Lipid oxidation in emulsified foods: An overview of recent progress

Charlotte Jacobsen
Research group for Bioactives – analysis and application
Division of Food Technology
chja@food.dtu.dk
Outline

• Introduction
• Factors affecting oxidation in complex emulsified foods
• Effect of oil quality
• Effect of ingredients
• Optimization of production process
• Introduction to antioxidants in emulsified foods
• Effect of antioxidant addition including lipophilisation
• Conclusions
• Acknowledgements
Lipid oxidation

Unpleasant flavors, Nutrient loss, Texture, Color, Functionality

Lipid oxidation is one of the most important chemical degradation processes

Oxidized lipids → Rancidity

Food emulsions: Mayonnaise, salads & dressing, yoghurt, milk

Bulk oil

Fish
Oxidation and analysis of oxidation

Hydroperoxides: peroxide value (PV) or conjugated dienes

Volatile oxidation products

Volatile oxidation products

Sensory evaluation

Rancid off-flavour

R•

RH

LH

L•

LOOH

LOO•

O2

L•

LH
Factors that can affect lipid oxidation in emulsified foods:

1. Ingredients (Amount, type and quality)

2. pH

3. The surface charge

4. Viscosity

5. Oil droplet size / surface area

6. Processing conditions

7. Antioxidants

- Emulsifier at the interface (Structure/thickness)
- Emulsifier in the aqueous phase (Antioxidative properties)
- Oil
- Water
- AO
- AO
- AO
- Fe
- Fe
- Fe
- AO
- AO
- AO
The role of emulsifiers

Effect of oil quality
Fish oil enriched milk

Fishy taste
[0-9]

Oil quality affects the food quality
→ seems that PV needs to be
≤ 0.1 meq/kg fish oil

Milk with a fish oil and rapeseed oil mixture

Effect of food composition
Effect of fish oil concentration and emulsifier type in fish oil enriched mayonnaise (20 °C)

- Oxidation increased with increasing fish oil conc.
- Previous data: Egg yolk and low pH main factors responsible for lipid oxidation
- Milk protein resulted in increased oxidation

- 1-penten-3-one [ng/g] vs. Storage time [days]
- Rancid (Flavour) vs. Storage time [Months]
Effect of the Ingredients: Mayonnaise-based Shrimp Salad

- Salads without asparagus were more oxidised
- Salads without shrimps were the least oxidised
- The panel could not discriminate between standard salad with or without fish oil

## Comparing Milk and Drinking yoghurt: Effect of Ingredients in Yoghurt

Intensity of fishy off-flavour (0-9):

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 1</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>5.4 ± 1.5</td>
<td>6.0 ± 2.4</td>
<td>7.4 ± 1.0</td>
</tr>
<tr>
<td>Yoghurt (CA+P+FS)</td>
<td>0.0 ± 0.1</td>
<td>0.5 ± 1.0</td>
<td>0.4 ± 0.7</td>
</tr>
<tr>
<td>Yoghurt (CA+P)</td>
<td>0.4 ± 0.5</td>
<td>0.9 ± 1.4</td>
<td>1.0 ± 1.3</td>
</tr>
<tr>
<td>Yoghurt (CA)</td>
<td>0.4 ± 0.5</td>
<td>0.7 ± 1.0</td>
<td>1.3 ± 0.9</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>0.5 ± 0.4</td>
<td>0.8 ± 1.0</td>
<td>1.6 ± 1.2</td>
</tr>
</tbody>
</table>

CA: Citric acid; P: Pectin; FS: Fruit preparation and sugar

- Fish oil enriched milk oxidised much faster than fish oil enriched yoghurt
- Ingredients added to yoghurt did not affect oxidation

Comparing Milk and Yoghurt: Antioxidant Assays on Peptide Fractions in Yoghurt

<table>
<thead>
<tr>
<th></th>
<th>Crude protein</th>
<th>10-30</th>
<th>3-10</th>
<th>&lt;3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radical scavenging (DPPH)</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Metal chelation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Reducing power</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

Comparing Milk and Yoghurt: Oxidative Stability of Milk with Peptide Fractions from Yoghurt

LC-MS/MS characterisation of peptides

Fractions > 3 kDa were dominated by peptides from caseins. Phosphorylated peptides were not found.

