MIC 2013

Analyzing lipid oxidation – opportunities and challenges

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FHF-Project 900552

"Low oxidized omega-3 oils and potential health benefits: Biological effects of omega-3 fatty acids of varying degrees of oxidation"

Report 33/2013

"Stability of selected marine oils. Effect of oil quality on cellular responses - Phase 2"

(Lagringsstabilitet av et utvalg marine oljer. Effekt av oljekvalitet på cellulære responser - Fase 2)

Based on a collaboration within the Nofima Lipid Platform

FHF
FISKERI- OG HAVBRUKSNÆRINGENS FORSKNINGSFOND

Nofima

Overview

- Novel methods to measure lipid oxidation
- Comparison of different methods for analyzing lipid oxidation on defined marine oils with increasing oxidation
- Effect of lipid oxidation on cellular systems

Lipid oxidation of marine oils

Formation of:

- Primary oxidation products

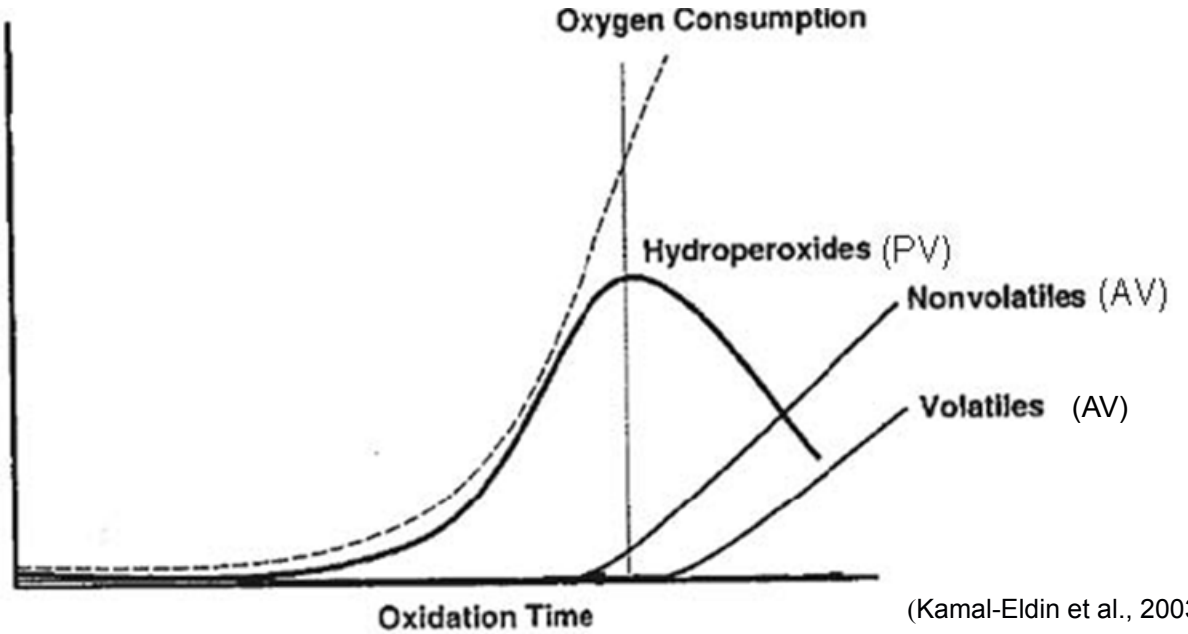
  - Free radicals
  - Lipid hydroperoxides

- Secondary oxidation products

  - aldehydes (alkanals, alkenals, OH-alkenals, core aldehydes)
  - ketones
  - alcohols
  - hydrocarbons

The established methods to measure lipid oxidation in marine oils

- PV – peroxide value, AOCS Method Cd 8-53 (primary oxidation products)
- AV – anisidine value, AOCS Method Cd 18-90 (secondary oxidation products)
- TOTOX- (2xPV+AV) -estimated total oxidation value

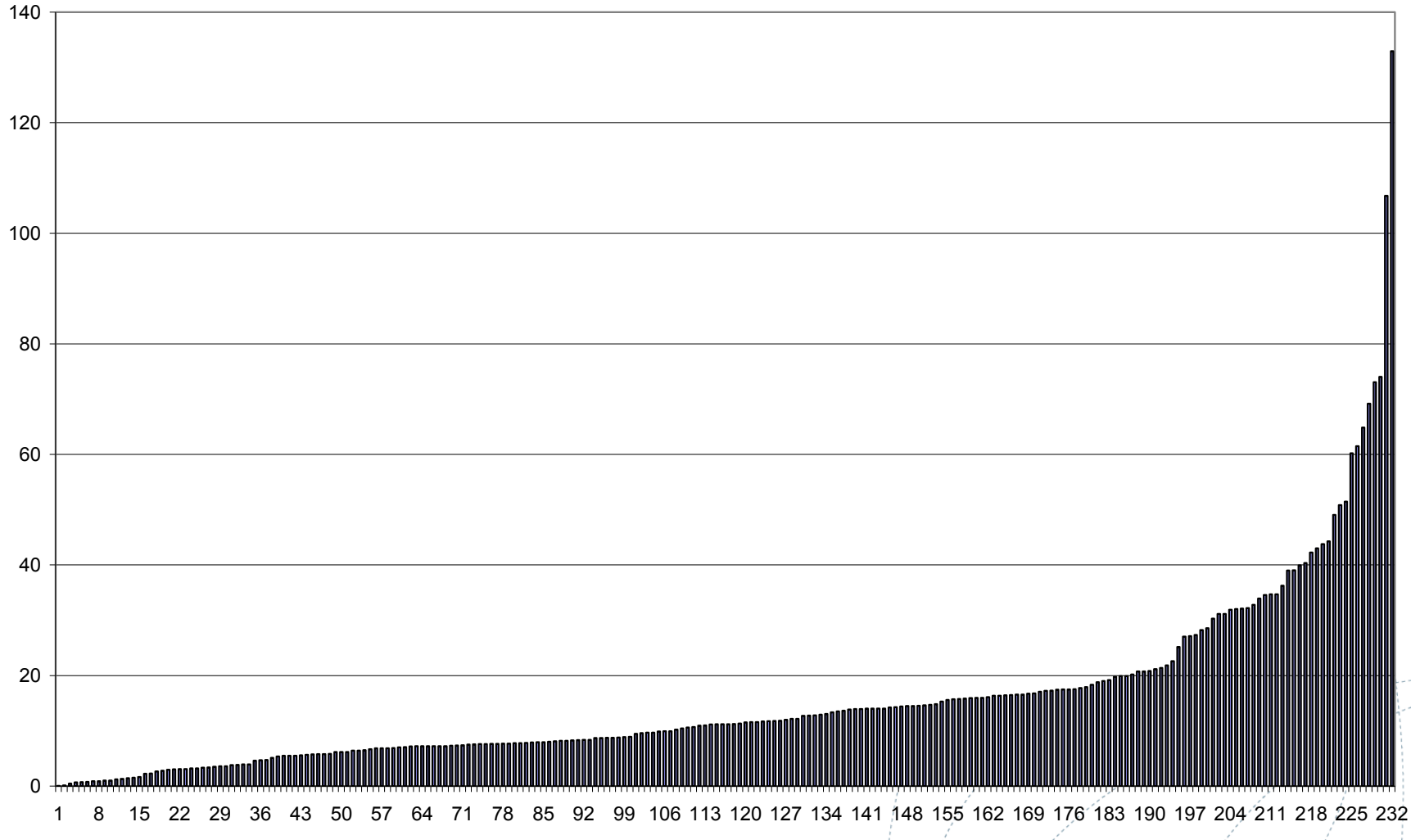


(Kamal-Eldin et al., 2003)

