The Maillard reaction:
Implications for the dairy industry

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Maillard browning

- The Maillard reaction is also known as non-enzymic browning
- Very different from enzymic browning which occurs when an apple is cut
- Involves several reactions; the formation of brown pigments is the last one
- Maillard browning is seen in many cooked foods, e.g., meat, bread, potato chips, roasted coffee
- Less visible in most milk and dairy products but still very important

The Maillard Reaction

- Named after Louis Camille Maillard who published a paper on the brown pigments, melanoidins, in 1912
- Starts with a reaction between a reducing sugar and a protein
- [A “reducing” sugar is one that can be oxidised, in chemical terms one with an aldehyde group which can be oxidised to a carboxylic acid, e.g., lactose, glucose, galactose but NOT sucrose]
- Sucrose-sweetened products, e.g., sweetened condensed milk, are not more susceptible to Maillard browning unless the sucrose contains some invert sugar (glucose+ fructose)

The Maillard Reaction 2

- Reaction requires heat; the higher the temperature, the faster it goes
- In the dairy industry it occurs during high-temperature processing
  - A major reason why UHT processing replaced in-container sterilisation – sterilised milk has a brownish colour
- And, importantly, it also occurs during storage, particularly at temperatures above ~30°C.
- It occurs in powders as well as liquid products
Significance in food processing

"Among the various processing-induced chemical reactions in proteins, the Maillard reaction (non-enzymic browning) has the greatest impact on sensory and nutritional properties" [Fennema, O.R. (1996) Food Chemistry (3rd edn)]

Brown colour formation and flavour change are the most significant effects
[WWII soldiers complained that dried egg was going brown and developing off-flavour]

Factors affecting the Maillard reaction

• Temperature
• Amount and type of reducing sugar
• Time
• Relative humidity (for powders)
• pH – faster reaction at higher pH
• Stabilisers used in sterilisation

Effects on milk and dairy products

Adverse effects

• Browning is the obvious effect but is only noticed in:
  o very highly heated products, e.g., sterilised milk
  o lactose-hydrolysed milk
    ⊡ The products of lactose hydrolysis, glucose and galactose, react much faster than lactose in the Maillard reaction
  o products stored at high ambient temperature (>30°C) for several weeks or months, e.g., milk powders, UHT milk

Skim milk, goat’s milk, full cream cow’s milk (X2) and lactose-reduced milk, after storage at 50 °C for 4 months
Deeth & Lewis (2017)
Lactose-reduced milk samples after storage at 4, 20, 35 and 50 °C for 4 months

Deeth & Lewis (2017)

Effects (cont)

- Off-flavour production, especially during storage of long-shelf-life products
- Reduces pH in UHT milk during storage due to production of formic and acetic acids
- Causes protein cross-linking which may reduce solubility of some powders
- Reduction in nutrient value through blocked lysine
  - a concern for products such as infant formulae

Beneficial effects

- Some Maillard products have antioxidant properties
  - CSIRO scientists showed heated casein-glucose mixture had antioxidant properties in whole milk powder and in encapsulated fish oil
  - The brown colour is beneficial in some foods, not dairy

Stages of the Maillard reaction

- The Maillard reaction is often considered to occur in 3 broad stages:
  - Early (lactosylation)
  - Mid or advanced - formation of numerous products
  - Late – formation of brown pigments

- Note: all stages can be occurring at the same time

Early stage Maillard Reaction: Lactosylation (glycation)
**Lactosylation**

- First stage of Maillard reaction in milk
  - No brown colour produced in this stage
  - Lactose reacts with the ε-NH₂ group of lysine

\[
\text{Lysine:} \quad \text{the amino acid}
\]

**Lactosylation 2**

- Forms lactosyl-lysine (protein-bound)
- Lactosyl-lysine is unstable and spontaneously converts to lactulosyl-lysine (by an Amadori rearrangement)
- Lactulosyl-lysine (the Amadori product) is a stable product

**Lactosylation 3**

- Formation of lactulosyl-lysine makes lysine nutritionally unavailable (lysine blockage)
- More than one lactose can react per protein molecule
  - Lactose molecules can react with different lysines
  - in mono- and di-lactosylated caseins, 7 of 14 lysines in αs1-casein and 5 of 11 lysines in β-casein were lactosylated
**Lactosylation**

- Initiated during processing but continues during storage
- Extent of lactosylation can be measured in various ways but commonly as furosine
  - furosine is formed from lactulosyl-lysine by digestion with acid
  - yield of furosine from lactulosyl lysine is ~32% so amount of blocked lysine can be estimated
- there is no furosine in milk

**Lactosylation (measured as furosine) increases during storage**

![Graph showing increase in furosine during storage](Elliott et al. (2005))

**Trial with MPC80**

Samples kept for up to 12 weeks at:
- 3 humidities and
- 4 temperatures

Lactosylation measured as furosine

Colour measured by Minolta colorimeter – change in colour ΔE was calculated from L*, a* & b*

**Furosine change of MPC during storage**

![Graph showing furosine change during storage](Le et al. (2011a))
**Colour change (ΔE) of MPC during storage for up to 12 weeks at:**
3 humidities, 4 temperatures

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
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<tbody>
<tr>
<td>44% RH</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>66% RH</td>
<td>0</td>
<td>500</td>
<td>1000</td>
<td>1500</td>
<td>2000</td>
<td>2500</td>
<td>3000</td>
<td>3500</td>
<td></td>
</tr>
<tr>
<td>84% RH</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