Fraction < 3 kDa dominated by free amino acids

Absorbance at 210 nm

Optimising processing conditions

- Temperature and pressure
- Type of equipment
Effect of Emulsification Conditions: Milk Affected by Temperature and Pressure

- Homogenization with a low temperature and a low pressure leads to more oxidation than a high temperature and a high pressure!

## Homogenisation Conditions Affect Protein Composition at the Interface

<table>
<thead>
<tr>
<th>Pressure [MPa]</th>
<th>Temperature [°C]</th>
<th>β-lactoglobulin</th>
<th>α\textsubscript{s1}-casein</th>
<th>α\textsubscript{s2}-casein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference, no treatment</td>
<td></td>
<td>4.92</td>
<td>5.18</td>
<td>4.19</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>8.01</td>
<td>5.94</td>
<td>5.14</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>10.17</td>
<td>4.28</td>
<td>4.09</td>
</tr>
<tr>
<td>15</td>
<td>50</td>
<td>9.36</td>
<td>5.21</td>
<td>5.08</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>14.56</td>
<td>4.55</td>
<td>4.59</td>
</tr>
<tr>
<td>22.5</td>
<td>50</td>
<td>9.45</td>
<td>4.56</td>
<td>4.23</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>13.72</td>
<td>3.68</td>
<td>3.74</td>
</tr>
</tbody>
</table>

Homogenisation conditions affect surface behaviour of proteins

Casein: green
Lactoferrin: red
Lipids: blue

Fish oil enriched milk
50 °C, 50 bar

Fish oil enriched milk
72 °C, 225 bar
Effect of emulsification equipments
Microfluidizer vs. two-stage Valve Homogenizer
Delivery emulsions
Effect of emulsification Equipment
Fish oil-in-water emulsion

- Ingredients:
  - 10% fish oil
  - 1% emulsifier
  - 89% buffer

- Storage with iron for 14 days

Type of emulsifier
Oil droplet size

Transition metal ions

Processing conditions

CAS_M
69 MPa, 3
passes

Microfluidizer

CAS_H
80 MPa
4 passes

Two-stage valve

homogenizer

50 MPa
3

WPI_M

WPI_H

CAS_H

WPI_M

WPI_H

Particle Size Distribution

Particle Size (µm)

Volume (%)

CAS_M

CAS_H

WPI_M

WPI_H

Particle Size (µm)

Volume (%)
Emulsification Equipment

Results: PCA Biplot

- CAS vs WPI: Higher PV in CAS but less increase in volatiles than WPI
  - Metal chelation
- WPI_M vs WPI_H: Protein at the interface
  - Structure and thickness of interfacial layer


Protein in aqueous phase:
WPI_M: 2.86 mg/mL
WPI_H: 4.96 mg/mL
Effect of antioxidants in omega-3 enriched foods
Antioxidants

Antioxidants = Compounds that prevent or delay lipid oxidation

1) Primary antioxidants / Radical scavenging
   → Inactivate reactive radicals

2) Secondary antioxidants
   → Bind oxygen
   Chelate metal ions
   Quench singlet oxygen
   Regenerate other antioxidants
Lipid oxidation

Under certain conditions, antioxidants are also found to be prooxidants...

