Does aroma composition allow to discriminate groups of dark chocolates categorized on the basis of their organoleptic properties? Inputs of direct-injection mass spectrometry (PTR-ToF-MS) and GC-Olfactometry

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Final Objective:
Find key aroma compounds typical for each sensory poles in chocolate

1st hypothesis:
The sensory classification of the chocolates is mainly based on their composition in volatile organic compounds (VOCs)

1st objective:
Obtain the volatile compounds fingerprints of the 187 chocolates
Obtain the VOCs fingerprints

Samples preparation

+ 1mL of artificial saliva *

Stirring in a water bath at 36.2°C

Equilibrating for 2h

* water + salt + alpha amylase + mucins

Samples analysis:
PTR-ToF-MS
Obtain the VOCs fingerprints

PLS-DA with chemical data (factors 1 and 2) 187 samples distributed in 4 sensory poles (Y variables) / 314 ions (X variables)

R-Square:
Calibration: 0.84
Validation: 0.80
Obtain the VOCs fingerprints

PLS-DA with **sensory data** (factors 1 and 2), 187 samples / 36 sensory descriptors

PLS-DA with **chemical data** (factors 1 and 2), 187 samples / 314 ions

The produced “chemical maps” showed that the headspace PTR-MS analyses of the chocolates allowed retrieving the classification of the 187 samples into the four sensory categories previously determined.
The 1st hypothesis is validated

2nd hypothesis:
There are volatile organic compounds typical for each pole

2nd objective:
Analyse compounds which have an olfactory impact, identify them and find key organic compounds for each pole
Identification of key aroma compounds

- Flavour extraction:
  - 30 g of chocolate + 300 µL internal standard + 100 mL of MiliQ water

- SAFE:
  - Aroma extract
  - Water bath (37°C)
  - Sample
  - Liquid nitrogen

- Liquid-liquid extraction:
  - Extraction with CH$_2$Cl$_2$ (3 x 15ml)

- Concentration with Kuderna-Danish:
  - Water bath (70°C)

- GC-O samples analysis:
  - Sample inlet

- GC-MS samples analysis:
  - Extract of concentrated aroma (400 µl)
Identification of key aroma compounds

One average index = one odorant area
Number of repetition = detection frequency

<table>
<thead>
<tr>
<th>Average index</th>
<th>Number of repetition</th>
<th>Common descriptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1072</td>
<td>4</td>
<td>Fruity, floral</td>
</tr>
<tr>
<td>1109</td>
<td>3</td>
<td>Roasted</td>
</tr>
<tr>
<td>1173</td>
<td>6</td>
<td>Solvent, fruity</td>
</tr>
<tr>
<td>1195</td>
<td>5</td>
<td>Fruity</td>
</tr>
<tr>
<td>1248</td>
<td>3</td>
<td>Unpleasant</td>
</tr>
<tr>
<td>1293</td>
<td>7</td>
<td>Butter, fruity</td>
</tr>
<tr>
<td>1296</td>
<td>4</td>
<td>Fruity, floral</td>
</tr>
<tr>
<td>1308</td>
<td>11</td>
<td>Mushrooms</td>
</tr>
<tr>
<td>1326</td>
<td>4</td>
<td>Roasted, peanuts</td>
</tr>
<tr>
<td>1346</td>
<td>8</td>
<td>Cereal, roasted</td>
</tr>
<tr>
<td>1384</td>
<td>8</td>
<td>Metal</td>
</tr>
</tbody>
</table>

Samples

Extract of the table with detection frequencies of 124 odorant areas (OAs) in 12 samples

Selection of discriminant OAs showing differences between higher and lower detection frequencies values in samples > 30%
Identification of key aroma compounds

Correspondence analysis (factors 1 and 3): detection frequencies of 34 OAs (grey dots) within 12 samples (diamonds).

Correspondence analysis (factors 1 and 2): detection frequencies of 34 OAs (grey dots) within 12 samples (diamonds).

Legend:
- Pole 1
- Pole 2
- Pole 3
- Pole 4

<table>
<thead>
<tr>
<th>OAs</th>
<th>Compounds</th>
<th>Odor attribute</th>
</tr>
</thead>
<tbody>
<tr>
<td>1126</td>
<td>Pentan-2-ol</td>
<td>Fruity</td>
</tr>
<tr>
<td>1461</td>
<td>Acetic acid</td>
<td>Vinegar</td>
</tr>
<tr>
<td>1795</td>
<td>Ethyl phenylacetate</td>
<td>Floral</td>
</tr>
<tr>
<td>1824</td>
<td>2-phenylethyl acetate</td>
<td>Fruity, floral</td>
</tr>
<tr>
<td>2204</td>
<td>δ-Decalactone</td>
<td>Fruity, floral</td>
</tr>
</tbody>
</table>
Take home messages

- The analysis of VOCs allows retrieving the classification of the 187 samples into the four sensory categories previously determined.
- Sensory classification of the chocolates could be explained chiefly by the profiles of flavour compounds released by the matrix but not in its entirety.
- There are OAs for each pole which have been identified thanks to correspondence analysis. Unidentified OAs due to coelutions will be resolved using a GC-2D analysis.

Yes, aroma composition allows to discriminate groups of dark chocolates categorized on the basis of their organoleptic properties.
Thanks

Jean Luc Le Quéré & Hélène Labouré & Renaud Boulanger

Isabelle Andriot & Karine Gourrat & Etienne Sémon

Marie Repoux & Florent Coste & Pierre Costet

Sébastien Preys

The International Cocoa Organization

Thanks for your attention