The Maillard Reaction: Radicals and Flavor

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History: The first report

- First investigation of reaction between sugars and amino acids by Louis Camille Maillard
- Reported reaction of excess d-glucose with glycine in aqueous conditions.
  - Liberation of CO$_2$ and loss of H$_2$O
  - Postulated incorporation of amino acid into sugar, forming heterocycles


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Some polycyclic molecules with nitrogen incorporated

Blackish substance

History: First Overall Reaction Pathway

• First overall transformation pathway was proposed in 1953 by John E. Hodge
  • Based on previous work with Carl Rist

• Proposed scheme “Hodge Scheme” is still currently accepted

• In 1979 Science Citation Index named “Chemistry of Browning Reactions in Model Systems” a Citation Classic.

Hodge, J. E., Journal of Agricultural and Food Chemistry, 1953, 1(15), 928–43
Modified Hodge Scheme

Reducing sugar + α-amino acid → N-Glycosalamines or N-fructosylamines

Amadori rearrangement → 1-Amino-1-deoxy-2-ketose (Amadori) or 1-amino-2-deoxy-2-aldose (Heyns)

Sugar dehydration → Hydroxymethyl furfural (hexoses)

Furfural (pentoses)

N-substituted 1-amino-2-deoxy-2-ketose

3-Furanones
4-Furanones
Pyrroles
Thiophenes

Retro-aldolization

Hydroxyacetone
Dihydroxyacetone

Glyoxal
Pyruvaldehyde
Glycolaldehyde
Glyceraldehyde

A reductone

+ Amino acids → Aldehydes + α-aminoketones (+ methional from methionine) (+ H2S and NH3 from cystine)

Strecker Degradation

Acetoin → Heterocyclization

Pyridines
Thiazoles
Pyrazines
Pyrroles
Oxazoles
Imidazoles
Common flavor compounds: Acyclic

Found in:

\[
\begin{align*}
\text{butanediione} & & \text{Coffee, bread crusts, stewed/ roasted meats, beer, wine} \\
\text{Methional} & & \text{Coffee, bread crusts, tea, stewed/ roasted meats, baked potato, chips, beer} \\
\text{phenylacetaldehyde} & & \text{Coffee, bread crusts, stewed meats, chocolate, wine}
\end{align*}
\]
Acyclic products from Maillard reaction

- Methional
- phenylacetylaldehyde
- 2-mercaptopyruvate
- (E)-2-((methylthio)methyl)but-2-enal
- glyoxal
- butanedione
- pyruvaldehyde
- prop-2-ene-1,1-diylbis(methylsulfane)
- glyceraldehyde
- 1,3-dihydroxyacetone
- acetoin
- glycolaldehyde

Hodge, J. E., Journal of Agricultural and Food Chemistry, 1953, 1(15), 928–43
Common flavor compounds: Cyclic

3,5,6-trimethylpyrazin-2(1H)-one

Found in:
Coffee, bread crusts, stewed/ roasted meats, toasted sesame, popcorn

4,5-dimethyl-2-(2-(methylthio)ethyl)oxazole

Found in:
Cocoa, coffee, meats, roasted barley, baked potato, roasted peanuts, French fries

1-(3H-imidazo[4,5-c]pyridin-4-yl)ethan-1-one

Found in:
Coffee, roasted barley, stewed/ roasted meats,
Heterocyclic products

5-(hydroxymethyl)furan-2-carbaldehyde (5-hydroxymethyl furfural)

3-hydroxy-2-methyl-4H-pyran-4-one

2-hydroxy-2,5-dimethyl-3(2H)-thiophenone

1-(2,3-dihydro-1H-pyrrolizin-5-yl)ethan-1-one

1-(3H-imidazo[4,5-c]pyridin-4-yl)ethan-1-one

3,5,6-trimethylpyrazin-2(1H)-one

4,5-dimethyl-2-(2-(methylthio)ethyl)oxazole

Sugar-Amine condensation

\[
\text{Aldose} + \text{RNH}_2 \xrightleftharpoons{} \text{H}_2\text{O} \rightarrow \text{Schiff base} \rightarrow \text{N-substituted glycosylamine}
\]

Hodge, J. E., *Journal of Agricultural and Food Chemistry*, 1953, 1(15), 928–43
Amadori rearrangement

N-substituted glycosylamine

Schiff base cation

enol form

keto form

N-substituted 1-amino-2-deoxy-2-ketose

Progress

Reducing sugar + α-amino acid → N-Glycosalamines or N-fructosylamines

Amadori rearrangement → 1-Amino-1-deoxy-2-ketose (Amadori) or 1-amino-2-deoxy-2-aldose (Heyns)

