Recent Developments in Coffee Flavour Formation Using In-Bean Experiments

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(Nestle PTC Orbe, Switzerland)
From the plant to the cup: Roasting is the critical step in flavour formation.

- Genetic, botanic
- Cultivation, harvesting
- Post-harvest treatment
- Coffee technology
- Roasting
- Instant coffee
- Cup preparation
- Roast & ground coffee

Diagram showing the process from plant to cup with stages including genetic, botanic, cultivation, harvesting, post-harvest treatment, coffee technology, roasting, instant coffee, cup preparation, and roast & ground coffee.
Coffee roasting: Some compounds formed from green bean precursors

**Coffee Flavour Precursors**

- **SUGARS**
  - Sucrose, glucose, fructose (arabinogalactans)

- **AMINO ACIDS**
  - (Strecker-active AA)

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  - Sucrose, glucose, fructose (arabinogalactans)

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  - (Strecker-active AA)

- **CHLOROGENIC ACIDS**

- **CAROTENOIDS**

- **LIPIDS**

**Caramelization**

- **Maillard, Strecker**

**Fragmentation**

- **Diketones**
- **Aldehydes**

**Pyrolysis**

- **Pyridine and derivatives**

**Hydrolysis**

- **Chlorogenic acid lactones**

**Cyclisation**

- **Chlorogenic acid lactones**

**Hydrolysis**

- **ORGANIC ACIDS**
- **CO₂**

**Oxidation**

- **Carbonyls**

**Oxidative cleavage**

- **E-β-Damascenone**

**3-Mercapto-3-methylbutyl-formate**

**Pyrazines**

**Nicotinic acid**

**Quinic acid lactone**

**Quinic acid**
Get a better understanding of the relative importance of flavour precursors in coffee under realistic conditions.
**Biomimetic in-bean study**

- Green coffee beans (GC)
- Exhausted beans (EB)
- Spiking with precursors
- Spiked green beans
- Water extraction at 95°C for 2 h
- Roasting
- Biomimetic recombinant (BR)
- Spiking with precursors
- Roasted beans spiked with precursors
- Spiking
- Incorporation of labelled precursors
- Omission of EB + BR in sugars/AA
- EB + BR + labeled precursors
- Roasting
## Composition of the biomimetic recombinate (BR)

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount (g)</th>
<th>Components</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenols</strong></td>
<td></td>
<td><strong>Amino acids (cont.)</strong></td>
<td></td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>8.849</td>
<td>L-Proline</td>
<td>0.105</td>
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<tr>
<td>Trigonelline</td>
<td>1.584</td>
<td>L-Tyrosine</td>
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<td>Caffeine</td>
<td>1.031</td>
<td>L-Valine</td>
<td>0.042</td>
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<td><strong>Organic acids</strong></td>
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<td>L-Isoleucine</td>
<td>0.063</td>
</tr>
<tr>
<td>D-( -)-Quinic acid</td>
<td>0.865</td>
<td>L-Leucine</td>
<td>0.052</td>
</tr>
<tr>
<td>L-(+)-Lactic acid</td>
<td>0.0034</td>
<td>L-Phenylalanine</td>
<td>0.076</td>
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<tr>
<td>Potassium acetate</td>
<td>0.081</td>
<td>L-Lysine</td>
<td>0.031</td>
</tr>
<tr>
<td>Malic acid</td>
<td>0.398</td>
<td><strong>Sugars</strong></td>
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<tr>
<td>Potassium citrate</td>
<td>2.620</td>
<td>D-( -)-Arabinose</td>
<td>0.011</td>
</tr>
<tr>
<td><strong>Amino acids</strong></td>
<td></td>
<td>L-(+)-Rhamnose</td>
<td>0.0036</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>0.028</td>
<td>D-(+)-Galactose</td>
<td>0.198</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.109</td>
<td>D-(+)-Sucrose</td>
<td>12.48</td>
</tr>
<tr>
<td>L-Serine</td>
<td>0.070</td>
<td>D-(+)-Mannose</td>
<td>0.029</td>
</tr>
<tr>
<td>L-Aspartic acid</td>
<td>0.286</td>
<td><strong>Metals</strong></td>
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<tr>
<td>L-Glutamic acid</td>
<td>0.205</td>
<td>Iron(III) chloride</td>
<td>0.0084</td>
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<tr>
<td>L-Glycine</td>
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<td>Manganese(II) chloride</td>
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<tr>
<td>L-Histidine</td>
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<td>Copper(II) sulfate</td>
<td>0.0053</td>
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<tr>
<td>L-Arginine</td>
<td>0.075</td>
<td>Zinc(II) sulfate, heptahydrate</td>
<td>0.0008</td>
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</tbody>
</table>
1. Formation of 2-furfurylthiol (FFT): Model studies

**Model System**

- Tressl et al. (1993): FFT is formed from arabinose/cysteine via 3-deoxypentosone (3-DP) and furfural while maintaining the intact carbon chain.

