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:
  - very highly heated products, e.g., sterilised milk
  - lactose-hydrolysed milk
    - The products of lactose hydrolysis, glucose and galactose, react much faster than lactose in the Maillard reaction
  - products stored at high ambient temperature (>30C) 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

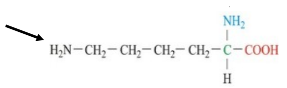
Stages of the Maillard reaction

- 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

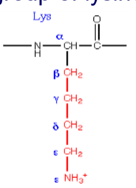
Early stage Maillard Reaction: Lactosylation (glycation)

Lactosylation

- First stage of Maillard reaction in milk
  - No brown colour produced in this stage
- Lactose reacts with the ε-NH_2 group of lysine



Lysine: the amino acid

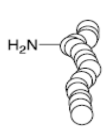


Lysine in a protein

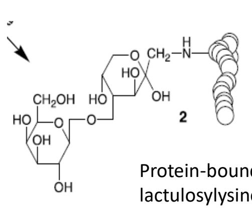
Lactosylation 2

- 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



Amine group of lysine side-chain attached to protein backbone



Protein-bound lactulosylysine (the Amadori product)

Lactosylation 4

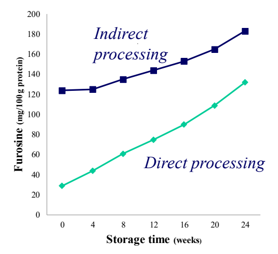
- Formation of lactulosyl-lysine makes lysine nutritionally unavailable (lysine blockage)
- More than one lactose can react per protein molecule
  - Lactose molecules can react with different lysines
  - in mono- and di-lactosylated caseins, 7 of 14 lysines in α_{s1}-casein and 5 of 11 lysines in β-casein were lactosylated

Lactosylation 5

- 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

UHT milk



Elliott *et al.* (2005)

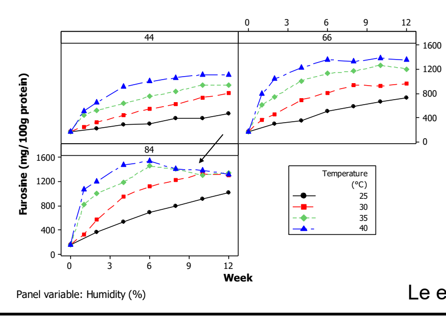
Trial with MPC80

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

Lactosylation measured as furosine

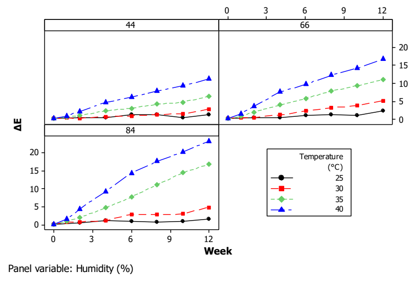
Colour measured by Minolta colourmeter – change in colour ΔE was calculated from L*, a* & b*

Furosine change of MPC during storage



Le et al. (2011a)

Colour change (ΔE) of MPC during storage for up to 12 weeks at:
3 humidities, 4 temperatures



A colour difference of ~ 3.5 can be noticed

(Le et al. 2011a)

Trial with 4 powders

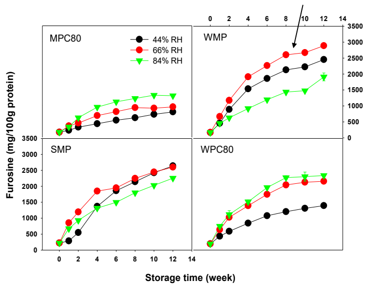
Samples of MPC80, WMP, SMP & WPC stored for up to 12 weeks at:

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



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

Product	% Blockage
Raw milk	0
Pasteurised milk	0-2
UHT milk	0-10
In-container sterilised	10-15
Evaporated milk	15-20
Condensed milk	14-36
Spray-dried powder	0-7
Roller-dried powder	10-50
Infant formulae	5-34

Mehta & Deeth (2016)

Mid/advanced stage Maillard Reaction

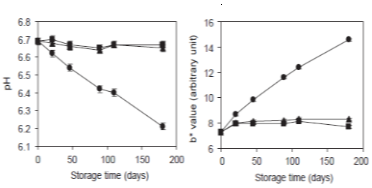
Advanced Maillard Reaction Products (AMRPs)

- 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



Stored UHT milk

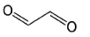
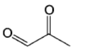
at 4 °C (■), 20 °C (▲) and 40 °C (●) for up to 180 days.

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-pyrraline, 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. 
Glyoxal

Methylglyoxal
+ 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 ΔE

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

Thank you for your attention

Any questions?

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