Condensation

3-Furanones
4-Furanones
Pyrroles
Thiophenes

NH₃
H₂S

Reductones and dehydroreductones

+ NH₃

Hydroxyacetone
Dihydroxyacetone

Glyoxal
Pyruvaldehyde
Glycolaldehyde
Glyceraldehyde

Retro-aldolization

Furfural (pentoses)

Hydroxymethyl furfural (hexoses)

- 3 H₂O

+ Amino acids

Strecker Degradation → Aldehydes + α-aminoketones (+ methional from methionine) (+ H₂S and NH₃ from cystine)

Acetoin

Heterocyclization → Pyridines
Thiazoles
Pyrazines
Pyrroles
Oxazoles
Imidazoles
Formation of reductones

N-substituted 1-amino-2-deoxy-2-ketose

Pathway may stop at this point or continue


Reductone path: Hydroxymethyl furfural

Nursten, H., The Maillard Reaction Chemistry, Biochemistry and Implications; Royal Society of Chemistry: Cambridge, 2005
Secondary reductone path

- This pathway is favored under neutral and basic conditions
- May form furones or go on to sugar fragmentations

Nursten, H., The Maillard Reaction Chemistry, Biochemistry and Implications; Royal Society of Chemistry: Cambridge, 2005
Sugar Fragmentation

- Only half of possible fragmentation paths shown
- Multiple pathways, all giving different products


Nursten, H., The Maillard Reaction Chemistry, Biochemistry and Implications; Royal Society of Chemistry: Cambridge, 2005
Progress

Reduction sugar + a-amino acid → N-Glycosalamines or N-fructosylamines

Condensation → Amadori rearrangement

1-Amino-1-deoxy-2-ketose (Amadori) or 1-amino-2-deoxy-2-aldose (Heyns) → Hydroxymethyl furfural (hexoses) or Furfural (pentoses) → 3 H₂O

Sugar dehydration

Reductones and dehydroreductones

3-Furanones 4-Furanones Pyrroles Thiophenes

NH₃ H₂S

Retro-aldolization

Hydroxyacetone Dihydroxyacetone Glyoxal Pyruvaldehyde Glycolaldehyde Glyceraldehyde

+ Amino acids Strecker Degradation

Aldehydes + α-aminoketones (+ methional from methionine) (+ H₂S and NH₃ from cystine)

Acetoin

Heterocyclization

Pyridines Pyrazines Oxazoles Thiazoles Pyrroles Imidazoles
Saccharinic Rearrangement

- Enolize into enediol
- One alcohol jumps onto other carbon, carbon losing OH gains H other carbon
- At same time carbonyl reforms
- As carbonyl reforms proton is transferred
- OH migration, carbonyl reformation and proton transfer are one concerted step

Isabell, H. S., J. Res. Natl. Stand. 1944, 32, 45-59
Progress

Reducing sugar + α-amino acid → N-Glycosalamines or N-fructosylamines

- Amadori rearrangement

1-Amino-1-deoxy-2-ketose (Amadori) or 1-amino-2-deoxy-2-aldose (Heyns)

- 3 H₂O

- Furfural (pentoses)

- Hydroxymethyl furfural (hexoses)

Sugar dehydration

3-Furanones, 4-Furanones, Pyrroles, Thiophenes

NH₃, H₂S

Reductones and dehydroreductones

- Retro-aldolization

Hydroxyacetone, Dihydroxyacetone

Glyoxal, Pyruvaldehyde, Glycolaldehyde, Glyceraldehyde

+ Amino acids (→ Aldehydes + α-aminoketones (+ methional from methionine) (+ H₂S and NH₃ from cystine))

Strecker Degradation

Acetoin

Heterocyclization

Pyridines, Thiazoles, Pyrazines, Pyrroles, Oxazoles, Imidazoles
Heterocyclic products

5-(hydroxymethyl)furan-2-carbaldehyde
(5-hydroxymethyl furfural)

3-hydroxy-2-methyl-4H-pyran-4-one

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3,5,6-trimethylpyrazin-2(1H)-one

4,5-dimethyl-2-(2-(methylthio)ethyl)oxazole
Formation of pyrazines is a path almost exclusive to asparagine, lysine and dipeptides


Pyrazines

- Formation of pyrazines is a path almost exclusive to asparagine, lysine and dipeptides
- Due to pyrazine ubiquity, this path is common in Maillard reaction