Limitations:

- Interferes with other substances – lack of specificity
- Not a quantitative measure of the oxidation status in the oils

Peroxide value of about 230 marine oils incl. mixtures



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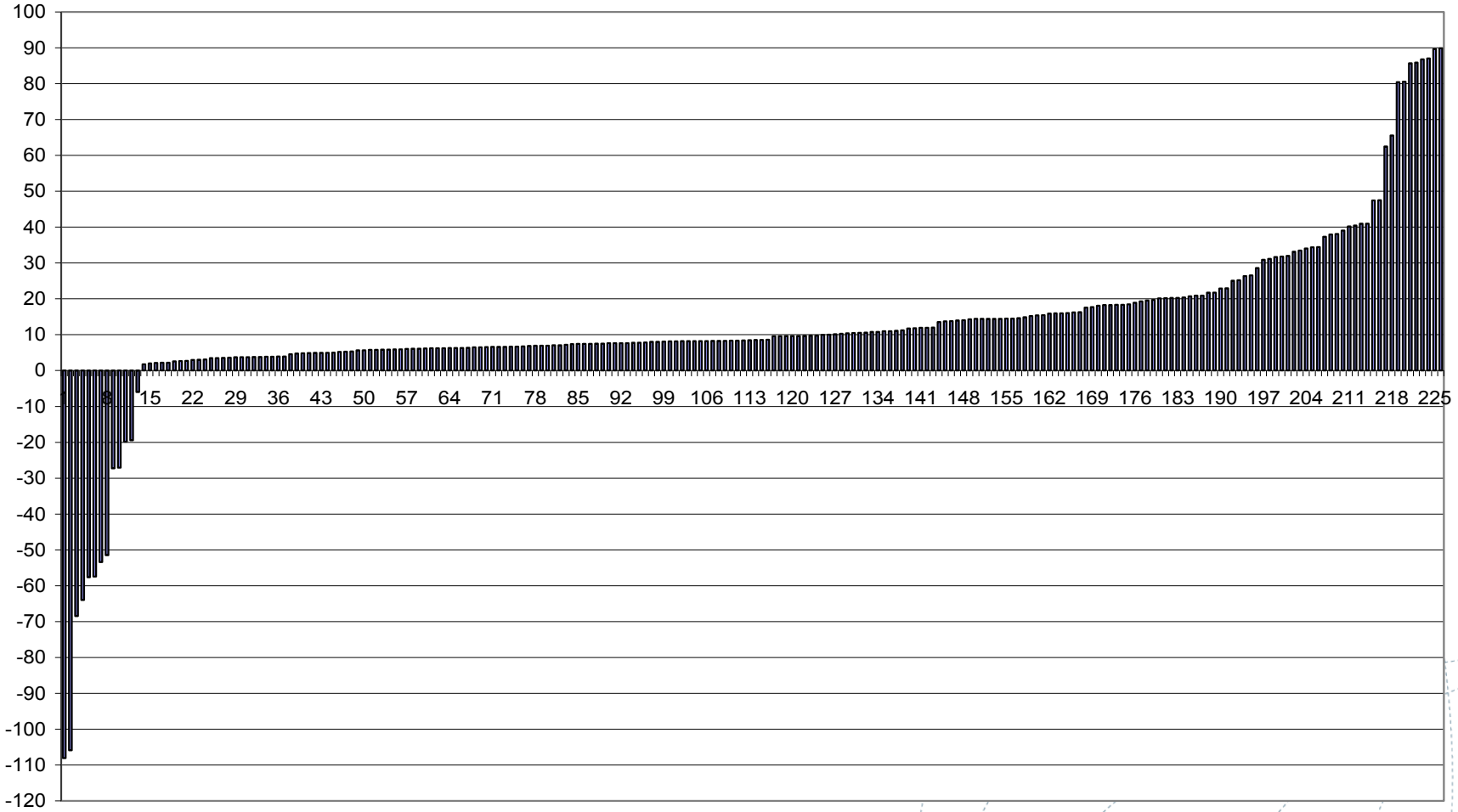
Anisidine value of 225 oils incl. mixtures



Novel methods to measure lipid oxidation

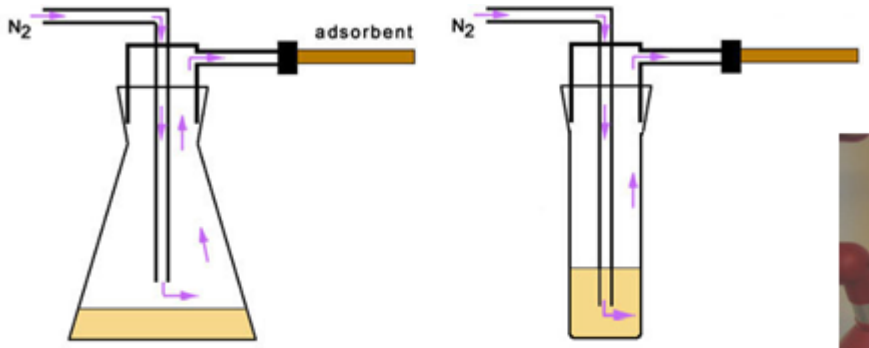
- Volatile secondary oxidation products by Headspace GC-MS
- Volatile aldehydes by Single Drop Micro Extraction (SDME) – GC/MS
- OH-alkenals by NCI-GC/MS

Aldehydes	Odor threshold in oil (ppm)		Description
	Nasal	Retronasal	
Hexanal	320	75	Tallowy, green, leafy
Heptanal	3200	50	Oily, fatty
Octanal	320	50	Oily, fatty, soapy
Nonanal	13500	260	Tallowy, soapy-fruity
Decanal	6700	850	Orange peel-like
2-t-pentenal	2300	600	Pungent, apple
2-t-hexenal	10000	400	Apple
3-c-hexenal	14	3	Green, leafy
2-t-heptenal	14000	400	Fatty, bitter almond
2-t-nonenal	900	65	Tallowy, cucumber
3-c-nonenal	250	35	Cucumber
2-t-decenal	33800	150	Tallowy, orange
2,4-t,c-heptadienal	4000	50	Frying odour, tallowy
2,4-t,t-heptadienal	10000	30	Fatty, oily
2,6-t,t-nonadienal	2500	460	Fatty, oily
2,6-t,c-nonadienal	4	1.5	Cucumber-like
2,4-t,c-decadienal	10	-	Frying odour
2,4-t,t-decadienal	180	40	Frying odour
2,4,7-t,t,c-decatrienal	-	24	Cut beans
4,5,2-t-epoxy-t-decenal	1.3	3	Metallic

Analysis of volatile secondary oxidation products by Headspace GC-MS

Nitrogen is blown over or through the samples, and the volatile compounds transported by the gas are collected on an adsorbent and analysed by GC-MS.

