From *Sensomics* to *AI smelling and Computer Vision*: Exploring the chemical sensory code of premium chocolate

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Foreword
...the boundaries between chemistry and biology are vanishing\(^1\)...

Food-omics domains and strategies of investigation

The role of multidimensional (gas) chromatography
*Artificial Intelligence Smelling* and *Computer vision*

Premium cocoa origin *identification* and aroma blueprint
✓ *Computer vision* a change of perspective
✓ *Artificial Intelligence smelling* for large screenings

Conclusive remarks

From *Sensomics to AI smelling and Computer Vision*: Exploring the chemical sensory code of premium chocolate

Prof. Irene Chetschik

Multidimensional Analytical platforms

1D/2D Chromatography Detection:
- (HR)-Mass Spectrometry
- Olfactometry
- Parallel detection

Food metabolomics
Chemical composition of food vs.
- crop botanical origin
- harvesting area
- climate impact
- post-harvest
- storage conditions

Nutrimetabolomics
Human metabolome by
- dietary patterns
- specific foods
- nutrients
- micro-organisms
- bioactives

Food safety
- Xenobiotics
- Non-intentionally added substances
- Contaminants
- MOSH/MOAH

Sensomics
Food hedonic profile
- potent odorants
- chemical odor code
- volatiles patterns
- odor activity value
- olfactometry

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Prof. Thomas Hofmann
J. Agric. Food Chem. 2015, 63, 32, 7095–7096

...the boundaries between chemistry and biology are vanishing...

Higher level information
Understanding

Data mining
machine learning
unsupervised/supervised

Data processing
targeted/untargeted
profiling/fingerprinting

Multidimensional
Analytical platforms

Comprehensive two-dimensional gas chromatography as a boosting technology in food-omic investigations
Federico Stillo, Carlo Ricchi, Stephen E. Reichenbach, Chiara Cordero

...the boundaries between chemistry and biology are vanishing...

Prof. Thomas Hofmann
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Artificial Intelligence **smelling machine**

**Context:** Sensomics\(^1\)

**Principle:** *key-odorants and odorants patterns evoke specific smells/aroma qualities while contributing to define the overall flavor perception of a food identity*

**Methods:** extract, isolate, quantify potent odorants by reliable and robust methodologies

**Outcome:** Sensomics-Based Expert System\(^2\) (SEBES) that predicts key-aroma signatures of food without using human olfaction.

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**Computer Vision**

"...is a field of artificial intelligence (AI) that enables computers and systems to derive meaningful information from digital images....— and take actions or make recommendations based on that information. If AI enables computers to think, **computer vision enables them to see, observe and understand.**"\(^3\)

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Separation power (peak capacity) is given by the product of the two chromatographic dimensions (GC×GC)

Independent (almost) displacement in both dimensions produces rational retention patterns for homologue series

Band compression (in space - for thermal modulators) produces signal-to-noise ratio enhancement - sensitivity

Bi-dimensional peak patterns exploits a 3D space where fingerprinting could be more accurate that in a 2D space (as for 1D-GC profiles)

Information dimensions
spectral signature (identity)
volutility/polarity
sensory descriptor (bio-assay)

Sample prep - GC×2GC-MS/FID
Sample prep - GC(O)×GC-MS

Profiling: detailed analysis of the chemical pattern

Target(ed) analysis:
GC-MS metadata (retention and spectra) analytes identity and amount

Chromatographic fingerprinting:
general and rapid high-throughput screening -> discriminate/classify samples

Limits:
high chemical dimensionality
complexity of food samples
isomers/isobars might co-elute and analytes discrimination becomes challenging
Need of multiple dimensions (separation / detection) to explore compositional complexity


Prof. Philip Marriott
“If you are not using GC×GC, you will never know what you are missing!”
J. Agric. Food Chem. 69, 11535–11537 (2021)
Conventional 1D GC

Comprehensive 2D GC

Prof. Philip Marriott
“If you are not using GC\times GC, you will never know what you are missing!”
J. Agric. Food Chem. 69, 11535–11537 (2021)

“High resolution” profiling
GC\times GC separation power
accurate quantitative profiling

2D/3D Chromatographic fingerprinting\textsuperscript{1}
pattern recognition (forensics)
comprehensive sample comparison

Group-Type Analysis
Rational retention logic
Ordered elution patterns

TrAC Trends in Analytical Chemistry, 134, 116133.
Roasted cocoa from Sao Tomé volatiles
HS-SPME (CAR/PDMS/DVB) - 500 mg - 50°C/50 min

About 700 detectable features (2D peaks) over 20 S/N
Of them 220 reliably identified by 70 eV spectrum and IT coherence
Various chemical classes highly correlated with post-harvesting practices, fermentation processes, technological impact, aroma compounds and potent odorants
220 targeted compounds