How butanedione combines with amine

Heterocyclic products

5-(hydroxymethyl)furan-2-carbaldehyde (5-hydroxymethyl furfural)

3-hydroxy-2-methyl-4H-pyran-4-one

2-hydroxy-2,5-dimethyl-3(2H)-thiophenone

1-(2,3-dihydro-1H-pyrrolizin-5-yl)ethan-1-one

1-(3H-imidazo[4,5-c]pyridin-4-yl)ethan-1-one

4,5-dimethyl-2-(2-(methylthio)ethyl)oxazole

3,5,6-trimethylpyrazin-2(1H)-one
Pyrroles and Pyrrolizidines

Addition of a second component

**Example of how viable molecules are still present after they have “left” the reaction**

Group Problem

Determine which fragmentation product combines with histidine

Propose a pathway for how your chosen starting material forms the shown product

(Hint: No secondary fragmentation products combine in later stages)

Choices:

- butanedione
- pyruvaldehyde
- Methional
Browning potential: melanoidins

Hodge, J. E., Journal of Agricultural and Food Chemistry, 1953, 1(15), 928–43
Melanoidins

- High molecular weight brown polymers, with unknown overall structure
- Masses identified from 1 kDa to >24 kDa
- Oligomers of heterocyclic compounds and/or sugar fragments
- $^{13}$C-, $^{15}$N-NMR, MALDI-TOF mass spec and IR have determined presence of pyridines, pyrazines, pyrroles and imidazoles
- Oligomers from 14 to >30 identified
- Normally 3-4% nitrogen

Nursten, H., The Maillard Reaction Chemistry, Biochemistry and Implications; Royal Society of Chemistry: Cambridge, 2005
Melanoidin constitution

- Constitution of melanoidins vary depending on aldehyde and amine condensing
- Rate of melanoid production is dependent on temperature

X = NR, O

Investigation toward melanoidin structures

- Constitution of melanoidins vary depending on aldehyde and amine condensing

**Observed content in melanoidin**

- High ether content
- High alcoholic hydroxyl content
- High enolic hydroxyl content, with low ether content

**Aldehyde and amine condensed**

- Furfural and glycine
- Glucose and glycine
- 2-oxopropanal and glycine

Electron spin resonance spectroscopy (ESR)

- In the presence of a magnetic field (H), the charged dipoles associated with the unpaired electrons line up either parallel or antiparallel to the direction of H and to precess about the field axis at a frequency proportional to the strength of H
- The difference in energy between the two state can be measured by the equation: \( \Delta E = \hbar \nu = g \mu_b H \)
- \( \hbar \) = Planck’s constant
- \( \mu_b \) = Bohr magneton constant
- \( \nu \) = precession frequency
- \( H \) = magnetic field strength
- \( g \) = spectroscopic splitting factor and changes with an electron’s proximity to magnetic nuclei (in absence of magnetic nuclei \( g = 2.0023 \) MHz/gauss)
ESR data

• In practice the difference in energy is reported as an absorption and plotted against field strength
• Spectra are normally of the first derivative of this absorption and entire area is measured
  • May be a second derivative
ESR spectra

$\nu = 9388.2 \text{ MHz}$

Absorbance

First Derivative

Magnetic Field Strength (G)
Gathering structural information

• If an electron is near a magnetic nuclei then there is hyper-fine splitting
• The signal of the electron is split by the magnetic nuclei by $2I+1$
  • $I$ = spin quantum number of the magnetic nucleus

• Think of ESR as NMR for electrons and hyperfine splitting as J-values
Hyperfine splitting
Radicals measured in ESR

• Electrons have a “spin” that describes the magnetic field (magnetic moment) developed by their rotation as charged particles

• Ordinary organic molecules have all their electrons paired up therefore all spins cancel
  • Non-magnetic or diamagnetic moments

• Free radicals are unpaired and not cancelled causing a paramagnetic moment that can be measured

• To measure a radical it must be stable enough to exist at a concentration of $>10^{-9}$ M at a time

Observation of radicals

• Stable free radicals first observed by Electron Spin Resonance spectroscopy (ESR) in sample of roasted coffee
• The concentration of free radicals is dependent on roasting times
• Radicals found to be present in grounds after brewing process with no change in concentration
  • Radical carrying species must not be water soluble

Studying radical stability

- The melanoidin’s original radical concentration was measured as $1.9 \times 10^{17}$ spin/gram
- After two weeks the radical concentration declined by only one third
  - Stored under dry air at room temperature

![Chemical reaction diagram](image_url)

Following radicals through the reaction

• ESR and UV-Vis was used to follow radicals as the Maillard reaction progressed from sugar to melanoidin