Measures volatile aldehydes, alcohols and ketones

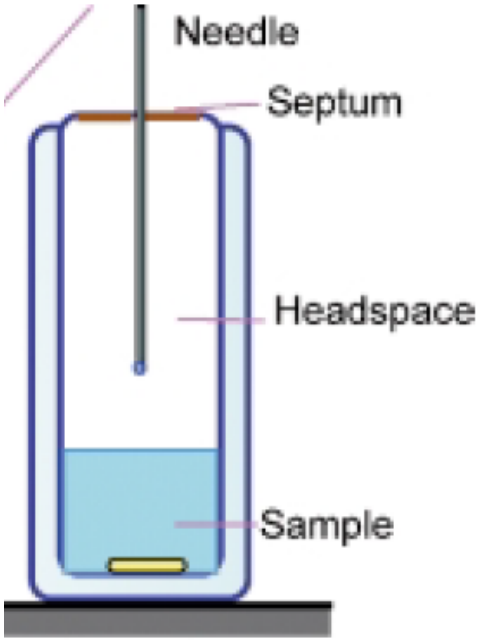


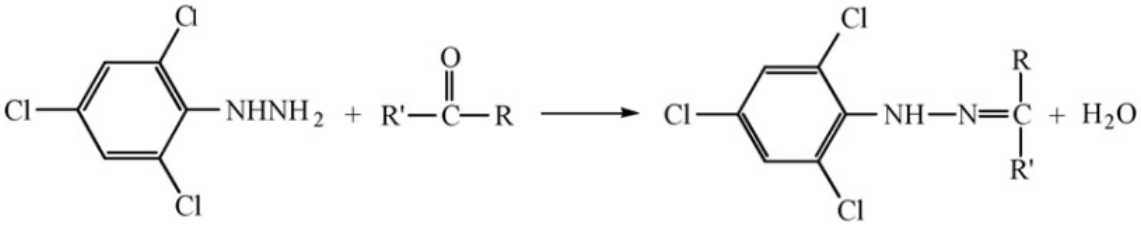
Correlates well with the sensory perception of oxidation

Analysis of volatile aldehydes by Single Drop Micro Extraction (SDME) – GC/MS: “The Droplet Method”

Fiamegos and Stalikas, (2008)

- Gas phase/Headspace in-droplet derivatisation
- Selective derivatisation and measurement of volatile aldehydes in a droplet
- Analysis with GC/MS
- Fully automated



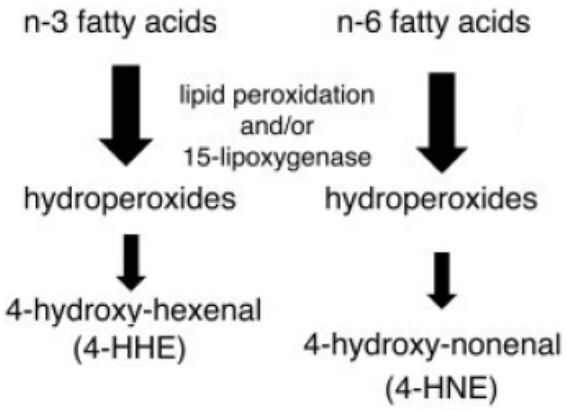


Analysis of OH-alkenals by NCI-GC/MS

LaFond et al., (2011)

Oxidation products from polyunsaturated fatty acids:

- n-3 oils give 4-hydroksy-(E)-2-heksenal (4-HHE)
- n-6 oils give 4-hydroksy-(E)-2-nonenal (4-HNE)



Comparison of different methods for analyzing lipid oxidation on defined marine oils

The effect of long time storage on the oxidative stability of different marine oils were tested

10 oils were stored with access to oxygen for 9 months at 20 degrees

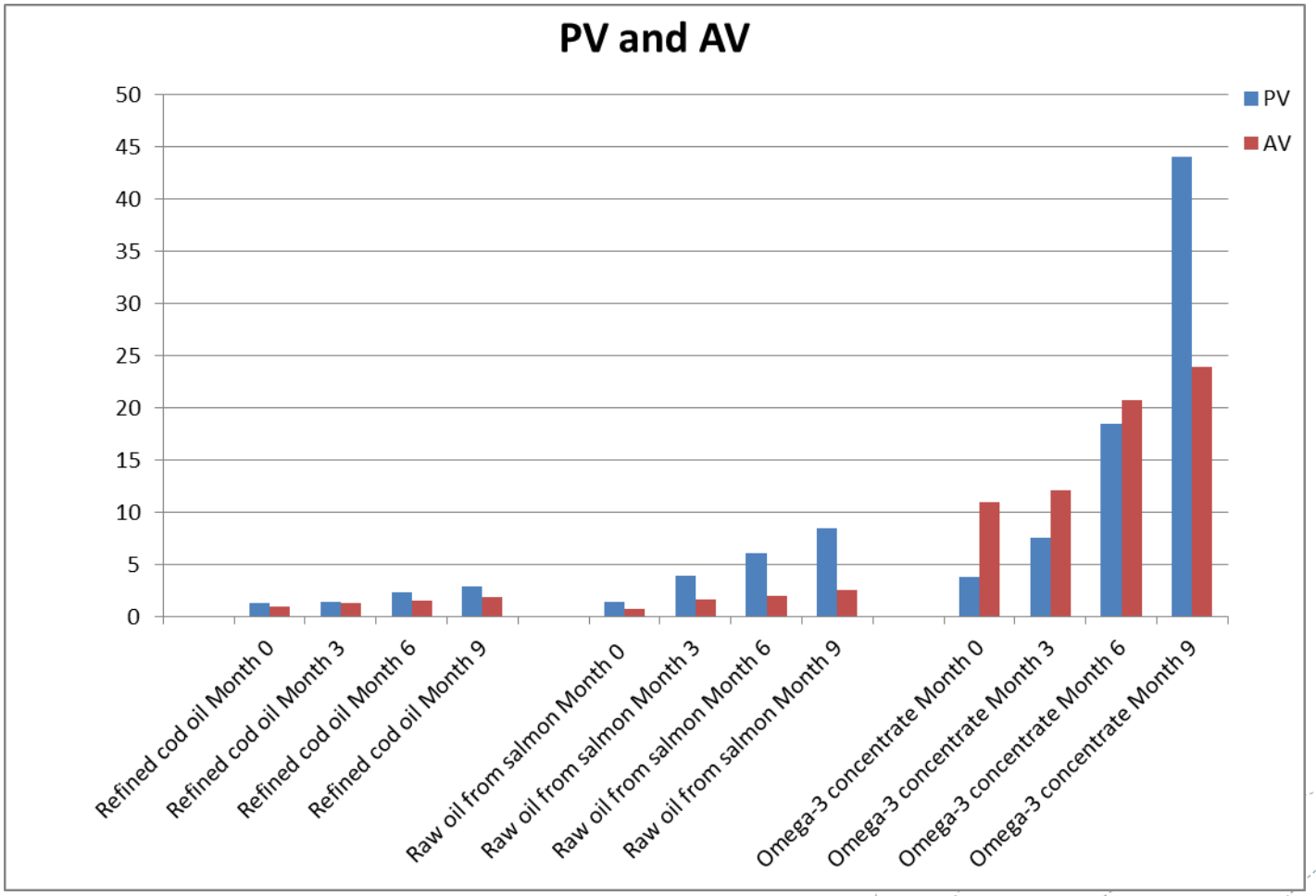
Samples were taken every three months.