Radical scavengers

Metal chelator
Polar paradox hypothesis

Interfacial phenomena in antioxidant activity

Bulk oil

Emulsion (o/w)

- Hydrophilic
- Amphiphilic
- Lipophilic

Antioxidants

Bulk oil – Physical structures

Schematic illustration of some association colloids formed by minor constituents:

Physical structures:

↑ Lipid oxidation reactions

Alter effectiveness of AO

AO in fish oil enriched mayonnaise

![Graph showing the effect of antioxidants on 2-Pentenal formation in mayonnaise over storage time.](image)

- No antioxidant
- Lactoferrin (700-2800 ppm) & Phytic acid (15-116 ppm)
- EDTA (6 & 24 ppm)

*Nielsen et al., 2004. J. Agric. Food Chem. 52:7690-7699*
AO in fish oil enriched dressing

1. Very good protective effect of EDTA – best single antioxidant
2. Limited but still protective effect of tocopherols
3. Interestingly, no effect of adding both EDTA and tocopherol
4. Prooxidant effect of ascorbyl palmitate in high concentration

AO in fish oil enriched milk

1. Prooxidant effects of tocopherols when added to fish oil alone
2. No effect of EDTA
3. Ascorbyl palmitate highly protective against oxidation
4. Rapeseed oil highly protective against oxidation

Additional exp: γ-tocopherol seems to work - α-tocopherol does not

Let et al., 2005. J. Agric. Food Chem. 53:5429-5437
Antioxidant effects in fish oil enriched foods

- Different antioxidants have very different effects in different foods
- Possibly due to different mechanisms of action and localizations

<table>
<thead>
<tr>
<th></th>
<th>Tocopherol</th>
<th>Ascorbyl palmitate</th>
<th>Ascorbic acid</th>
<th>EDTA</th>
<th>Propyl gallate/ Gallic acid</th>
<th>Lactoferrin</th>
<th>Caffeic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk 1.5% fat</td>
<td>Weak anti</td>
<td>Anti</td>
<td></td>
<td>Anti to no</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk drink 5% fat</td>
<td></td>
<td>Pro</td>
<td></td>
<td>Anti</td>
<td>Weak anti to pro</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking yoghurt</td>
<td></td>
<td></td>
<td></td>
<td>Anti</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5% fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dressing 25% fat</td>
<td>Weak anti</td>
<td>Pro</td>
<td></td>
<td>Anti</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayonnaise 80% fat</td>
<td>Weak anti to pro</td>
<td>Pro</td>
<td>Pro</td>
<td>Anti</td>
<td>Pro</td>
<td>Weak anti to pro</td>
<td></td>
</tr>
<tr>
<td>Energy bars 6.2% fat</td>
<td>Anti to weak pro</td>
<td>Pro</td>
<td>Pro</td>
<td>Pro</td>
<td>Pro</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pro: Prooxidative; Anti: Antioxidative
Cut-off effect (Model emulsions, o/w)

Conjugated Autoxidizable Triene (CAT) Assay

Emulsion (o/w): Phosphate buffer (pH 7.2), Brij (17 µM), Tung oil (stripped, 115 µM)
AAPH (1 mM) as initiator


Optimal alkyl chain - Rosmarinates & Chlorogenates: C₈ & C₁₂

Chlorogenic acid

Rosmarinic acid

Laguerre et al., 2010. J. Agric. Food Chem., 58:2869-2876
Cut-off effect (Model emulsions, o/w)

Antioxidant (CCL) location in an emulsion

Decreased Oxidation

Increased lipophilicity

CCL = Critical chain length

Modified from Laguerre et al., 2013. In: Lipid Oxidation: Challenges in Food systems, 261-295
Caffeates as antioxidants

Conjugated Autoxidizable Triene (CAT) Assay

Emulsion (o/w): Phosphate buffer, Brij, Tung Oil. AAPH as initiator

Effect of lipophilisation of caffeic acid on oxidation in fish oil enriched milk

Milk enriched with 0.5% of fish oil (total fat 1.5%)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentration [μM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>100</td>
</tr>
<tr>
<td>Methyl caffeate</td>
<td>100</td>
</tr>
<tr>
<td>Butyl caffeate</td>
<td>100</td>
</tr>
<tr>
<td>Octyl caffeate</td>
<td>100</td>
</tr>
<tr>
<td>Dodecyl caffeate</td>
<td>100</td>
</tr>
<tr>
<td>Hexadecyl caffeate</td>
<td>100</td>
</tr>
<tr>
<td>Eicosyl caffeate</td>
<td>100</td>
</tr>
</tbody>
</table>

