Analysis of Furan by Headspace GC/MS

Vural Gökmen
Furan in foods

• Furan has been known for a long time as a food constituent, for example, in caramel, coffee, bread, canned meat, etc.

• The formation of furan has been studied in model systems revealing three main precursor classes,
  – ascorbic acid and related compounds,
  – Maillard type systems containing amino acids and reducing sugars,
  – lipid oxidation of unsaturated fatty acids
Furan formation

Figure. Hypothetical formation pathways based on Maillard precursors, ascorbic acid, and/or polyunsaturated lipids leading to furan by thermally induced reactions.
Figure.
Furan formation from sugars, ascorbic acid or fatty acid
Table 1. Formation of furan from various reaction systems based on ascorbic acid, Maillard type precursors and lipids (*adapted from* Märk et al 2006)

<table>
<thead>
<tr>
<th>Model system</th>
<th>Furan (µmol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ascorbic acid</td>
<td>9950</td>
</tr>
<tr>
<td>dehydroascorbic acid</td>
<td>270</td>
</tr>
<tr>
<td>erythrose</td>
<td>1674</td>
</tr>
<tr>
<td>glucose + alanine + threonine</td>
<td>749</td>
</tr>
<tr>
<td>linoleic acid (C18:2; 9,12)</td>
<td>681</td>
</tr>
<tr>
<td>trilinolein</td>
<td>1727</td>
</tr>
<tr>
<td>linolenic acid (C18:3; 9,12,15)</td>
<td>3270</td>
</tr>
<tr>
<td>trilinolenin</td>
<td>4747</td>
</tr>
</tbody>
</table>
Foods at risk!

Foods
• high in ascorbic acid
  – Fruits and vegetables
• high in PUFA
  – Formulated foods
• Foods heated in closed jars
## Most relevant foods

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Number</th>
<th>Furan µg/kg</th>
<th>Average of positives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Negative</td>
<td>Minimum</td>
</tr>
<tr>
<td>Baby foods</td>
<td>273</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Infant foods</td>
<td>71</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Infant formulae</td>
<td>42</td>
<td>14</td>
<td>2.5</td>
</tr>
<tr>
<td>Coffee beans/powder</td>
<td>19</td>
<td>0</td>
<td>239</td>
</tr>
<tr>
<td>Coffee brewed</td>
<td>38</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Beers</td>
<td>14</td>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>Bread</td>
<td>13</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>Bread, toasted</td>
<td>6</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Crisp breads/crackers</td>
<td>4</td>
<td>0</td>
<td>4.2</td>
</tr>
<tr>
<td>Candy and chocolate</td>
<td>20</td>
<td>8</td>
<td>0.5</td>
</tr>
<tr>
<td>Desserts/puddings</td>
<td>12</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Fish</td>
<td>9</td>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>Fruit</td>
<td>28</td>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>Fruit juices</td>
<td>51</td>
<td>27</td>
<td>0.5</td>
</tr>
<tr>
<td>Fruit preserves</td>
<td>47</td>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>Gravies</td>
<td>8</td>
<td>0</td>
<td>13.3</td>
</tr>
<tr>
<td>Malt</td>
<td>6</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Convenience meals</td>
<td>32</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Meats</td>
<td>15</td>
<td>9</td>
<td>1.7</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>47</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Nutrition drinks/shakes</td>
<td>22</td>
<td>2</td>
<td>1.1</td>
</tr>
<tr>
<td>Sauces</td>
<td>41</td>
<td>4</td>
<td>3.3</td>
</tr>
<tr>
<td>Soups</td>
<td>58</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Soy sauces</td>
<td>12</td>
<td>0</td>
<td>17.2</td>
</tr>
<tr>
<td>Maple syrup</td>
<td>3</td>
<td>0</td>
<td>8.6</td>
</tr>
<tr>
<td>Tortilla and potato chips</td>
<td>6</td>
<td>1</td>
<td>4.4</td>
</tr>
<tr>
<td>Vegetable juices</td>
<td>8</td>
<td>0</td>
<td>3.2</td>
</tr>
<tr>
<td>Vegetables (in cans and jars)</td>
<td>50</td>
<td>9</td>
<td>0.8</td>
</tr>
<tr>
<td>Baked beans</td>
<td>26</td>
<td>0</td>
<td>23.3</td>
</tr>
</tbody>
</table>
Molecule Properties