Analysed with 4 different methods

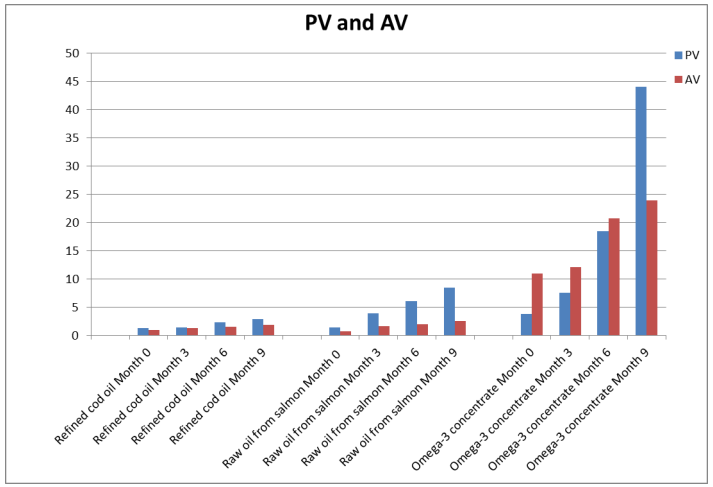
Present results of 3 oils

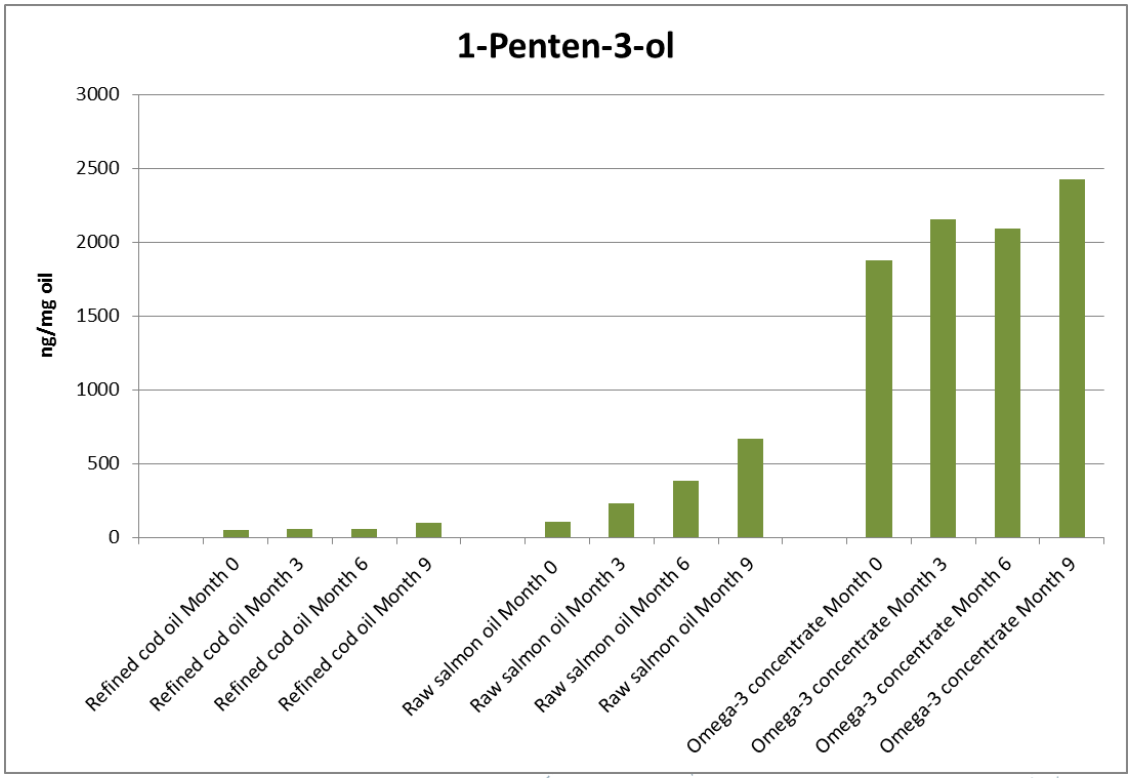
- Refined cod oil
- Raw salmon oil
- Omega-3 concentrate

The established methods PV and AV



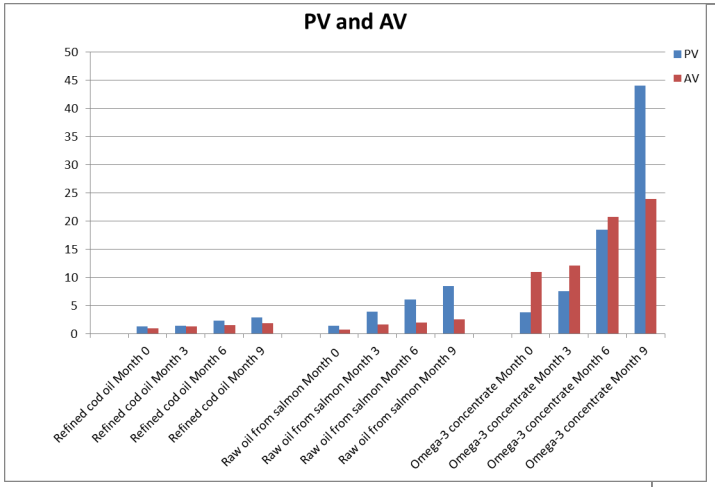
Volatile secondary oxidation products by Headspace GC-MS (1)