• Two distinct radicals were observed
  • One novel radical with a hyper-fine peak structure
  • One radical corresponding to known melanoidin radical character

• α- and β-amino acids gave significantly different splitting patterns
  • β-amino acids splitting pattern was found to be analogous to when n-butylamine was used

\[
\text{arabinose} + \text{alanine} \xrightarrow{\text{Water}} \text{Melanoidins}
\]

Melanoidin free radicals

- Model reactions of glucose with glycine, glutamine and lysine gave different melanoidins all with unique radicals
- Higher weight melanoids gave higher concentration of radicals
- All radicals were stable including carbon centered radicals
  - Radicals were observable with no appreciable decrease in concentration after 1 year of storage at 4°C

The novel radical peak

Hyper-fine peaks in ESR spectra were found to be due to 1,4-disubstituted pyrazinium radical cations.

Structure deduced from ESR studies on various Maillard condition:
  - Found as a result of aliphatic amines, and alanine reacting with any group containing an enediol group or potential for an enediol group.

Structure Deduction

- Almost all hyperfine structures gave splitting pattern shown
- Coupling to neighbors was shown and structure was elucidated from spectra
- Compared and matched to spectra of synthesized compound

The novel radical peak: creation

- Progression of radical followed via ESR
- Radical cation observed half-life of 10 minutes
- Dication rapidly converted into dihydrohydroxy and dihydrodihydroxy derivatives in water
  - Presence confirmed via LC-MS
- Amine must be a primary amine
Radicals in the Maillard pathway

- Two different reaction paths emerge: 1) Classic Hodge route; 2) Radical path

**Hodge route:**

sugars + amino acids $\rightarrow$ glycosylamines $\rightarrow$ Amadori compounds $\rightarrow$ reductones $\rightarrow$ melanoids

**Radical Path:**

sugars + amino acids $\rightarrow$ Glycosylamines and/or Amadori compounds $\rightarrow$ C2 and/or C3 fragments $\rightarrow$ free radicals $\rightarrow$ melanoids


Determination of dominant path

• Relative proportion of ionic (Hodge) and radical involvement investigated
• DPPH used to quantify both ionic and radical content via UV-Vis
  • Upon reaction with ions or radicals absorbance of DPPH (529nm) is lost
• Ionic intermediates also quantified with ferric ion transformed into ferrous ion
  • Formation of colored (505nm) phenanthroline-ferro(II) complex followed by UV-Vis
• Screened various amino acid and sugar combinations and pH: 5, 7 and 8

Radical pH dependence

- Ionic path is generally dominant at low pH
- Radicals present at 60-80% in early reaction stages at pH 7 and above

Radical dependence on amino acids

- Structure of amino acids had small effect on ion.radical ratios

Radical dependence on sugar

- Sugar structure plays a key role in radical content
- Disaccharides give lowest amount of radicals: cleavage of glycoside bond is rate limiting
- Monosaccharides with higher proportion of acyclic structures lead to higher radical formation

(at pH=7)

glucose > fructose >> maltose

Proposed radical mechanism

- Mechanism investigated in systems with iron and copper
  - Either metals are available in most food systems
  - Electron transfer is observed in reaction systems under metal and oxygen free environments

\[
\text{R} = \text{amino acid residue} \\
\text{R}_1 = \text{sugar residue}
\]

Free radicals and coffee: redux

• Unroasted coffee beans have a concentration of free radicals (CFR) of $10^{15}$ spins/gram
  • Possibly due to sun exposure during bean drying process or metal ions in the bean
• Roasted coffee has increased CFR to $10^{16}$ to $10^{17}$
• CFR values increased as degree of roast increased
  • Dark roast has more free radicals than light roast
• Radicals found to be especially stable
  • Long lived even after multiple aqueous extractions
  • High proportion of carbon centered radicals to hydroxyl radicals
A look back at coffee’s radicals

• Radical extinction of dry coffee with CFR of $7.5 \times 10^{16}$ was observed
• Over the course of 10-15 min hydroxyl radical singlet rapidly disappeared
• Secondary radical signals persisted with only slow changes
• Secondary signals proposed to correspond to the lysine derived radical cation involved in protein cross-linking and melanoidin formation
  • CROSSPY radical
• Only hydroxyl radicals reacted with radical spin traps

Conclusion

• Maillard reaction: a complex compilation of reactions competing and converging to create the flavors in food
• Hodge path is still accepted, competing radical pathways are possible and probable
• Radical pathways may give insight into formation of heterocycles and antioxidant activity of foods
References