### Alcohols
- Ethanol
- 2-Methyl-3-buten-2-ol
- 2-Butanone
- 2-Pentanol
- 2-Heptanol
- 2-Pentanol
- 2-Methyl-3-pentanol
- 2,4-Hexanediol
- 2-Butanone
- 2-Methyl-2-buten-3-ol
- 2-Butanol
- 2-Methyl-2-buten-3-ol
- 2-Butanol
- 2-Pentanol
- 2-Pentanol
- 2-Pentanol
- 2-Butanol
- 2-Hexanol
- 1-Octanol
- 4-Isopropoxybutanol
- 2-Furan methanol
- 4-Buty1-2-butanol
- 1-Phenyl ethanol
- Geraniol
- Benzyl alcohol
- Phenylethyl Alcohol
- 1-Butanediol
- Phenol
- 2-Pheinoxy-ethanol
- 2-Pentanol
- 2-Pentanol
- 1-Butanol
- 2-Hexanol

### Ketones
- Acetone
- 2-Butanone
- 2,3-Butanedione
- 2,3-Pentanedihone
- 3-Penten-2-one
- 4-Methyl-3-penten-2-one
- 2-Heptanone
- 3-Hydroxy-2-butanone
- 2-Octanone
- 1-Hydroxy-2-propanone
- 3-Heptanone-1
- 2,3-Octanediene
- 6-Methyl-5-hepten-2-one
- 4-Hydroxy-4-methyl-2-pentanone
- Nonanone
- 1-Acetoxy-2-propanone
- 2-Undecanone
- Acetophenone
- Geranylacetone

### Aldehydes
- Acetaldehyde
- Propanal
- 2-Methyl propanal
- 3-Methyl butanal
- Pentanal
- Hexanal
- 2-Methyl-2-butanal
- Heptanal
- Octanal
- 2-Ethyl-2-hexenal
- 5-Methyl isopropyl-2-hexenal
- Nonanal
- Fusel oil
- Decanal
- Benzaldehyde
- 2-Nonenal
- Undecanal
- Phenyl acetaldehyde
- Dodecanal
- Benzy1 acetate
- Tridecanal
- 3-Phenylpropenal
- 2-Phenyl-2-propanal
- Triadecanal
- 2-Phenyl-2-butenal
- 1H-Pyrrole-2-carboxaldehyde

### Acids
- Acetic acid
- Propanoic acid
- 3-Hydroxy-Butanoic acid
- 2-Methyl propanoic acid
- Butanoic acid
- 3-Methyl butanoic acid
- Hexanoic acid
- Octanoic acid
- Nonanoic acid

### Esters
- Methyl acetate
- Ethyl acetate
- Ethyl propanoate
- Ethyl butanoate
- Ethyl 3-methylbutanoate
- Ethyl 2-methylpropanoate
- Butyl acetate
- 2-Pentyl acetate
- 1-Butan0le, 3-methyl-acetate
- Ethyl pentanoate
- Butyl 2-methylpropanoate
- 4-Methyl-2-pentyl acetate
- Ethyl 4-methylpentanoate
- 4-Pentenyl acetate
- Butyl butanoate
- 2-Pentenyl acetate
- Ethyl hexanoate
- Ethyl triglate
- Prenyl acetate
- 2-Heptyl acetate
- Hexyl acetate
- Methyl 2-hydroxypropanoate
- Ethyl heptanoate
- Ethyl 2-hydroxypropanoate
- 2-Propanoyl hexanoate
- 1,1-Ethanediol diacetate
- Ethyl 2-hydroxy-3-methylbutanoate
- Ethyl octanoate
- Octyl acetate
- 2,3-Butanediol diacetate
- Ethyl 2-hydroxy-4-methylpentanoate
- Linalyl acetate
- Menthol acetate
- 1-Methoxy-2-propyl acetate
- Isobornyl acetate
- Ethyl isobutyrate
- Tetraydrofurfuryl acetate
- Ethyl 2-methylpropanoate
- Ethyl decanoate
- Ethyl benzoate
- Linalyl propanoate
- 2-Phenyl ethyl acetate
- Ethyl dodecanoate
- 2-Methyl propyl benzoate
- Ethyl 3-phenoxypropionate
- y-actalactone
- delta-2-decenolactone
- y-nonalactone
- Pentyl acetate
- Triacetin