Storage: 5°C, 12 days (0, 3, 6, 9 and 12)

Alemán et al. 2015. Food Chemistry 167, 236-244.
Volatile development in Milk

**Induction time**
- 3 days: Con, CA C₀
- 6 days: CA C₁₂, CA C₁₆, CA C₂₀
- 9 days: CA C₈, Not defined, CA C₁, CA C₄

**General trend of volatile development**
- Lowest concentration: CA C₄ and CA C₁

**Day 12**
- Con
- CA C₀, CA C₁₆
- CA C₁₂
- CA C₈
- CA C₂₀
- CA C₄
- CA C₁
Experimental design: Mayonnaise

**Fish Oil Enriched Mayonnaise**

- 80 % Fat (64% rapeseed oil, 16% Fish Oil, w/w), Egg yolk as emulsifier
- Antioxidants (solubilized in MeOH), Conc. 100 µM
  - Con, CA C₀, CA C₁, CA C₄, CA C₈ (+200), CA C₁₂, CA C₁₈
- Storage at 20°C for 4 weeks (Sampling days: 0, 3, 9, 12, 15, 21 and 28)

**Efficacy of the antioxidants**

- Lipid hydroperoxides (Spectrophotometric)
- Secondary volatile oxidation products (GC-MS)
- Tocopherols (HPLC)

*Alemán et al. 2015. Food Chemistry 167, 236-244.*
Volatiles - Mayonnaise

9 days
CA C₀
CA C₁
CA C₈ 200

12 days
Con
CA C₄
CA C₈
CA C₁₂
CA C₁₈

Induction time

Day 28
Con
CA C₀
CA C₁₈
CA C₁

General trend of volatile development
Lowest concentration: CA C₈ (200), CA C₁₂, CA C₄ and CA C₈

CA C₈
CA C₄
CA C₁₂
CA C₄ 200
CA C₁₂

Discussion

Mayonnaise

No effect

CA C0, CA C1, CA C18

Less efficient antioxidants

Most efficient antioxidants

CA C4, CA C8, CA C12

Increased conc. increased effect

Milk

No effect

CA C0, CA C8 - C20

Less efficient antioxidants

Most efficient antioxidants

CA C1, CA C4

The “cut-off” effect is influenced by the food system

It is not possible to predict optimal chain length in food products based on CAT assay
Inhibition effects of ferulates in fish oil enriched milk

Natural antioxidants in mayonnaise salads (tuna)

Formation of volatiles:
Reference > Thyme > Rosemary > Oregano

Antioxidant effects of extracts from seaweed (*Fucus vesiculosus*) in mayonnaise

**Peroxide value**

**1-Penten-3-ol**

***Honold et al. Submitted to Food Chemistry***
Evaluation of compounds contributing to activity in mayonnaise

Phenolic compounds

Astaxanthin

Fucoxanthin

Oxidation products – later stage of storage
Conclusions

• Ingredients can affect lipid oxidation

• Oil quality is more important in some systems than in others

• The emulsifiers used as ingredients and the composition of the interface in emulsions affect lipid oxidation

• Emulsification processes must be optimised to minimise oxidation

• Type of emulsification equipment can affect oxidation

• The same antioxidant can have very different effects in different food systems

• Polar antioxidants can be modified to have an optimal chain length for each food system

• Natural antioxidants from seaweed have promising effects
Acknowledgements

• Caroline Baron
• Nina Skall Nielsen
• Mette Bruni Let
• Anna F. Horn
• Ann-Dorit Moltke Sørensen
• Anne S. Meyer
• Anna Klein
• Sabeena Farvin
• Lis Berner
• Inge Holmberg
• Trang Vu
• Jane Jørgensen
• Mercedes Aleman
• Ditte B Hermund
• Philipp J Honold