FLAVOUR INGREDIENTS FROM FERMENTED DAIRY STREAMS

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Abstract

Using a combination of enzyme and microbial fermentation technologies, dairy streams can be converted into complex flavour ingredients for food applications. The advantage of this process is faster and more controllable flavour development than in traditional food fermentations. The resulting flavour ingredients have a more diverse range of flavours, and better balance, than enzyme-modified cheeses (EMCs). For the consumer, these products can be labelled as ‘all dairy’ and are perceived as ‘clean label’. Here we describe processes and flavours for two examples of dairy-derived flavour ingredients: (i) a milk fermentation that uses a selected combination of bacterial cultures to produce a savoury flavour ingredient; and (ii) the microbial fermentation of an enzymatically treated cheese to produce a flavour ingredient that contains complex sulphury notes that are reminiscent of surface-smear-ripened cheese.

Introduction

For food manufacturers to satisfy consumer preferences, products need natural ingredients as flavourings. Fermentation, in which microbial enzymes catalytically bio-transform foods, is a long-established natural method for providing foods with storage stability, as well as improving flavour. Enzyme-modified cheese (EMC) technology is one means by which a cheese slurry is incubated with various enzymes to generate flavours rapidly, and at a far greater intensity than would be the case for naturally ripened cheese (1). However, unlike naturally ripened cheese, which has an overall flavour arising from the balance of a wide variety of flavour compounds, EMC often contains mainly a single class of flavour compound (e.g. short-chain fatty acids) and so may suffer from a poor flavour balance. Our colleagues have developed flavour ingredients with better flavour balance, whereby dairy substrates are fermented using bacterial cultures in combination with additional enzyme treatments, while maintaining pH and temperature control (2, 3). Here we describe the process flow and components of two such flavour ingredients. The first example embodies a milk fermentation to produce an all-dairy savoury flavour ingredient (SFI). The second example embodies an all-dairy complex sulphury flavour ingredient (CSFI).

Experimental

Production of flavour ingredients. For the SFI, made from whole milk (or reconstituted whole milk powder), unwanted organism growth during fermentation is prevented by controlled preheating (2). The preheated milk is then fermented with two distinct strains of bacteria for 48 h at controlled pH and temperature. For the CSFI, a cheese slurry is hydrolysed by a protease enzyme and is fermented for 100 h at controlled
pH and temperature (3). Heat inactivation terminates the fermentation for both examples. The SFI is either used as a paste or is dried to produce a powdered flavour product. The CFSI can be mixed with cheese curd to give a flavoured paste.

**Identification of the important flavour compounds in the flavour ingredients.** Known methods were used: to analyse L-glutamic (4), L-aspartic (4) and succinic (5) acids; to extract volatile aroma compounds (6); and for gas chromatography (GC) (7). For semi-quantitative analysis with GC–flame ionization detection, the CSFI was spiked with D₇-butyric acid as an internal standard. Unless otherwise stated, the retention time of each aroma compound was checked against that of an authentic standard - obtained either from a commercial source or from chemical synthesis. Potent odorants were screened using aroma extract dilution analysis (AEDA). The extract was sequentially diluted twofold, and the sniffing runs were repeated, until no odorants were detected. Each odorant's final dilution corresponds to the flavour dilution (FD) value. Methanethiol was identified separately using headspace solid-phase microextraction (SPME) with a carboxen fibre (75 μm coating, manual holder).

**Results and Discussion**

**SFI Process.** Fermentation of whole milk (or reconstituted whole milk powder) at controlled pH and temperature (see below) gives an SFI with an intense and very palatable savoury flavour. The final ingredient may be in the form of either a concentrated paste or a spray-dried powder.

<table>
<thead>
<tr>
<th>whole milk (or reconstituted whole milk powder)</th>
<th>cultures</th>
<th>fermentation</th>
<th>heat inactivation</th>
<th>evaporation</th>
</tr>
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<tbody>
<tr>
<td>pH control / temperature control / ~48 h</td>
<td>savoury flavour ingredient</td>
<td></td>
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</table>

**SFI characteristics.** The SFI has a complex savoury flavour and may comprise 0.5–5% of the final product, depending on the application. Taste compounds were deduced (Table 1).

**Table 1.** *Putative taste compounds and their taste activity values (TAVs).*

<table>
<thead>
<tr>
<th>Taste compound</th>
<th>Concentration (mM) in 5% formulation</th>
<th>Taste threshold (mM)</th>
<th>TAV</th>
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<tr>
<td>succinic acid</td>
<td>15</td>
<td>0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.50</td>
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<td>L-glutamic acid</td>
<td>1.3</td>
<td>3.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.30</td>
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<tr>
<td>L-aspartic acid</td>
<td>0.02</td>
<td>4.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
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</table>


The principal taste compound of SFI was succinic acid with a salty character, similar to monosodium glutamate (8, 9), making reduced-salt applications possible.

