Challenges when developing marine lipids for functional foods

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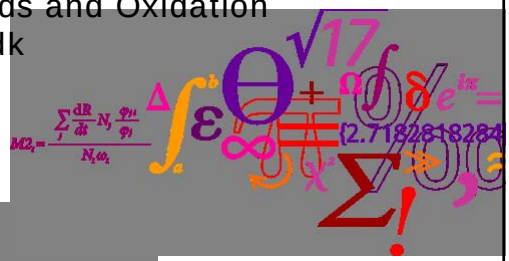


DTU Aqua
National Institute of Aquatic Resources

Agenda

- **Thanks to co-authors : Nina Skall Nielsen, Eva Falch, Turid Rustad, Stig Jansson, Ivar Storrø**
- **Products with healthy omega-3 lipids**
- **Quality parameters – lipid oxidation**
- **Raw material deterioration**
- **Processing of marine omega-3 lipids**
- **Prevention of lipid oxidation in omega-3 rich foods**

Marine lipid products

- ω-3 fatty acid:
  - **Ethyl esters**
  - **Triacylglycerides**
  - **Phospholipids**

- Oils
- Emulsions
- Microencapsulated powders

Applications

- ω-3 fatty acids:
  - **Pharmaceuticals**
  - **Food supplements**
  - **Food fortification**
  - **Infant formulas**
  - **Pet nutrition**

DHA-Product

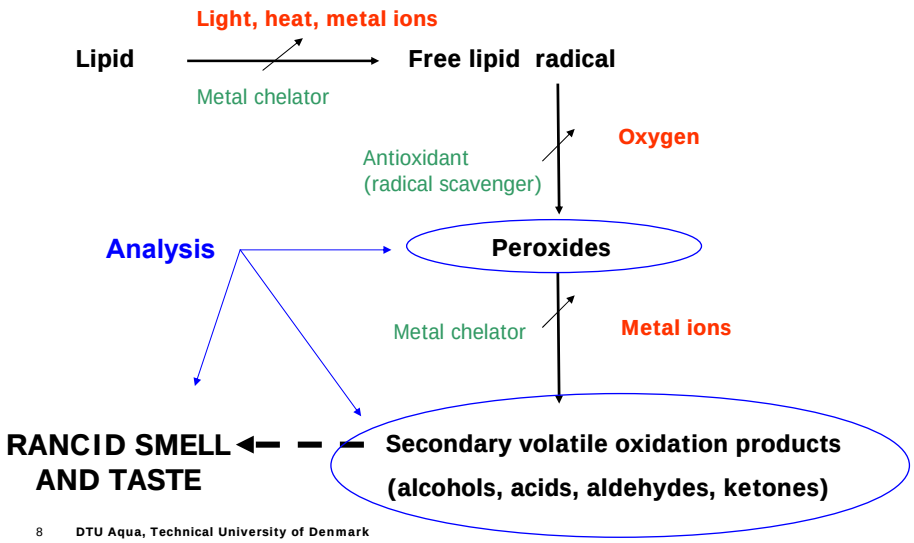
Quality parameters

1) Level of long chain omega-3 polyunsaturated fatty acids (PUFA)
2) Level of different lipid classes (phospholipids, triglycerides, free fatty acids, other lipids such as squalene)
3) Contamination by lipophilic compounds such as dioxin, PCB and other environmental pollutants
4) Lipid oxidation

