Impact of ensiling on the traceability and authentication of foods of animal origin

Frank Monahan, Aidan Moloney, Olaf Schmidt and Padraig O’Kiely
Definitions

• Food traceability - “the ability to follow the movement of a food through specified stages of production, processing and distribution” (WHO/FAO, 2007).

• Food authenticity – “the process by which a food is verified as complying with its label description” (Dennis, 1998)"
Interest in traceability/authenticity?

- Consumers want assurance about mode of production, country of origin
- High value food products, require traceability/authentication
- Food fraud
“Tracers” in meat and milk → evidence of grass/silage feeding?
Potential tracers of animal feed in meat and milk

- Fatty acids
- Volatiles, terpenes, aldehydes
- Carotenoids
- Vitamin E
- Stable isotopes
- Genes expressed
- Fingerprinting
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We are what they eat....
Feed fatty acids

Moloney et al, 2013, Meat Sci., 95, 608
Fatty acids in beef

Moloney et al, 2013, Meat Sci.,95,608
Fatty acids in beef

Moloney et al, 2013, Meat Sci., 95, 608
Human platelet n-3 fatty acids

- 20:5n-3 EPA
- 22:5n-3 DPA
- 22:6n-3 DHA
- LC n-3 PUFA
- Total n-3 PUFA

McAfee et al., 2011, Brit J Nutr, 105, 80–89
Alfaia et al., 2009, Food Chemistry 114, 939–946
Canonical discriminant analysis

Röhrle et al, 2015
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We are what they eat....
Volatile terpenes:

Monoterprenes:
- pinene
- sabinene
- limonene

Sesquiterpenes:
- β-gurjunene
- β-caryophyllene
- germacrene
- γ-cadinene
Terpenes in sheep fat

- Suzuky & Bailey, 1985, JAFC, 33, 343
- Young et al., 1997, Meat Sci., 45, 183
- Priolo et al., 2004, Meat Sci., 66, 475

![Graph](chart.png)

- Grass-fed
- Concentrate-fed

*Arbitrary units*
Terpenes in beef

![Bar chart showing terpenes in beef](chart)

Larick et al., 1987, IJFST, 38, 445
Terpenes in milk

Fernandez et al., 2003, IJFST, 38, 445
Factorial discriminant analysis using four terpenes to discriminate lamb

<table>
<thead>
<tr>
<th>Pasture outdoor</th>
<th>Grass silage indoor</th>
<th>Grass silage indoor</th>
<th>Concentrate indoor</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 month NOV - APRIL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasture outdoor</td>
<td>Pasture outdoor</td>
<td>Pasture/Concentrate outdoor</td>
<td>Concentrate indoor</td>
</tr>
<tr>
<td>6 month MAY - OCT</td>
<td>Silage - Pasture SiP</td>
<td>Silage – Pasture/Concentrate SiPC</td>
<td>Concentrate C</td>
</tr>
</tbody>
</table>
## Discriminating volatile compounds

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>SiP</th>
<th>SiPC</th>
<th>C</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>skatole</td>
<td>2.84</td>
<td>2.81</td>
<td>2.76</td>
<td>2.52</td>
<td>0.003</td>
</tr>
<tr>
<td>3-undecanone</td>
<td>2.97</td>
<td>0.80</td>
<td>1.60</td>
<td>0.41</td>
<td>0.011</td>
</tr>
<tr>
<td>cuminic alcohol</td>
<td>3.43</td>
<td>2.85</td>
<td>2.95</td>
<td>3.16</td>
<td>0.075</td>
</tr>
<tr>
<td>2-methyl butanol</td>
<td>4.32</td>
<td>4.24</td>
<td>4.46</td>
<td>4.61</td>
<td>0.012</td>
</tr>
<tr>
<td>ethenyl benzene</td>
<td>3.59</td>
<td>3.67</td>
<td>3.60</td>
<td>3.55</td>
<td>0.313</td>
</tr>
<tr>
<td>2-buten-1-ol</td>
<td>4.12</td>
<td>4.09</td>
<td>4.14</td>
<td>3.78</td>
<td>0.002</td>
</tr>
<tr>
<td>Unidentified</td>
<td>2.55</td>
<td>0.89</td>
<td>2.94</td>
<td>3.60</td>
<td>0.003</td>
</tr>
</tbody>
</table>

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We are what they eat....
Proportion of light reflected

Wavelength, nm

Concentrate fed

Pasture fed

Silage-Pasture fed

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Vitamin E stereoisomers in animal feeds

Röhrle et al. 2010, Food Chemistry 124, 935–9403
Pasture - Silage
Silage - Concentrate
Pasture/Conc.

Production system

Röhrle et al. 2010, Food Chemistry 124, 935–9403
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$^{2}\text{H}/^{1}\text{H}, \ ^{13}\text{C}/^{12}\text{C}, \ ^{15}\text{N}/^{14}\text{N}, \ ^{18}\text{O}/^{16}\text{O}, \ ^{34}\text{S}/^{32}\text{S}$

$\delta^{2}\text{H} \quad \delta^{13}\text{C} \quad \delta^{15}\text{N} \quad \delta^{18}\text{O} \quad \delta^{34}\text{S}$
$^{13}\text{C}/^{12}\text{C}$ ratio ($\delta^{13}\text{C}$) differs in different feeds

<table>
<thead>
<tr>
<th>High $^{13}\text{C}/^{12}\text{C}$ ratio</th>
<th>Low $^{13}\text{C}/^{12}\text{C}$ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>Grass (temperate areas)</td>
</tr>
<tr>
<td>Sugar cane</td>
<td>Barley, wheat</td>
</tr>
<tr>
<td>Tropical grasses</td>
<td>Soya bean</td>
</tr>
<tr>
<td><strong>C$_4$ plants</strong></td>
<td><strong>C$_3$ plants</strong></td>
</tr>
<tr>
<td>$-5.6$ to $-18.6%$</td>
<td>$-23.2$ to $-34.3%$</td>
</tr>
</tbody>
</table>
$^{15}\text{N}/^{14}\text{N}$ ratio ($\delta^{15}\text{N}$) in feed is affected by fertilizers used in growing the feed.