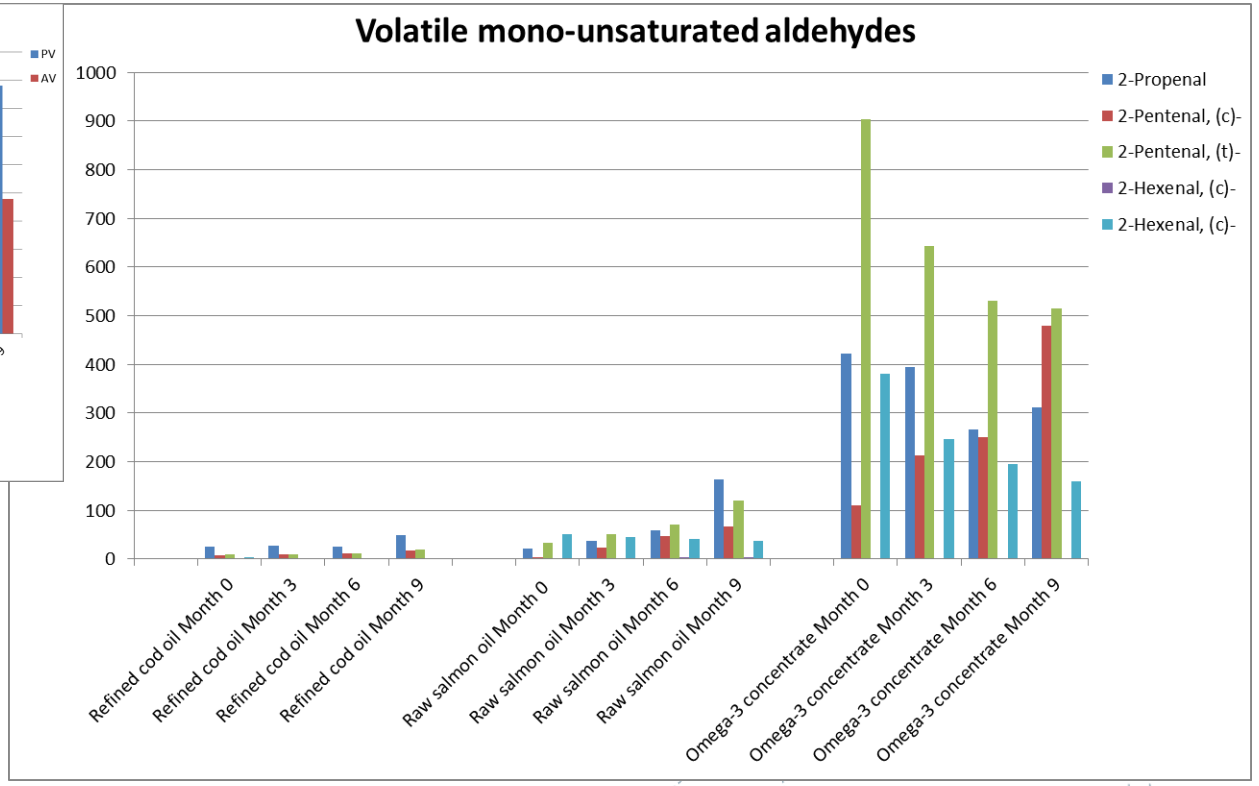




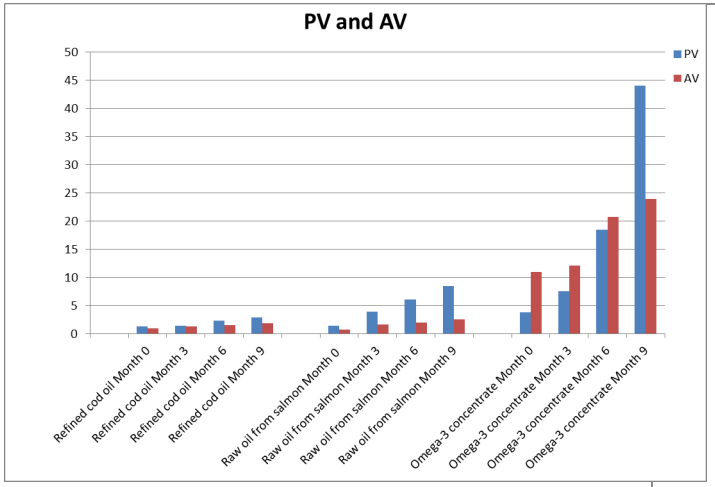
1-penten-3-ol:
Evaporates less
Are not converted
Accumulates more easily in the oil
A good marker for oxidation

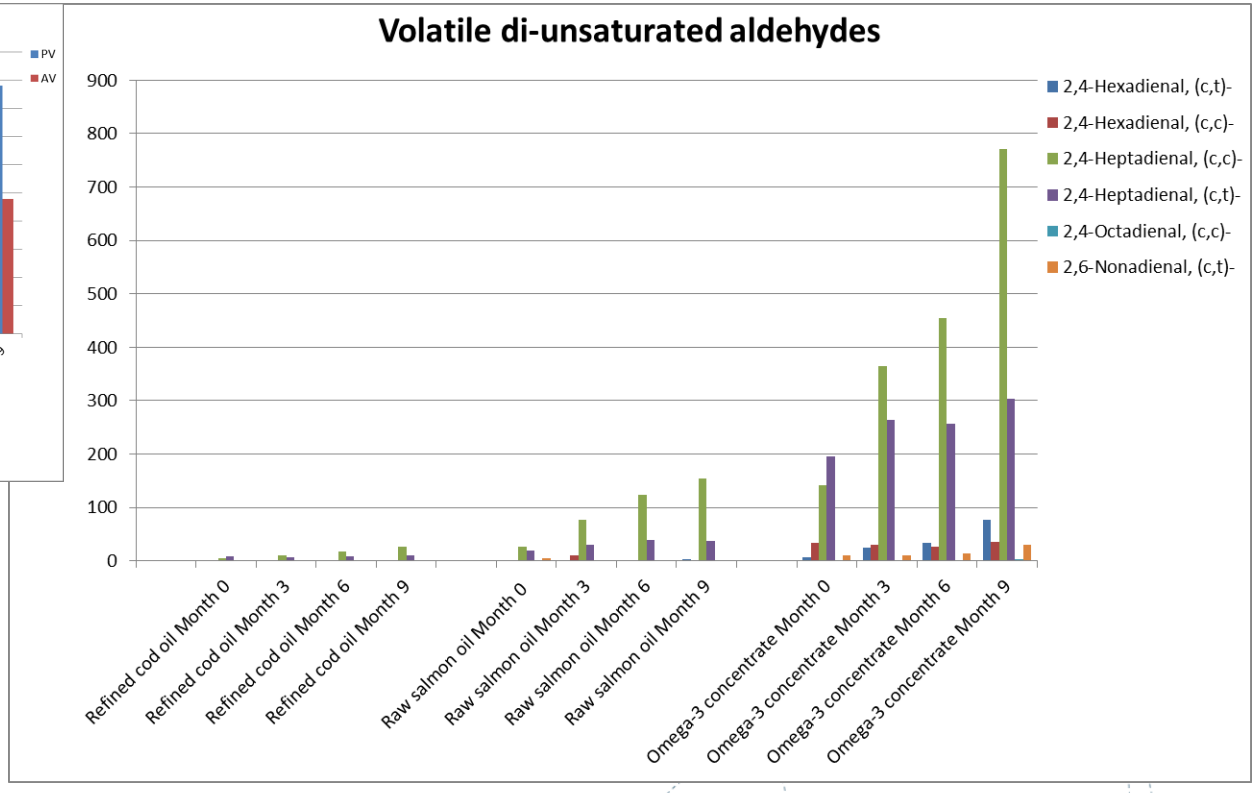
Volatile secondary oxidation products by Headspace GC-MS (2)





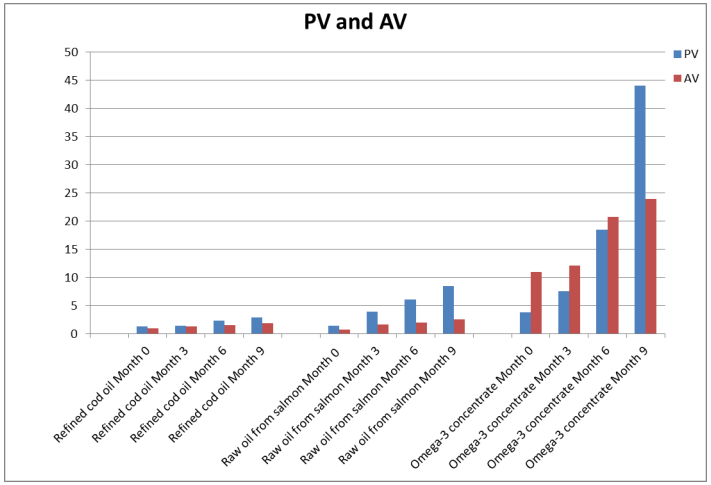
Volatile secondary oxidation products by Headspace GC-MS (3)