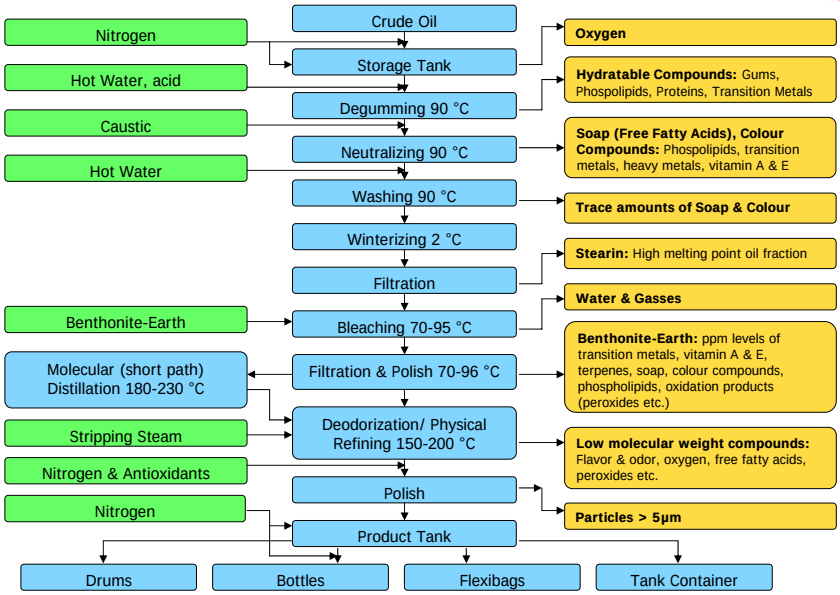
Raw material quality

- Environment (contaminants, temperature/feed)
- Species and seasonal variation
- Lipid oxidation
  - Catalysed by: lipoxygenase, heme-iron
  - Oxidation susceptibility:
    white muscle < dark muscle < skin
  - Antioxidant enzymes: superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase
  - Endogeneous antioxidants: tocopherol, astaxanthin, ubiquinol and vitamin C
- Lipolytic activity

Lipid oxidation



Processing of marine oils



Effects of marine oil processing

- *Degumming by treatment with acid:* **The removal of phospholipids and trace metals will improve the oxidative stability**
- *Neutralisation by addition of an alkali solution:* **Removal of free fatty acids improve the sensory properties and oxidative stability. FFA should be < 0.1-0.2 %. Also removes antioxidants**
- *Bleaching* : **Adsorption of coloured compounds, peroxides and some volatile oxidation compounds and other impurities. Improve the oxidative stability**
- *Active carbon treatment:* **Removes dioxins but not PCBs**
- *Deodorisation step:* **Decomposition of peroxides and removal of volatiles, but also removes antioxidants. Thin film deodorisation removes PCBs and dioxins**

Oxidative stability

The oxidative stability of bulk oils and n-3 enriched products depends on different factors:

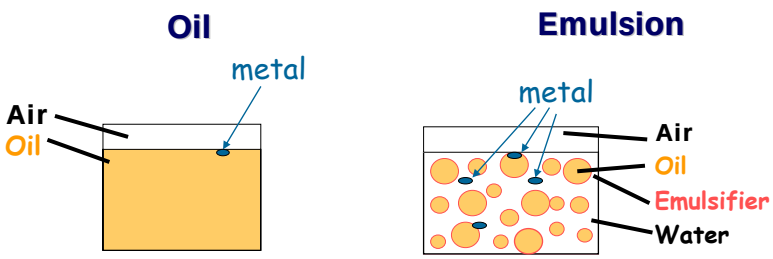
Oil and food products

- Oil type
- Metal
- Oxygen
- Temperature
- Light
- Endogenous antioxidants

Food products

- Processing condition
- Distribution of pro/antioxidants (pH, emulsifier)
- Emulsifier charge (attraction/repulsion)
- pH
- Viscosity
- Proteins

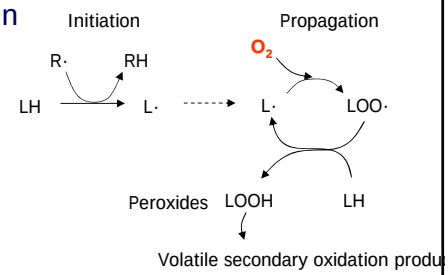
Metals



- **Metals are in the water phase or on the interface**
- **Metals are more important in the oxidation of emulsions than in the oxidation of bulk oil**