**CSFI process.** The two-stage CSFI fermentation process (see below) includes both enzymatic and fermentation steps to give a flavour ingredient with a smear-ripened and savoury type of flavour much more rapidly than with traditional fermentation.

cheese slurry → protease cultures → fermentation → heat inactivation → complex sulfury flavour ingredient
<table>
<thead>
<tr>
<th>RI</th>
<th>Identity</th>
<th>FD value</th>
<th>Conc. (μg/g)</th>
<th>Some descriptors</th>
<th>RI</th>
<th>Identity</th>
<th>FD value</th>
<th>Conc. (μg/g)</th>
<th>Some descriptors</th>
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<td>935</td>
<td>ethyl 2-methylpropanoate</td>
<td>4</td>
<td>0.05</td>
<td>fruity</td>
<td>1650</td>
<td>acetophenone</td>
<td>4</td>
<td>0.05</td>
<td>rolled oats</td>
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<tr>
<td>970</td>
<td>pyruvaldehyde</td>
<td>32</td>
<td>trace</td>
<td>butter</td>
<td>1680</td>
<td>3-methylbutanoic acid</td>
<td>64</td>
<td>5</td>
<td>marmite</td>
</tr>
<tr>
<td>1005</td>
<td>methanesulfenic acid</td>
<td>64</td>
<td>trace</td>
<td>chives</td>
<td>1750</td>
<td>dimethyl tetrasulfide</td>
<td>1</td>
<td>trace</td>
<td>cheesy</td>
</tr>
<tr>
<td>1030</td>
<td>ethyl butanoate</td>
<td>32</td>
<td>0.2</td>
<td>fruity</td>
<td>1775</td>
<td>4-methylpentanoic acid</td>
<td>16</td>
<td>50</td>
<td>rancid, sweaty</td>
</tr>
<tr>
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<td>8</td>
<td>trace</td>
<td>fruity</td>
<td>1830</td>
<td>1,2,4-trithiolane</td>
<td>4</td>
<td>trace</td>
<td>sweaty</td>
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<td>unknown</td>
<td>32</td>
<td>seen</td>
<td>burnt wool</td>
</tr>
</tbody>
</table>

Compounds are listed in order of their GC retention index, using an EC-1000 column. Retention indexes (RI) are included (based on n-alkanes). Unless otherwise stated, each aroma compound was identified by comparing it with an authentic standard based on the following criteria: matching retention times on the same column; mass spectrum; descriptions of its aroma attribute. An authentic standard was not available. Tentative identification was based on the mass spectrum. The identification is speculative.
**CSFI characteristics.** The CSFI is a potent flavour ingredient that can be used in applications at 0.1% concentration. The majority of potent aroma compounds from the CSFI arise from degradation of the amino acids methionine, phenylalanine, leucine, isoleucine and valine. Sulphur compounds contribute particularly to the profile of flavour compounds (Table 2). Many of these are already known as cheese flavour compounds including: dimethyl disulphide, S-methyl thiopropanoate, S-methyl thiobutanote, S-methyl thio-3-methylbutanoate, 2,4-dithiapentane and dimethyl trisulphide (10). Other known flavour compounds, not previously described in the cheese flavour literature (to the authors’ knowledge), include: S-methyl thio-2-methylpropanoate and dimethyl tetrasulphide (11); dimethyl thiosulfinate and dimethyl thiosulfonate (12); and 1,2,4-trithiolane and 1,2,4,6-tetrathiepane (13). These, and other compounds listed in Table 2, have previously been seen only in a non-commercial Cheddar cheese that developed astonishingly intense flavours after maturation for 21 years (J.G. Bendall, unpublished results). AEDA revealed the presence of a potent odorant, with a chive character and a retention index of 1005, for which no peak was observed by GC–mass spectrometry. By the nature of its aroma character, this odorant is likely to be a sulphur-containing compound. Few sulphur-containing chemical structures with such a short retention time are possible, and other plausible sulphur-containing compounds were eliminated as candidates after checking their retention indexes against those of authentic standards. It is therefore speculated that the odorant may have been methanesulphenic acid (CH$_3$S-OH), which is known as an intermediate in the generation of other sulphur-containing flavour compounds from methionine (12).

By combining enzyme and microbial fermentation technologies, while maintaining pH and temperature control, dairy substrates can be fermented into ‘clean label’ flavour ingredients that contain succinic acid, which imparts a savoury taste, as well as a wide range of potent aroma compounds that can impart a more complex and balanced flavour than could otherwise be generated using EMC technology.

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**References**