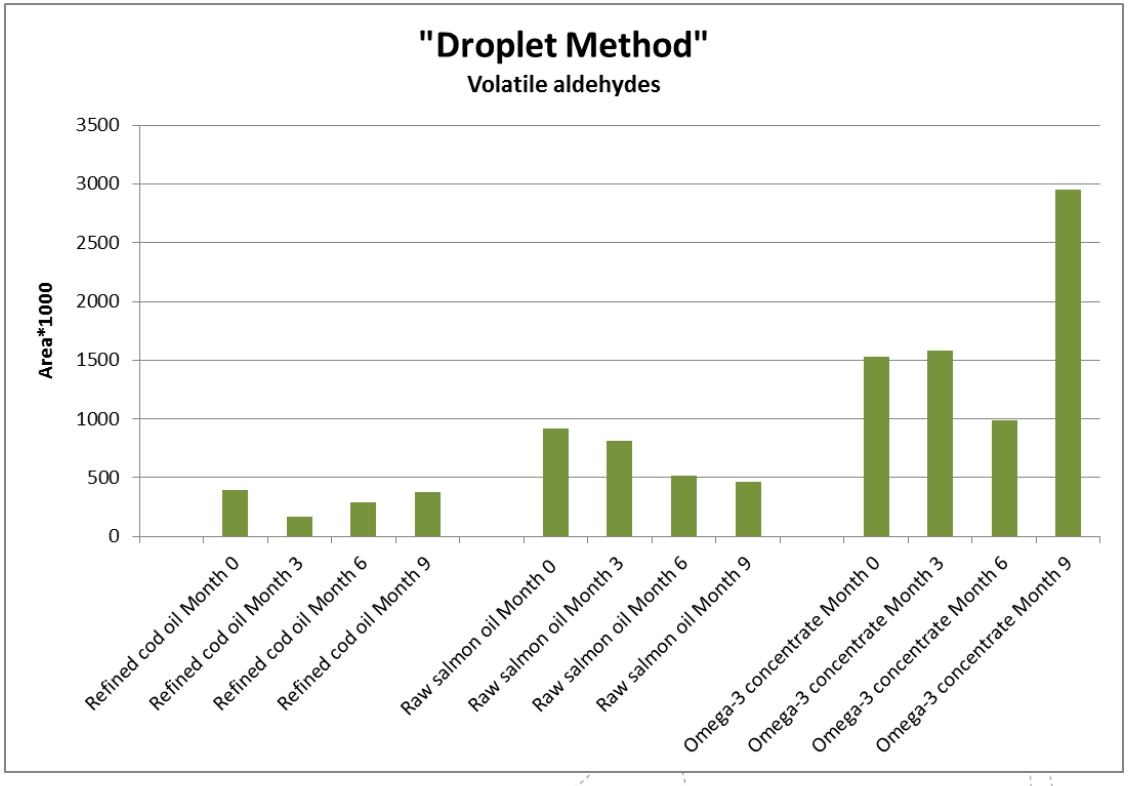




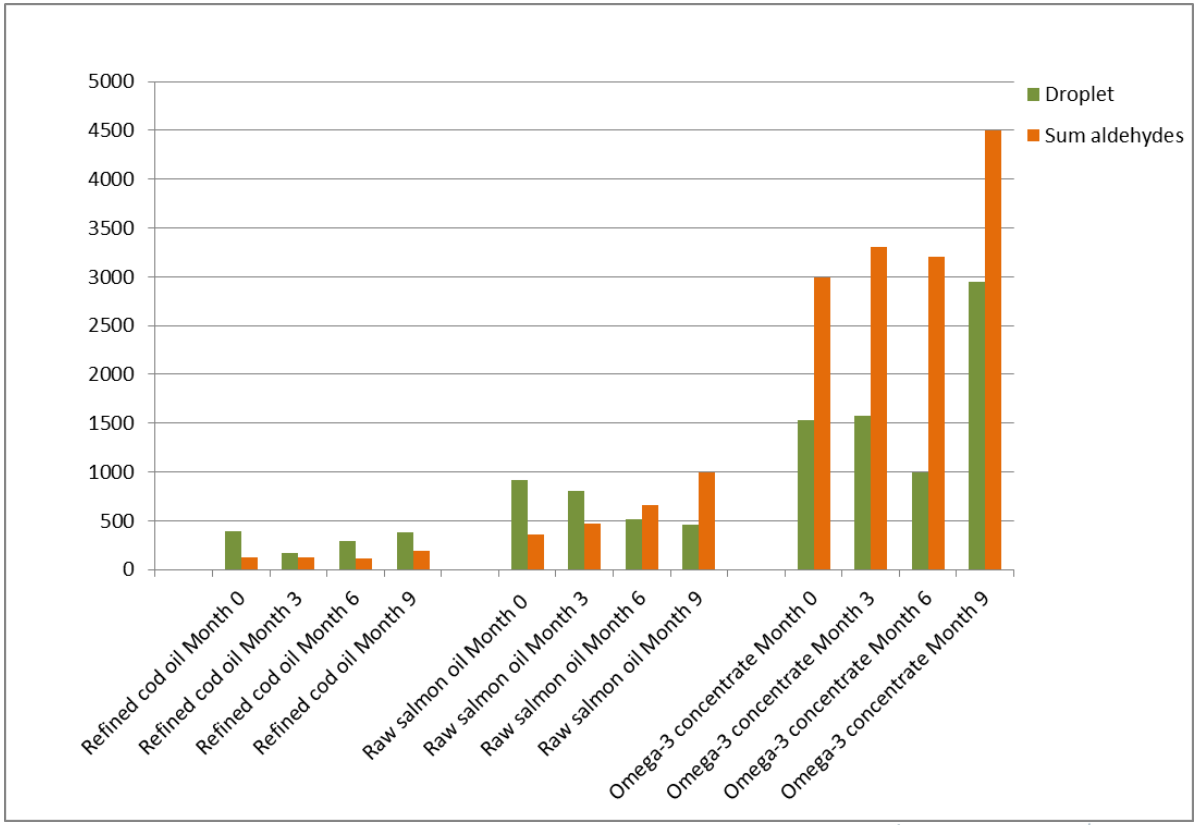
Volatile di-unsaturated aldehydes do not correspond very well with AV.
These aldehydes contribute to the unwanted smell and taste.

Volatile aldehydes by Single Drop Micro Extraction – GC/MS; “Droplet Method”