• Highly volatile
  – Boiling point 31°C
  – Suitable analyte for GC-MS analysis

• Low molecular weight, MW=68
## Overview of Methods

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Pretreatment</th>
<th>Sample Preparation</th>
<th>Equilibration temperature</th>
<th>Internal Standard</th>
<th>Detection</th>
<th>Column</th>
<th>LOD [µg/kg]</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Various foods</td>
<td>Solid or semi-solid samples are diluted with water of saturated NaCl.</td>
<td>Static headspace sampling</td>
<td>80°C, adopted 60°C later</td>
<td>d4-furan</td>
<td>GC-MS</td>
<td>HP PLOT Q</td>
<td>-</td>
<td>US FDA</td>
</tr>
<tr>
<td>Various foods</td>
<td>Solid samples are homogenized and diluted with water.</td>
<td>Static headspace sampling</td>
<td>50°C</td>
<td>d4-furan</td>
<td>GC-MS</td>
<td>HP PLOT Q</td>
<td>2.0</td>
<td>Hasnip et al., 2006</td>
</tr>
<tr>
<td>Coffee and fruit juices</td>
<td>Solid samples are homogenized and diluted with water.</td>
<td>Static headspace sampling</td>
<td>40-70°C</td>
<td>d4-furan</td>
<td>GC-MS</td>
<td>PLOT HT-Q</td>
<td>0.1</td>
<td>Şenyuva and Gökmen, 2005</td>
</tr>
<tr>
<td>Jars, canned foods, coffee</td>
<td>Samples are blended with water.</td>
<td>Static headspace sampling</td>
<td>30°C</td>
<td>d4-furan</td>
<td>GC-MS</td>
<td>CP-PoraBOND Q</td>
<td>0.1</td>
<td>Becalski et al., 2005</td>
</tr>
<tr>
<td>Various foods</td>
<td>Solid samples are homogenized.</td>
<td>Solid phase microextraction</td>
<td>50°C</td>
<td>d4-furan</td>
<td>GC-MS</td>
<td>PLOT Q</td>
<td>0.2-0.6</td>
<td>Goldmann et al., 2005</td>
</tr>
<tr>
<td>Baby foods</td>
<td>Samples are homogenized.</td>
<td>Solid phase microextraction</td>
<td>30°C</td>
<td>d4-furan</td>
<td>GC-MS</td>
<td>HP-INNOWAX</td>
<td>&lt;0.1</td>
<td>Bianchi et al., 2006</td>
</tr>
<tr>
<td>Water</td>
<td>Samples are homogenized.</td>
<td>Solid phase dynamic extraction (Liquid and headspace)</td>
<td>30°C</td>
<td>d4-furan</td>
<td>GC-MS</td>
<td>HP PLOT Q</td>
<td>1.5</td>
<td>Ridgway et al., 2006</td>
</tr>
</tbody>
</table>
Scheme for Furan Analysis

1. **Sampling**
2. **Homogenization at cold**
3. **Equilibration in a closed vial**
4. **Headspace sampling**
5. **Detection**

- Add labelled internal standard
  - $d_4$-furan
  - $d_6$-Acetone

- Static/dynamic
- Solid phase microextraction

- GC/MS
Two potential approaches

- Static/Dynamic Headspace Sampling
- Solid Phase Microextraction

Solid Phase Microextraction (SPME) is an innovative, solvent free technology that is fast, economical, and versatile. SPME is a fiber coated with a liquid (polymer), a solid (sorbent), or a combination of both. The fiber coating removes the compounds from your sample by absorption in the case of liquid coatings or adsorption in the case of solid coatings. The SPME fiber is then inserted directly into the Gas Chromatograph for desorption and analysis.
Furan analysis by headspace sampling

- Sampling at cold conditions: T~4°C
- Equilibration in a closed vial at warm conditions: T~30°C
- Headspace sampling for GC/MS analysis
- GC/MS analysis
SPME for headspace sampling

1. Drill down septum piercing needle to avoid breakage
2. Insert needle into container
3. Adjust needle depth for headspace sampling
4. Extend plunger to expose fiber
5. Retract fiber before removing to avoid damaging the fiber
6. Drill down septum piercing needle to avoid breakage
7. Remove SPME device
Factors to be considered

• Ascorbic acid has been shown to be major precursor responsible for furan formation.
  – It is known that furan derivatives may form even at room temperatures during prolonged storage. Increasing the temperature accelerates this formation!