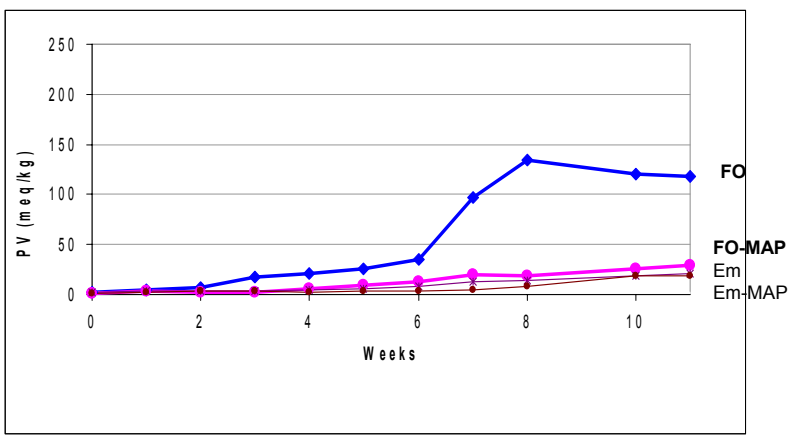
Oxygen

- Oxygen is vital for the oxidation reaction
- Reduction of access to oxygen slows oxidation
- Reduction of oxygen during production may be difficult
  - Vacuum
  - Nitrogen atmosphere
- Reduction of oxygen during storage
  - Exclude headspace oxygen
  - Modified atmosphere
  - Air-tight packaging material



Oxygen

Oxidation in fish oil enriched fitness bar



Stability: **FO-MAP**, **Em**ulsion, **Em**ulsion-MAP > bulk **FO**

Temperature

- Oxidation increases with increasing temperature
- A rule of thumb (based on activation energy) is that:
  The oxidation rate doubles for every 10°C increase in temperature
- For light sensitised oxidation the rate is not much influenced by temperature
- Keep as cold as possible

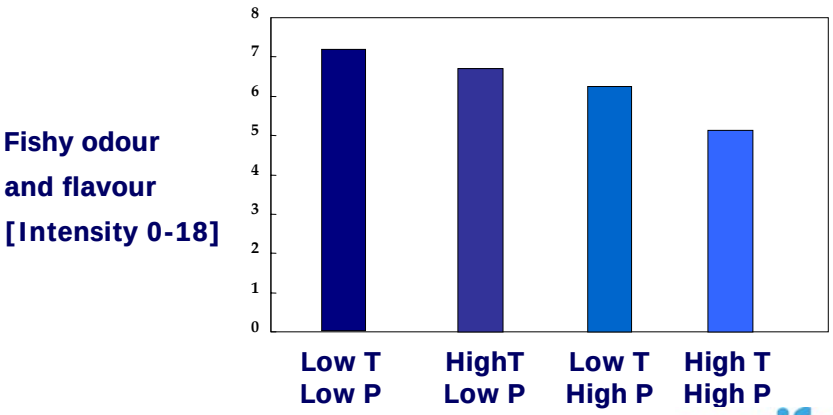
Food processing conditions

- Processing may involve
  - Access to oxygen
  - Heating
  - Contamination with metal

Food processing conditions

Processing conditions – Temp and Pressure

Sensory off-flavour after 7 days of storage of milk with fish oil



Emulsifier type

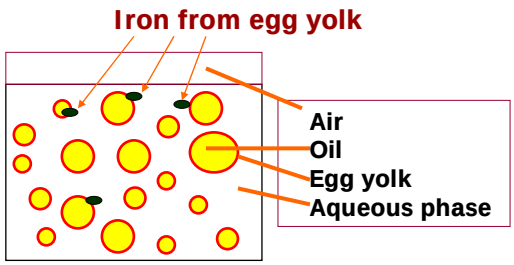
Emulsifiers:

- needed to make physically stable emulsions.
- surface active molecules with amphiphilic properties that reduces surface tension.