<table>
<thead>
<tr>
<th>High $^{15}\text{N}/^{14}\text{N}$ ratio</th>
<th>Low $^{15}\text{N}/^{14}\text{N}$ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic fertilizer</td>
<td>Artificial fertilizer</td>
</tr>
<tr>
<td>Animal/fish protein</td>
<td></td>
</tr>
</tbody>
</table>

e.g. animal manure

$\delta^{15}\text{N} \sim 5\%$

$\delta^{15}\text{N} \sim 0\%$
$^{34}\text{S}/^{32}\text{S}$ ratio (δ$^{34}\text{S}$) in feed is affected by sea spray / inclusion of fishmeal?

<table>
<thead>
<tr>
<th>High $^{34}\text{S}/^{32}\text{S}$ ratio</th>
<th>Low $^{34}\text{S}/^{32}\text{S}$ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Close to the sea</td>
<td>Inland</td>
</tr>
<tr>
<td>Marine plants (seaweed)</td>
<td></td>
</tr>
</tbody>
</table>
$\delta^{34}\text{S} \text{ Ireland in Sheep's wool}$

Hydrogen and oxygen ratios ($\delta^2\text{H}$, and $\delta^{18}\text{O}$) in water (and feed) are affected by climate, proximity to the sea, altitude, latitude

<table>
<thead>
<tr>
<th>High $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ ratios</th>
<th>Low $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>At high temperatures</td>
<td>At low temperatures</td>
</tr>
<tr>
<td>In lowlands</td>
<td>At mountains</td>
</tr>
<tr>
<td>Close to the sea</td>
<td>Inland</td>
</tr>
<tr>
<td>Western Europe</td>
<td>Eastern Europe</td>
</tr>
</tbody>
</table>
Stable isotopes
Feed $\rightarrow$ Beef

Osorio et al. 2011, J Agric Food Chem, 59, 3295
### Muscle stable isotope ratios: C, N, H, S

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>SiP</th>
<th>SiPC</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta^{13}C$ ‰</td>
<td>-27.7</td>
<td>-27.6</td>
<td>-26.4</td>
<td>-25.0</td>
</tr>
<tr>
<td>$\delta^{15}N$ ‰</td>
<td>9.2</td>
<td>8.9</td>
<td>7.9</td>
<td>6.3</td>
</tr>
<tr>
<td>$\delta^{2}H$ ‰</td>
<td>-122.4</td>
<td>-121.1</td>
<td>-117.1</td>
<td>-112.4</td>
</tr>
<tr>
<td>$\delta^{34}S$ ‰</td>
<td>4.6</td>
<td>4.7</td>
<td>4.8</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Osorio et al. 2011, J Agric Food Chem, 59, 3295
Grass Silage
$\delta^{13}C = -29.6\%\circ$

Maize Silage
$\delta^{13}C = -11.8\%\circ$

Bahar et al., 2005, RCMS, 19, 1937-42.
Irish vs non-Irish beef

Osorio et al. 2011, J Agric Food Chem, 59, 3295
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### Genetic markers?

<table>
<thead>
<tr>
<th>Authors</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassar-Malek et al. (2009)</td>
<td><strong>Selenoprotein W</strong> up-regulated in outdoor pasture vs indoor concentrate</td>
</tr>
<tr>
<td>Duckett et al. (2009)</td>
<td><strong>Stearoyl CoA desat</strong> and <strong>FAS</strong> up-regulated in concentrate finished vs pasture</td>
</tr>
<tr>
<td>Shibato et al. (2009)</td>
<td>Differential expression attributed to changes in fibre type and metabolic enzymes</td>
</tr>
<tr>
<td>Lejeune et al. (2015)</td>
<td>21 genes differentially expressed in outdoor pasture vs indoor concentrate-fed beef cattle</td>
</tr>
</tbody>
</table>
PCA – gene expression data

PC2
Scores

Concentrate-fed

Pasture-fed

RESULT2, X-expl: 53%, 13%

Lejeune et al. (2015)
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We are what they eat....
NIR spectroscopy (on milk)

<table>
<thead>
<tr>
<th>Comparison</th>
<th>% Milk samples correctly classified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasture vs Maize Silage</td>
<td>95.5</td>
</tr>
<tr>
<td>Pasture vs Hay</td>
<td>91.5</td>
</tr>
<tr>
<td>Pasture vs Silage/Haylage</td>
<td>93.3</td>
</tr>
</tbody>
</table>

FTIR spectroscopy (on butter)

Key messages

• Strong tracers exist in animal-derived food products (e.g. meat, milk) to underpin authenticity-based traceability systems

• Enabling
  → Unique product signatures based on production system
  → Links to geographical origin, regional assignment
  → Consumer assurance
  → Market opportunities
Acknowledgements

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