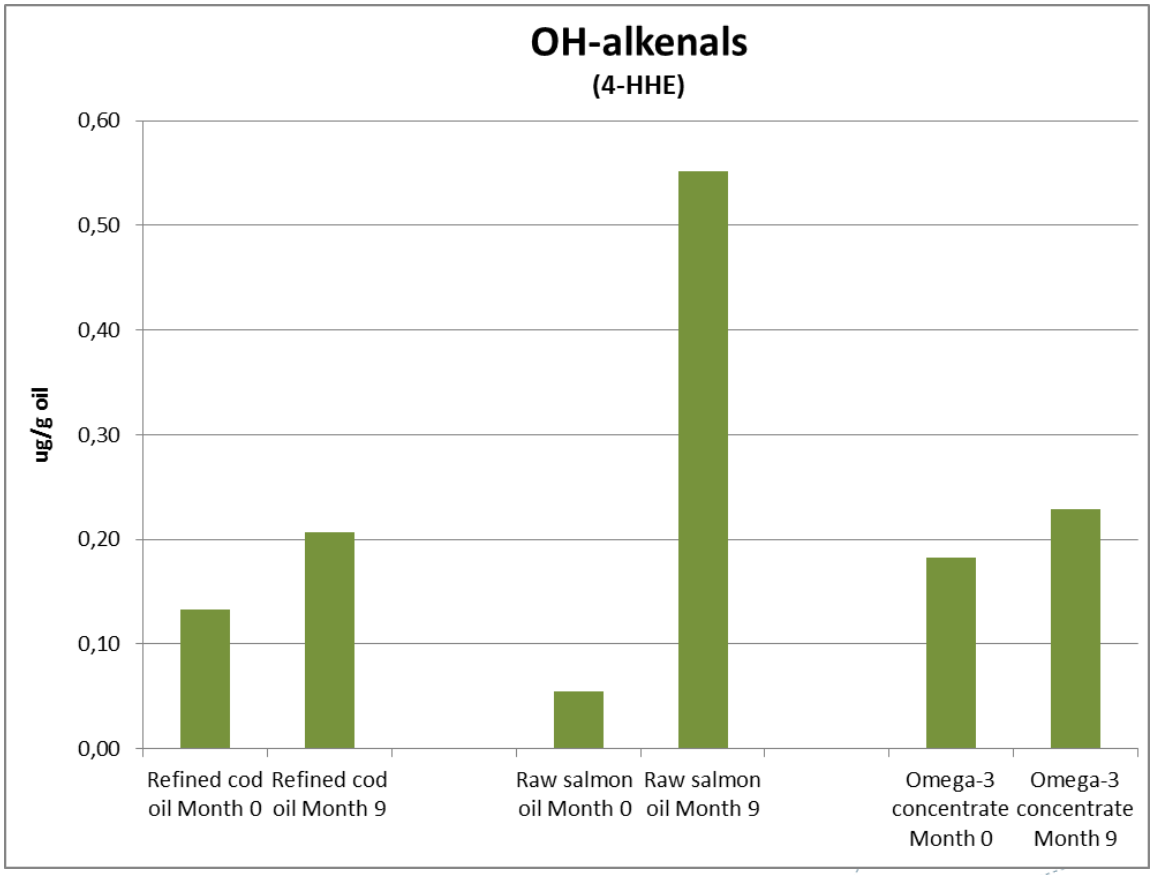


Correlation between amount of volatile aldehydes measured with Headspace-GCMS and Droplet Method



There is a certain variation between total amount volatile aldehydes measured with Headspace GC-MS and amount derivatised volatile aldehydes measured by the Droplet Method

OH-alkenals by NCI-GC/MS



Summary analysis of lipid oxidation

- PV and AV methods have limitations:
  - Interferes with other substances – lack of specificity
  - Not a quantitative measure of the oxidation status in the oils
- Measurements of volatile oxidation components and specific OH-alkenals may be good alternatives
- The content of volatile aldehydes generally do not correlate with the PV and AV values.
- One single method will not give a complete picture of the oil quality. A spectre of different analysis methods are needed to get a correct measurement of the oil quality.
- Development of good analysis methods for non-volatile oxidation components is needed.

Effect of lipid oxidation on cellular systems

There is growing concern whether oxidation products can reduce the positive health effects of marine omega-3 fatty acids (VKM 2011).

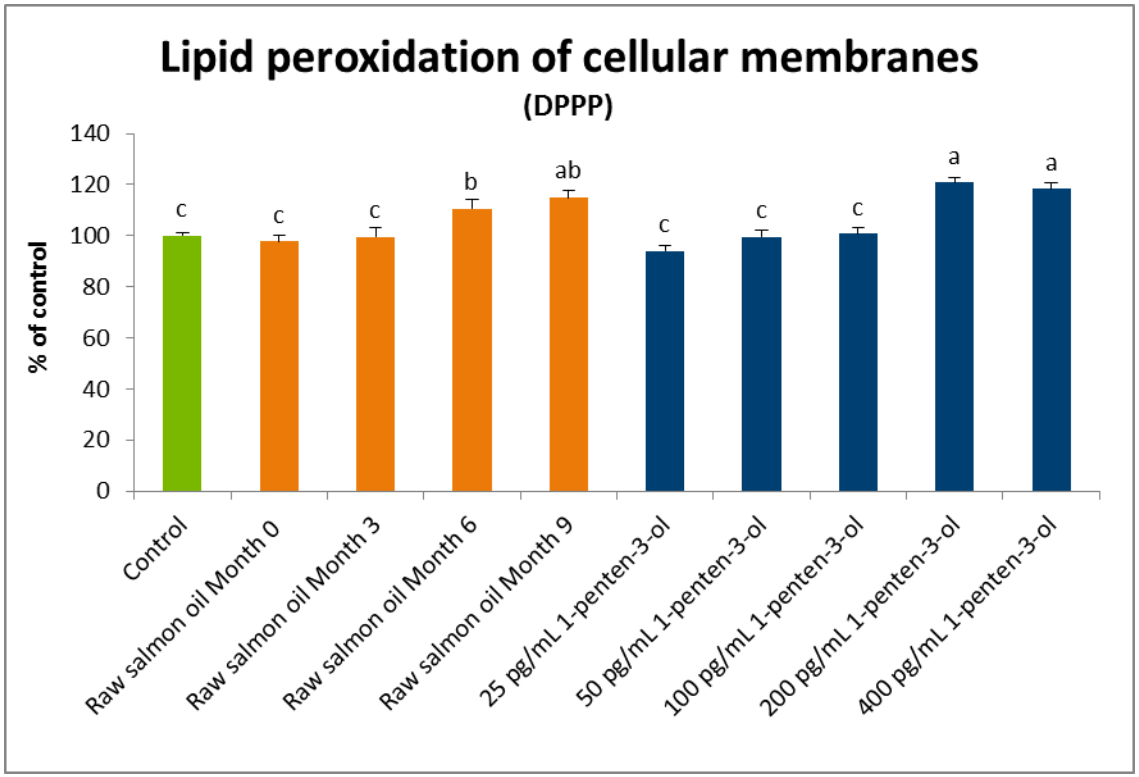
Not much research on health effects of oxidised marine omega-3

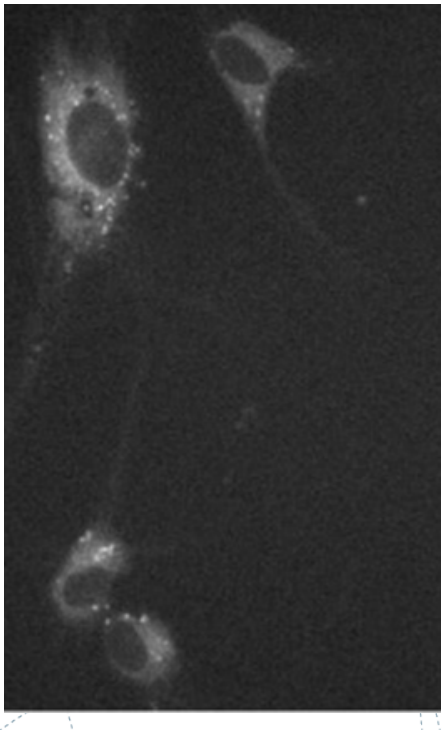
- No changes in a variety of *in vivo* markers of oxidative stress, lipid peroxidation or inflammation were observed after daily intake of oxidized fish oil for three or seven weeks, indicating that intake of oxidized fish oil may not have unfavorable short-term effects in healthy humans. *Ottestad et al, 2011 Br. J. Nutr.*
- Intake of oxidised omega-3 capsules had a negative effect on the cholesterol level of women with dyslipemia. *García-Hernández et al, 2013, Int. J. Food Sci. Nutr.*