![Chemical diagram](image)

**Figure**: Hypothetical formation of FFT in coffee from arabinogalactans or arabinose (R=H) and cysteine (protein-bound).

- Grosch (1999): Arabinogalactans suggested as precursor of FFT by isolating the polysaccharide from green coffee and roasting it in the presence of cysteine.
Formation of 2-Furfurylthiol (FFT): Omission and spiking experiments

**In-bean experiment**

- Omission of sugars favored the generation of FFT, whereas furfural content was highly suppressed
- Spiking with sucrose increased furfural amounts but considerably decreased concentrations of FFT
- Spiking experiment with cysteine resulted in enhanced FFT amounts, thus indicating cysteine as a suitable sulphur source
Formation of 2-furfurylthiol (FFT): Labelling experiments

**In-bean experiment**

![Graph showing relative intensity of furfural and FFT](image)

Incorporation of D-[U-\(^{13}\)C\(_5\)]-arabinose did not yield fully labelled furfural nor FFT, but partially labelled FFT with \(^{13}\)C\(_1\), \(^{13}\)C\(_2\) and \(^{13}\)C\(_3\)-moieties

2-Furfurylthiol (FFT) is most likely not generated *via* the furfural pathway.
2. Formation of alkyl pyrazines: Model studies

**Model System**

- *Amrani-Hemaimi et al. (1995):* Potential precursors are C6 and C5 sugars (e.g. fructose, glucose, arabinose) and degradation compounds form key intermediates (e.g. α-amino carbonyls) through the Strecker reaction.

- *Amrani-Hemaimi et al. (1995):* In addition, alanine and glycine play a key role as they are incorporated into the side chain of the alkyl pyrazine.

**Figure:** Precursors and key intermediates of pyrazines
Formation of alkyl pyrazines: Omission and spiking experiments

**In-bean experiment**

Omission of amino acids or sugars

- Omission of free amino acids and spiking with alanine confirmed their importance
- Sugars omission resulted in highly increased pyrazine concentrations

Spiking of green coffee with sugars (equimolar amounts) or alanine

- Amino acids, in particular alanine, are key precursors in the formation of ethyl pyrazines
- Spiking of green beans with sugars had a suppressing effect
Formation of alkyl pyrazines: Labelling experiments

**In-bean experiment**

Spiking with L-[3-13C]-alanine

<table>
<thead>
<tr>
<th>Compound</th>
<th>relative intensity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3-diethyl-5-methylpyrazine</td>
<td>24%</td>
</tr>
<tr>
<td></td>
<td>43.6%</td>
</tr>
<tr>
<td></td>
<td>32.3%</td>
</tr>
<tr>
<td>2-ethyl-3,5-dimethylpyrazine</td>
<td>64%</td>
</tr>
<tr>
<td></td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>3%</td>
</tr>
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<td></td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>16%</td>
</tr>
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<td>0%</td>
</tr>
<tr>
<td></td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td>80%</td>
</tr>
</tbody>
</table>

Incorporation of [U-13C6-fructose]-sucrose (16% of natural content)

<table>
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<th>Compound</th>
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<tr>
<td></td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>0%</td>
</tr>
</tbody>
</table>

C3-Fragments are formed from free sugars as well as from non-water extractable polysaccharides:

- Labeled alanine is efficiently incorporated into ethyl pyrazines
- Pyrazine formation implies competition between bound and free sugars for the water extractable N-source
3. Formation of Furaneol®: Model studies

Model System

- **Hofmann & Schieberle (2001):** Furaneol is mainly formed from the intact C6-glucose skeleton under roasting conditions (dry heating).

- **Hofmann & Schieberle (2001):** Under aqueous conditions, furaneol is also formed by recombination of reactive C₃ sugar fragments (ca. 60%).
Formation of furaneol: Omission experiments

**In-bean experiment**

- **Free sugars (sucrose) play a major role in the formation of Furaneol**
  - Omission of free sugars → significantly decreased concentrations (-55%).
  - Omission of amino acids → promoting generation (+62%): less trapping of reactive C₃

- **Spiking with \(^{13}\text{C}_6\)-Fru-sucrose led to exclusive generation of fully labeled and fully unlabelled molecules**
  - In agreement with previous model studies: Furaneol is formed from intact C6-glucose

- **Fructose is a much more efficient precursor as compared to glucose**
  - 16% labeled Furaneol formed, with only 1/6 of the total sucrose amount added as labeled isotopomer
4. Formation of diketones: Model studies

Model System

- Yaylayan et al. (1999): Diketones are formed from mono-, di- & oligosaccharides by Maillard-type reactions in the presence of amino acids like glycine and alanine.
- Schieberle et al. (2003): CAMOLA experiments indicated that the recombination of C3 and C1 intermediates is the predominant pathway in 2,3-butanedione formation.