- Examples:
  - macromolecules, such as proteins unfolding at the interface
  - smaller surfactant molecules, such as phospholipids, diacylglycerols, monoacyl-glycerols and free fatty acids

Oxidation mechanisms in mayonnaise

Mayonnaise with pH < 4



- **Low pH and iron from egg yolk cause oxidation in mayonnaise**
- **Oxidation may perhaps be reduced by choosing another emulsifier with lower iron content than egg yolk**

Packaging

- **Many products are packaged in transparent material**
- **However, exposure of omega-3 PUFA to light will result in photooxidation (photosensitized oxidation)**
- **Light of shorter wavelength has more detrimental effect on oils than longer wavelengths**
- **The incorporation of UV-absorbing compounds in oil, packed in transparent plastic bottles, improves sensory and oxidative stability of the oil**
- **Important to protect the omega-3 PUFA enriched foods from light during production and especially during storage**

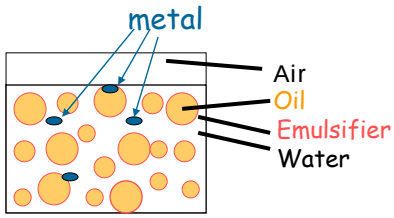
Effects of antioxidants in n-3 PUFA enriched products

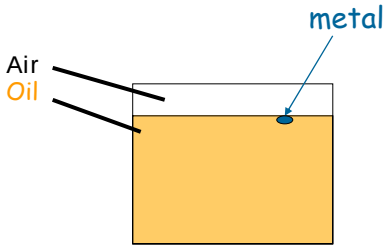
The effect of antioxidants depends on

- ”Polar” paradox
- Distribution of pro/antioxidants (pH, emulsifier)
- Emulsifier charge (attraction/repulsation)
- pH (protonation, stability, ionisation)
- Water (hydrogen binding)
- Viscosity
- Proteins
- Interactions with emulsifiers, ingredients

Oil versus emulsions

The Polar Paradox!





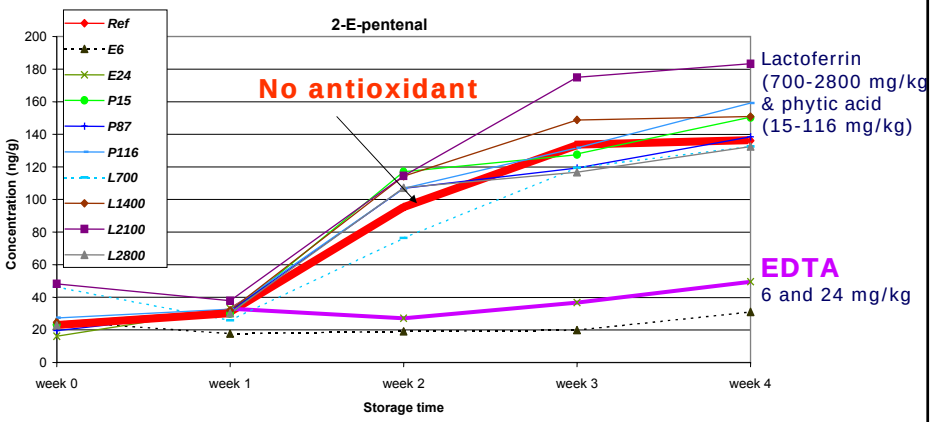
Antioxidant mechanisms

Antioxidant mechanism	Examples of antioxidants
Metal chelation	Citric acid, EDTA,[1] lactoferrin, phytic acid (phosphates)
Oxygen scavenging	Ascorbic acid, glucose oxidase-catalase
Singlet oxygen quenching	Carotenoids, tocopherols
Active oxygen scavenging	Superoxide dismutase, catalase, mannitol
Primary radical chain-breaking	Tocopherols, ascorbic acid and derivatives, gallic acid and gallates,[1] BHA,[1] BHT,[1] several natural polyphenols, rosemary and sage antioxidants
Alkoxyl radical interruption	Tocopherols (some rosemary compounds)
Secondary chain-breaking	Glutathione peroxidase and glutathione–S-transferase, thiodipropionic acid and its derivatives[1]

Note: EDTA: ethylene diamine tetraacetic acid; antioxidants marked[1] are all 'synthetic' antioxidants. Thiodipropionic acid and its derivatives are permitted as food additives in the USA, but not in the EU.