• Among food items analyzed, furan levels are greater in vegetable based foods than fruit based ones
  – Is pH of food critical for furan formation?

• What is the critical temperature for furan formation to begin?
Analytical Conditions

• Conditions reported by US FDA is followed with little modifications;
  – HS conditions
  – GC conditions
  – MS conditions

• Equilibration temperature and time were varied in order to understand the behaviour of furan during HS sampling.
Sample Preparation

• Test matrices (raw materials of widely consumed food products)
  – Green coffee (ground & blow-up under nitrogen)
  – Tomato juice (freshly squeezed)
  – Orange juice (freshly squeezed)

• Sampling prior to HS
  – 3 g sample + 2 ml water in 20 mL vial
Static HS Sampler Conditions

- Oven temperature
  - 40-80 °C

- Equilibration time
  - 0-60 min

  - Pressurization : 0.15 min
  - Injection : 0.15 min (splitless)
  - Transfer line temperature : 120°C
  - Needle temperature : 100°C
GC Conditions

• GC Column
  – PLOT HT-Q (12.5 m x 0.32 mm i.d., 0.45 μm film thickness)

• Column flow
  – 2.2 ml/min helium (constant flow)

• Oven temperature
  – 50°C, 10°C/min to 230°C and hold 12 min (run time 30 min)
MS Conditions

• Ionization : EI (70 eV)
• MS source temp : 280°C
• MS quad temp : 150°C
• MS transfer line temp : 225°C
• Scan range : m/z 30 to 350
• MS confirmation of furan
  – ratio of m/z 68 / m/z 39 (±10%)
  – retention time of furan standard
GC conditions resolves furan well!

Sample: ground Turkish coffee
Equilibration: 70°C x 30 min
GC conditions resolves furan well!

Sample: instant coffee
Equilibration: 70°C x 30 min
MS database confirms furan!

Matching quality 93%!

Library comparison of mass spectrum confirms furan ...
Green coffee generates furan response!

Sample: green coffee
Equilibration: 70°C

Why is furan present in green coffee? Is it naturally present or formed during analysis?
Even at 40°C!

The response increases with time!
Furan derivatives forms ...

• Furan derivatives begins to appear in the headspace of green coffee samples during equilibration at 70°C.
• The responses of furfural or HMF is less than that of furan in the headspace.
• This is most probably due to the differences in boiling points rather than the differences in their rates of formation.
Fresh tomato juice generates furan response too!

The same is true for freshly squeezed tomato juice ...
Furan response increases with time during the equilibration of green coffee!
temperature versus furan response

Is this kind of behaviour pH-dependent?

Furan response of raw foods: **green coffee >> tomato juice > orange juice**
Conclusions

**Scenario I**
If there is a fixed amount of furan in the sample.

\[ m_{\text{furan}} = \text{constant} \]
\[ T = \text{constant} \]
Conclusions

**Scenario II**
If there is a generation of furan in the sample.

The cases for green coffee and tomato juice are some like this!

The time required to reach equilibrium takes longer!
Equilibrium time and concentration shift.

**rate of generation**

\[
\frac{dm_{\text{furan}}}{dt} = k.C^n
\]

\[ T = \text{constant} \]
Matrix-matched calibration

Figure. Matrix matched calibration curve of furan (matrix: green coffee)
Further readings

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REVIEW

Analysis of heat-induced contaminants (acrylamide, chloropropanols and furan) in carbohydrate-rich food

Thomas Wenzl • Dirk W. Lachenmeier • Vural Gökmen
Analysis of furan in foods. Is headspace sampling a fit-for-purpose technique?

HAMIÈDE Z. ŞENYUVA¹ & VURAL GÖKMEN²