![Chemical structure](image.png)

**Figure**: Precursors and key intermediates of coffee diketones.
Formation of diketones: Omission and spiking experiments

**In-bean experiment**

Omission of amino acids or sugars

- Omission of sugars affect the formation of both diketones, in particular of 2,3-pentanedione
- Omission of free amino acids and spiking with alanine in green coffee do not influence the formation of diketones

Spiking of green coffee with sugars (equimolar amounts) or alanine

- Addition of sucrose increases particularly 2,3-pentanedione (+60%), to a lesser extent 2,3-butanedione (+10%)
Formation of diketones: Labelling experiments

- Incorporation of $[U^{13}C_6]$-fructose]-sucrose led also to fully labelled 2,3-pentanedione (ca. 10%)

- 2,3-Butanedione is only formed from the glucose moiety

- Free sugars play a key role in diketones formation

- Amino acids are not the limiting factor in diketone formation (←)

### 2,3-butanedione (6)

<table>
<thead>
<tr>
<th>ion</th>
<th>m/z</th>
<th>BREB$^d + [^{13}C_6]$-SUC</th>
<th>RGB$^c + [^{13}C]$-ALA</th>
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<tbody>
<tr>
<td>M</td>
<td>86</td>
<td>96</td>
<td>99</td>
</tr>
<tr>
<td>M+1</td>
<td>87</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>M+2</td>
<td>88</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M+3</td>
<td>89</td>
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<td>0</td>
</tr>
<tr>
<td>M+4</td>
<td>90</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M+5</td>
<td>105</td>
<td>10</td>
<td>M+5</td>
</tr>
</tbody>
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### 2,3-pentanedione (7)

<table>
<thead>
<tr>
<th>ion</th>
<th>m/z</th>
<th>BREB$^d + [^{13}C_6]$-SUC</th>
<th>RGB$^c + [^{13}C]$-ALA</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>100</td>
<td>83</td>
<td>95</td>
</tr>
<tr>
<td>M+1</td>
<td>101</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>M+2</td>
<td>102</td>
<td>2</td>
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</tr>
<tr>
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<td>103</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>M+4</td>
<td>104</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>M+5</td>
<td>105</td>
<td>10</td>
<td>M+5</td>
</tr>
</tbody>
</table>

Diketones show different formation pathways

In-bean experiment
Conclusions

• **Biomimetic in-bean experiments**: A powerful tool for the elucidation and verification of proposed formation pathways of aroma compounds in complex food systems such as coffee.

• **Combination of omission, spiking, and labelling experiments** under real food matrix conditions are very useful in providing more precise insights into Maillard-type formation mechanisms.

• **Great diversity of precursors** and other co-reaction agents present in the green bean lead to competing and even different pathways in flavor formation (e.g. 2-furfurylthiol, diketones).

• **Model versus Food**: Results obtained in model systems need to be validated under food processing conditions.
Study on the Role of Precursors in Coffee Flavor Formation
Using In-Bean Experiments

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JOSEF KERLER*†

†Nestlé Product Technology Centre Orbe, Nestec Ltd., CH-1350 Orbe, Switzerland, and §University of
Hohenheim, Institute for Food Chemistry, Stuttgart, Germany

The formation of several key odorants, such as 2-furfurylthiol (FFT), alkylpyrazines, and diketones,
was studied upon coffee roasting. The approach involved the incorporation of potential precursors in
green coffee beans by means of biomimetic in-bean and spiking experiments. Both labeled and
unlabeled precursor molecules were used, and the target analytes in the roasted coffee samples
were characterized in terms of their isotope labeling pattern and abundance. The biomimetic in-bean
experiments ruled out the 2-furaldehyde route to FFT as suggested by model studies. Furthermore,
no evidence was found for the incorporation of the arabinose C5 skeleton into FFT. Pathways
proposed for the formation of alkylpyrazines and diketones were confirmed, and a new mechanism
is suggested for the formation of 2-ethenyl-3-ethyl-5-methylpyrazine. The role of amino acids, for
example, alanine, and free sugars was substantiated. The results underscore the potential of this
methodology to provide better understanding of the formation pathways occurring in complex food
systems, which may be different from those obtained in model experiments.