*Le et al. (2011a)*

A colour difference of ~ 3.5 can be noticed

**Trial with 4 powders**

Samples of MPC80, WMP, SMP & WPC stored for up to 12 weeks at:

- 3 humidities and 4 temperatures

**Lactosylation** measured as furosine

**Lactosylation (measured as furosine) increases during storage at 30°C**

*In powders: MPC80, WMP, SMP, WPC80*

<table>
<thead>
<tr>
<th>Storage time (week)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPC80</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
</tr>
<tr>
<td>WMP</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
</tr>
<tr>
<td>SMP</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
</tr>
<tr>
<td>WPC80</td>
<td>SLS K</td>
<td>SLS K</td>
<td>SLS K</td>
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<td>SLS K</td>
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<td>SLS K</td>
</tr>
</tbody>
</table>

*Le et al. (2011b)*

**Effect of stabilisers/pH**

In-container sterilised 25% reconstituted skim milk samples

- SHMP
- TSC
- DSHP
- DHSP
- Control

TSC (trisodium citrate) & DSHP (disodium hydrogen phosphate) increase pH

*Lewis, unpublished*
**Blocked lysine: the result of the early stage Maillard Reaction**

- The lysine in lactulosyl-lysine is not available for digestion
- The percentage of lysine blocked by the Maillard reaction can be determined from furosine analysis

<table>
<thead>
<tr>
<th>Product</th>
<th>% Blockage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
<td>0</td>
</tr>
<tr>
<td>Pasteurised milk</td>
<td>0-2</td>
</tr>
<tr>
<td>UHT milk</td>
<td>0-10</td>
</tr>
<tr>
<td>In-container sterilised</td>
<td>10-15</td>
</tr>
<tr>
<td>Evaporated milk</td>
<td>15-20</td>
</tr>
<tr>
<td>Condensed milk</td>
<td>14-36</td>
</tr>
<tr>
<td>Spray-dried powder</td>
<td>0-7</td>
</tr>
<tr>
<td>Roller-dried powder</td>
<td>10-50</td>
</tr>
<tr>
<td>Infant formulae</td>
<td>5-34</td>
</tr>
</tbody>
</table>

Mehta & Deeth (2016)

**Mid/advanced stage Maillard Reaction**

**Advanced Maillard Reaction Products (AMRPs)**

- Sometimes called Advanced Glycation End-Products (AGEs)
  - Particularly in the medical field
  - Maillard reaction takes place in the human body as well as in food we eat
  - Medical interest because some AGEs have been implicated in some illnesses
    - May be pro-inflammatory but ‘jury is still out’ (Davis et al. 2016)
    - Discussion beyond scope of this presentation

**Advanced Maillard Reaction Products (AMRPs) 2**

- Includes many compounds
- All due to decomposition of lactulosyl-lysine

1. Formic & acetic acids – the reason for the decrease in pH of UHT milk during storage

![Graph](image.png)  
*Stored UHT milk*

Gaucher et al. (2008)
Advanced Maillard Reaction Products (AMRPs) 3

3. Flavour compounds, e.g. aldehydes
   - Major contributor to flavour of high-temperature treated products, UHT milk, milk powders
   - Lysyl-pyrroline, maltosine, maltol, β-pyranone, 3-furanone, cyclopentanone, galactosyl-isomaltol, acetylpyrrole, pentosidine found in heated milk (van Boekel 1998)

4. HMF (hydroxymethyl furfural)
   - Often used as index of heat treatment or Maillard reaction
   - When measured, HMF (total) includes free HMF and HMF formed from lactulosyl-lysine by reaction with oxalic acid during analysis
   - free HMF is much lower than total HMF

More AMRPs

4. Dicarbonyl compounds:
   - e.g. Glycerol + several others
   - Can cross-link proteins by acting as linking agents
     - React with amine groups on adjacent protein molecules
     - Cross-linking occurs during storage of long-life milk and powders – forms high-molecular-weight proteins
     - Role in cross-linking was demonstrated by incubation of a reconstituted MPC powder with methyl glyoxal;
       - it reproduced the same high-molecular-weight proteins seen in stored powder and UHT milk (Le et al. 2013)
     - Cross-linking can:
       - stabilise the casein micelle and protect it in UHT milk
       - Increase the viscosity of yogurt
       - reduce the solubility of powders

Late Stage Maillard Reaction

Late stage products

- Formation of brown pigments, melanoidins
  - Responsible for colour of many foods, e.g., coffee, cocoa, honey, malt
  - Molecular weights of 3,500 to 14,000 Da
  - Have antioxidative and antimicrobial properties
  - Do not appear to have adverse health effects

Wang et al. 2011
Inhibition of Maillard Reaction

Can we stop or inhibit the Maillard Reaction

- Probably not but it can be slowed down in some circumstances
- By adding reducing compounds such as cysteine
- By adding polyphenols such as those in tea, e.g., epicatechin
  - Epicatechin (0.1%) added to milk before UHT processing markedly reduced Maillard-derived flavour compounds and the cooked flavour of the UHT milk
  - At 0.2% added epicatechin, UHT milk tasted similar to pasteurised milk
- Tea polyphenols also reduced browning in UHT milk as measured by colour difference ∆E

(Colahan-Sederstrom & Peterson 2005; Schamberger & Labuza 2007)

References


Thank you for your attention

Any questions?