### Terpenoids
- Linalool
- Myrcene
- Limonene
- Eucalyptol
- Terpinene-4-ol
- Trans-linalool oxide

### Heterocycles
- Acetophenone
- 2,5-Dimethylpyrazine
- 2,3-Dimethylpyrazine
- 2,4,6-Triethylpyridine
- 2-Ethyl-6-methylpyridazine
- 2-Ethyl-3,5-dimethylpyrazine
- 6-Methyl-2-vinylpyrazine
- 2,3-Diethyl-5-Methylpyrazine
- 3,4,5,6-Tetramethyl-2-pyridone
- 2,3,6-Tetramethyl-4-piperidine
- Dihydro-5-methyl-2(3H)-furanone
- Dihydro-2(3H)-furanone
- Dihydro-5-ethyl-2(3H)-furanone
- 2-Acetyl-3,5-dimethylpyrazine
- 6-Methyltetrahydro-2H-pyran-2-one
- 4-Hydroxy-2,5-dimethyl-3(2H)-furanone
- 1(2H)-Isobenzofuranone
- Pyridine
- Methyl pyrazine
- Ethylpyrazine
Pyrazines signature: this chemical group of volatiles, formed through Maillard reaction of di-carbonyls and amino acids, is informative about geographical origin of cocoa. Pyrazines are also key-odorants imparting earthy and roasty notes.

Short chain fatty acids signature: linear and branched chain FAs derived from beans fermentation during post-harvest.

Linear saturated aldehydes and ketones signature.
"... Computer vision enables them to see, observe and understand..."\(^1\)

How can we see compositional differences? -> **Comprehensive Chromatographic Fingerprinting**

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**Targeted - fingerprinting**

- lake
- bifurcation
- termination
- spur
- point

**Untargeted Targeted\(^2\) - fingerprinting**

- targeted
- untargeted

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1. [https://www.ibm.com/topics/computer-vision](https://www.ibm.com/topics/computer-vision)
"...Computer vision enables them to see, observe and understand..."¹

How can we see compositional differences? -> Comprehensive Chromatographic Fingerprinting

²

³

¹

²

³

DATA PROCESSING
"... Computer vision enables them to see, observe and understand..."¹
How can we see compositional differences? -> **Comprehensive Chromatographic Fingerprinting**

Unknown feature
Target analytes

Unknown feature
Untargeted analytes
"... Computer vision enables them to see, observe and understand..."\(^1\)

How can we see compositional differences? -> **Comprehensive Chromatographic Fingerprinting**

Targeted and untargeted peak(-region) features are cross-aligned between all samples and metadata collected for further processing.

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**Comprehensive Chemical Fingerprinting of High-Quality Cocoa at Early Stages of Processing: Effectiveness of Combined Untargeted and Targeted Approaches for Classification and Discrimination**

*Journal of Agricultural and Food Chemistry*

*Comprehensive two-dimensional gas chromatography coupled with time of flight mass spectrometry featuring tandem ionization: Challenges and opportunities for accurate fingerprinting studies*
Advanced fingerprinting of high-quality cocoa: Challenges in transferring methods from thermal to differential-flow modulated comprehensive two dimensional gas chromatography

Federico Magagna\textsuperscript{a}, Erica Liberto\textsuperscript{a}, Stephen E. Reichenbach\textsuperscript{b}, Qingping Tao\textsuperscript{c}, Andrea Carretta\textsuperscript{d}, Luigi Cobelli\textsuperscript{d}, Matthew Giardina\textsuperscript{e}, Carlo Bicchi\textsuperscript{a}, Chiara Cordero\textsuperscript{a,*}

Step 1 - UT fingerprinting - all samples

Construction of a untargeted and targeted peaks UT template

Step 2 - Composite Class-images

Construction of Composite-class images for each group

UT template matching to composite class images

Step 3 - Machine Learning - Chemometrics

Unsupervised statistics (HC and PCA)
Supervised statistics (PLS-DA and Classification tree)

Step 4 - Validation

Confirmation of the discriminant role of markers using classic fingerprinting based on single chromatograms

Can we easily SEE pattern differences even if chemical dimensionality is high?
Pair-wise comparison datapoint features UT peak-regions mapped

Generation of cumulative class-images by re-aligned images and datapoints fusion. Cocoa origins have distinctive "class-images"

Class-images can be "pair-wise" compared to delineate diagnostic chemical patterns
We can SEE chemical signatures through the chromatographic fingerprint while investigating samples' chemical diversity and peculiar aroma properties.
By quantitative fingerprinting, the identification achieves high robustness and transferability over time and over analytical platforms.
AI smelling machine -> Sensomic-based expert system acting as ...

