounds compete for binding sites</td>
</tr>
<tr>
<td>Stir-bar sorptive extraction</td>
<td>Immersible in liquid samples</td>
<td>Extra handling required, only polydimethylsiloxane coating available</td>
</tr>
</tbody>
</table>

**Table 4.** Most-cited papers describing extraction of volatiles from dairy products by headspace methods

<table>
<thead>
<tr>
<th>Technique</th>
<th>Product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purge-trap</td>
<td>Hard cheeses</td>
<td>Engels et al. 24</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>Povolo and Contarini 35</td>
</tr>
<tr>
<td></td>
<td>Butter</td>
<td>Povolo and Contarini 26</td>
</tr>
<tr>
<td></td>
<td>Yogurt</td>
<td>Ott et al. 20</td>
</tr>
<tr>
<td></td>
<td>Ice cream</td>
<td>Chung et al. 37</td>
</tr>
<tr>
<td></td>
<td>Whey protein powder</td>
<td>Lee et al. 38</td>
</tr>
<tr>
<td></td>
<td>Cheddar cheese</td>
<td>Milo and Reineccius 39</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>Christensen and Reineccius 40</td>
</tr>
<tr>
<td></td>
<td>Butter</td>
<td>Peterson and Reineccius 41</td>
</tr>
<tr>
<td></td>
<td>Yogurt</td>
<td>Ott et al. 42</td>
</tr>
<tr>
<td></td>
<td>Ice cream</td>
<td>González-Tomas et al. 43</td>
</tr>
<tr>
<td>Static</td>
<td>Whey protein powders</td>
<td>Fabre et al. 44</td>
</tr>
<tr>
<td></td>
<td>Hard cheeses</td>
<td>Frank et al. 45</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>Marsili 46</td>
</tr>
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<td></td>
<td>Yogurt</td>
<td>Sostaric et al. 47</td>
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<td></td>
<td>Ice cream</td>
<td>Welty et al. 48</td>
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<td></td>
<td>Whey protein powders</td>
<td>Fabre et al. 49</td>
</tr>
<tr>
<td>Solid-phase microextraction</td>
<td>Bitto cheese</td>
<td>Panseri et al. 50</td>
</tr>
<tr>
<td>Stir-bar sorptive extraction</td>
<td>Human milk</td>
<td>Buettner 50</td>
</tr>
</tbody>
</table>

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CONCLUSIONS

The ability to obtain reliable lists of all of the significant volatile compounds and their concentrations in dairy product samples remains elusive. Extraction of volatiles may introduce errors, and instrumental analysis also has drawbacks. The human nose is the most sensitive device for measuring odors but it has its own idiosyncrasies. An all-instrumental technique akin to the electronic nose will eventually be developed, but until then scientists will have to rely on careful experimentation to achieve good results.

REFERENCES


<table>
<thead>
<tr>
<th>Table 5. Comparison of gas chromatographic detectors for analyzing volatiles from dairy products</th>
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</thead>
<tbody>
<tr>
<td><strong>Technique</strong></td>
</tr>
<tr>
<td>Flame ionization</td>
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<tr>
<td>Mass spectrometry</td>
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<tr>
<td>Olfactometry</td>
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