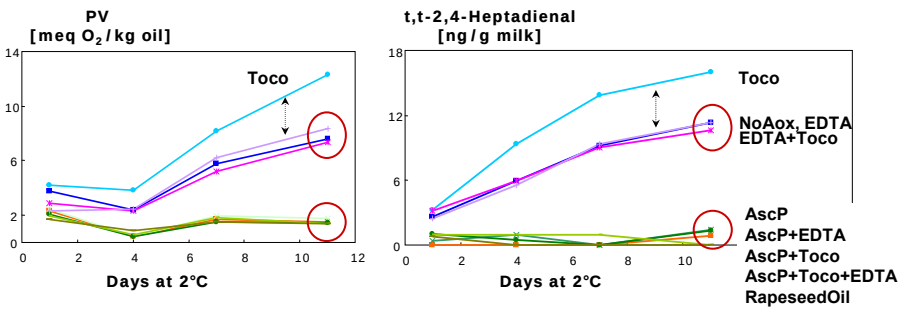
Effect of EDTA as antioxidant in mayonnaise

Only EDTA (6 and 24 mg/kg) prevented lipid oxidation in mayonnaise



Nielsen et al., J. Agric Food Chem (2004)

Effect of Tocols, EDTA and AscPalm in 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, Jacobsen, Pham, and Meyer, J. Agric Food Chem, 53, 5429-5437

Effects of different antioxidants in different foods

- Food systems:
- Mayonnaise (pH 4)
- Dressing
- Milk (1.5 % fat, pH 6.6)
- Milk drink (5 % fat, pH 6.6)

Antioxidants:
- EDTA
- Tocopherol
- AP (Ascorbyl palmitate)
- Ascorbic acid
- PG (Propyl gallate)
- Gallic acid

Food system	EDTA	Toco	Asc	AP	PG	Gallic acid
Mayonnaise	AO	Weak AO or Pro	PRO	PRO	PRO	PRO
Dressing	AO	AO (weak)	-	PRO	-	-
Milk		AO	-	AO	-	-
Milk drink	AO	-	-	PRO	-	

Jacobsen, Let, Nielsen and Meyer, Trends Food Sci. Technol., 19, 76-93

Oxidative stability - conclusions

Omega-3 oils are oxidatively unstable due to their content of unsaturated fatty acids

Products enriched in omega-3 PUFA have different stabilities depending on the composition of the product i.e. ingredients and their physical conditions

It is often impossible to predict the stability of one product by extrapolating chemical and sensory results from one product to another

Oxidative stability – future research

Continued research on the effect of chemical and physical composition of omega-3 enriched products on the oxidative stability is necessary to be able to understand the coherence between mechanisms affecting the oxidative stability of the lipids

- Prediction of stability of complex food systems using mathematical modelling
- Examination of the structure of the interface
- Improve our understanding of coherence of the level of volatiles and fishy off-flavour in specific products

Effects of antioxidants - conclusions

- Antioxidants may work differently depending on their concentration – antioxidative/ prooxidative
- Antioxidants may work additively or synergistic or opposing with other antioxidants
- Antioxidants work differently in different omega-3 enriched products depending on the composition of the products i.e. ingredients and their physical properties
- Factors affecting antioxidant activity:
  - Partitioning
  - pH
  - Oil type
  - Presence of metal ions
  - Emulsifier

Effects of antioxidants – future research

- Continued research necessary to understand the mechanisms by which antioxidants and metal chelators affect the oxidative stability of the lipids
- Localisation and interactions with emulsifiers
- Mobility and localisation in solid matrices
- Interaction with other ingredients
- Modelling to predict antioxidant efficacy in different foods

Acknowledgements

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