Odor Activity Value (OAV) = concentration / odor threshold

Table 4. Odor Thresholds and OAVs of Important Aroma Compounds in Unroasted and Roasted Cocoa Beans

<table>
<thead>
<tr>
<th>Odorant</th>
<th>Odor Threshold (µg/kg)</th>
<th>OAV&lt;sup&gt;o&lt;/sup&gt; Unroasted Beans</th>
<th>OAV&lt;sup&gt;o&lt;/sup&gt; Roasted Beans</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetic acid</td>
<td>124&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8870</td>
<td>2660</td>
</tr>
<tr>
<td>3-methylbutanoic acid</td>
<td>22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>404</td>
<td>440</td>
</tr>
<tr>
<td>ethyl 2-methylbutanoate</td>
<td>0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>138</td>
<td>135</td>
</tr>
<tr>
<td>3-methylbutanal</td>
<td>13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>123</td>
<td>2610</td>
</tr>
<tr>
<td>methylpropanoic acid</td>
<td>190&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51</td>
<td>73</td>
</tr>
<tr>
<td>3-hydroxy-4,5-dimethyl-2(5H)-furanone</td>
<td>0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43</td>
<td>65</td>
</tr>
<tr>
<td>ethyl 2-methylpropanoate</td>
<td>1.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>2-methylbutanoic acid</td>
<td>208&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>2-phenylethanol</td>
<td>211&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17</td>
<td>36</td>
</tr>
<tr>
<td>2-phenylacetic acid</td>
<td>360&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14</td>
<td>16</td>
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<tr>
<td>2-methoxyethanol</td>
<td>16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9</td>
<td>14</td>
</tr>
<tr>
<td>2-heptanol</td>
<td>263&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.4</td>
<td>4.1</td>
</tr>
<tr>
<td>butanolic acid</td>
<td>135&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.2</td>
<td>4.2</td>
</tr>
<tr>
<td>2-methylbutanal</td>
<td>140&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0</td>
<td>32</td>
</tr>
<tr>
<td>2-phenylethyl acetate</td>
<td>233&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>dimethyl trisulfide</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6</td>
<td>21</td>
</tr>
<tr>
<td>linalool</td>
<td>37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2</td>
<td>3.5</td>
</tr>
<tr>
<td>phenylacetaldehyde</td>
<td>22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0</td>
<td>250</td>
</tr>
<tr>
<td>2,3-diyethyl-5-methylpyrazine</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4</td>
<td>6.6</td>
</tr>
<tr>
<td>5-octenoic acid</td>
<td>4730&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3</td>
<td>2.4</td>
</tr>
<tr>
<td>2-ethyl-3,5-dimethylpyrazine</td>
<td>2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3</td>
<td>7.6</td>
</tr>
<tr>
<td>2-isobutyl-3-methoxy pyrazine</td>
<td>0.8</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td>4-hydroxy-2,5-dimethyl-3(2H)-furanone</td>
<td>25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1</td>
<td>46</td>
</tr>
<tr>
<td>4-methylphenol</td>
<td>68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2-ethyl-3,6-dimethylpyrazine</td>
<td>75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2-methyl-3-(methylthio) furan</td>
<td>0.4</td>
<td>&lt;1</td>
<td>1.0</td>
</tr>
<tr>
<td>5-octalactone</td>
<td>2490&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2,3,5-trimethylpyrazine</td>
<td>290&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>1-occt-3-one</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>
AI smelling machine potentials

Sensomics-based expert system acting as AI smelling machine

Odorants quantitation in high-quality cocoa by multiple headspace solid phase micro-extraction: Adoption of FID-predicted response factors to extend method capabilities and information potential

Chiara Cordero a,*, Alessandro Guglielmetti a, Barbara Sgorbini a, Carlo Bicchi a, Elena Allegrucci a, Guido Gobino b, Lucie Baroux c, Philippe Merle c
AI smelling machine potentials

Cocoa samples at different processing stages

Accurate quantification of potent odorants responsible of sensory quality (i.e., positive attributes and defects)

*AI smelling SEBES principle*

Comprehensive mapping of all detectable analytes - origin identification

**HS SPME - HS linearity conditions**

- **ISTDs Pre-loading**
  - α and β-Thujone 100 mg/L (5.0 μL)
  - Methyl-2-octynoate 1000 mg/L (5.0 μL)

- **Sampling**
  - EVOO 0.100 g

- 5 min 50°C
- 60 min 50°C

**Accuracy results - MS vs. FID and FID vs. predicted RRFs**

Predicted Relative Response Factors (RRFs) based on combustion enthalpies and molecular structure¹ - FID quantification without ESTD

---

...the boundaries between chemistry and biology are vanishing\(^1\) ...

**Chromatographic Fingerprinting and Computer vision Identification**

**Fingerprinting/profiling to unreveal marker patterns**

**Artificial Intelligence smelling machine molecular resolution tool**

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1. Prof. Thomas Hofmann J. Agric. Food Chem. 2015, 63, 32, 7095-7096
Thank you for your attention

Acknowledgments

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