OH-alkenals, alcohols and aldehydes may have toxic effects

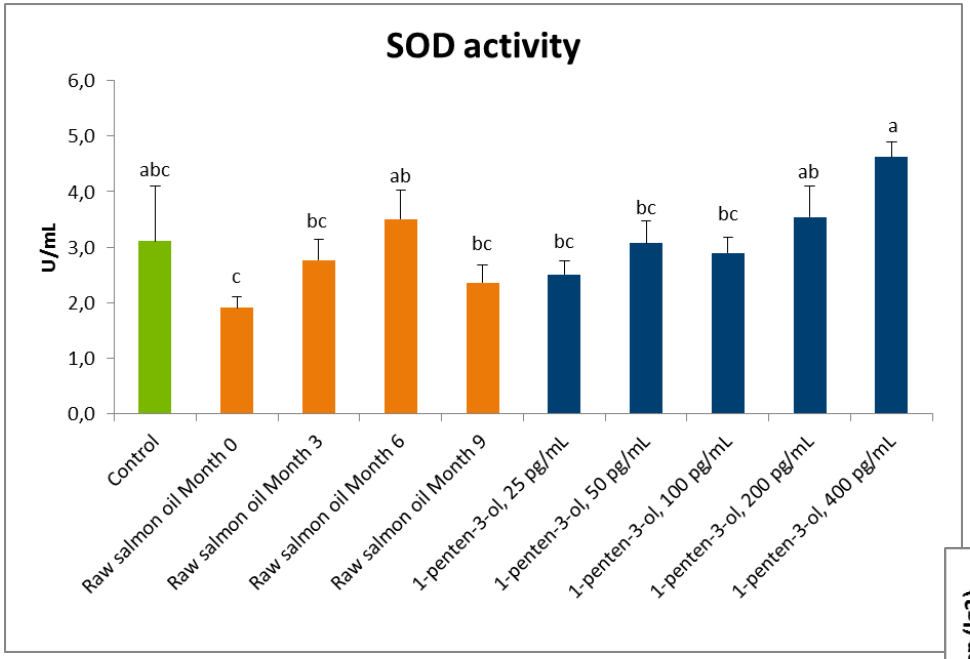
Tested the effect of an oil with increasing oxidation and an oxidation product (1-penten-3-ol) on various cellular processes involved in oxidative defense and inflammation

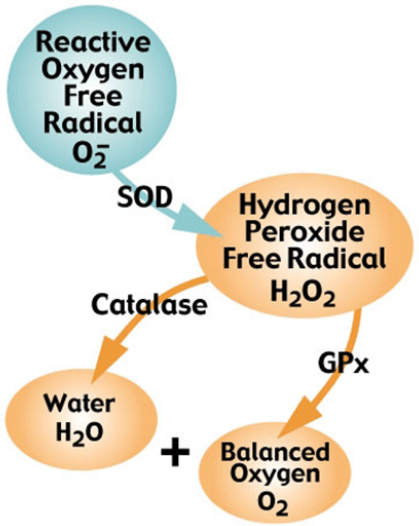
Effect on the cellular membrane

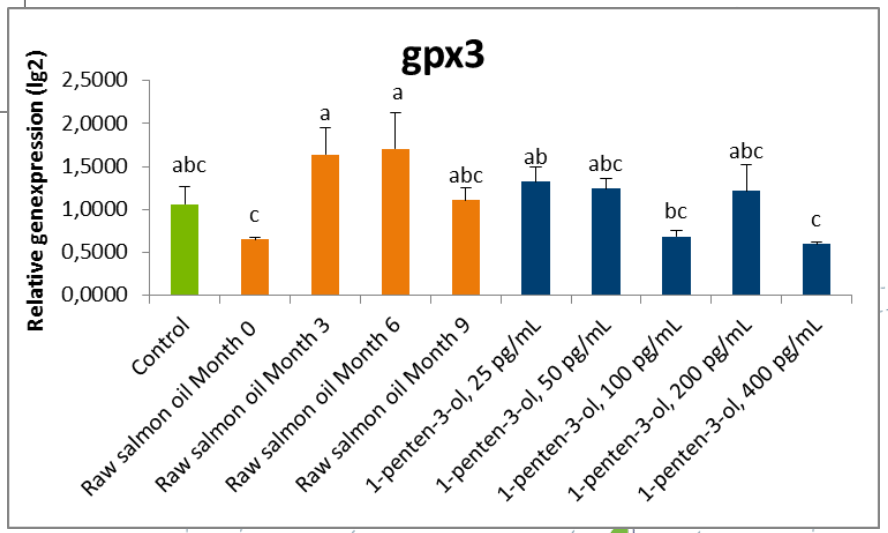




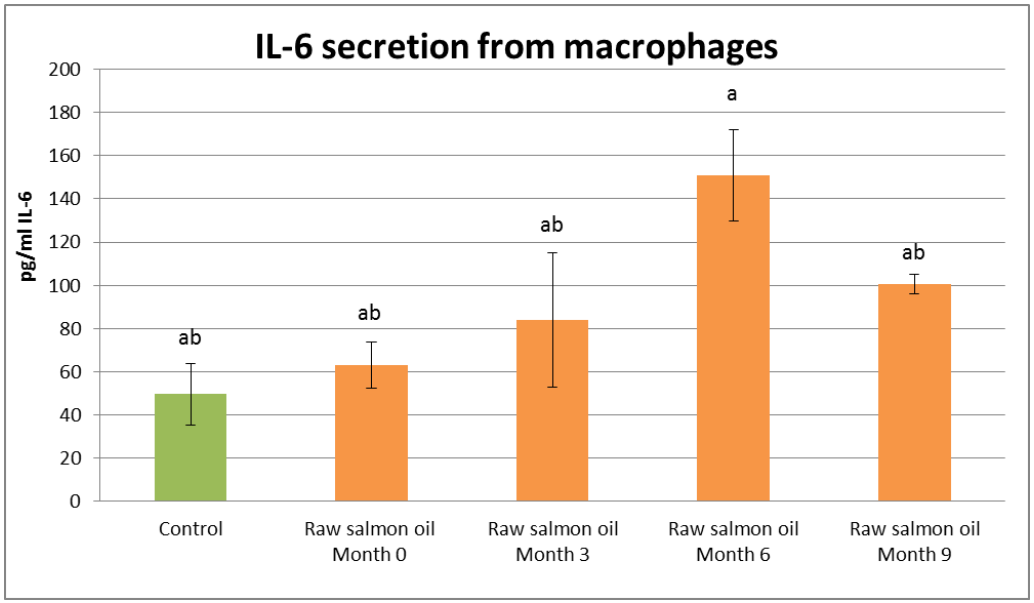
Effect on intracellular antioxidants







Effect on inflammation responses



Summary cellular responses

- Oxidation products present in oils can influence cellular responses involved in oxidative stress and inflammatory processes.

- The results show the importance of gaining knowledge about how the oxidation level and the individual oxidation products in the oil (and interaction between the various products) can affect cellular responses

- The preliminary results from cell models must be verified in more relevant biological systems such as mammalian models and human studies, before it is possible to conclude about potential health effects in humans

Thank you for your attention

- Bente Ruyter
- Gjermund Vogt
- John-Erik Haugen
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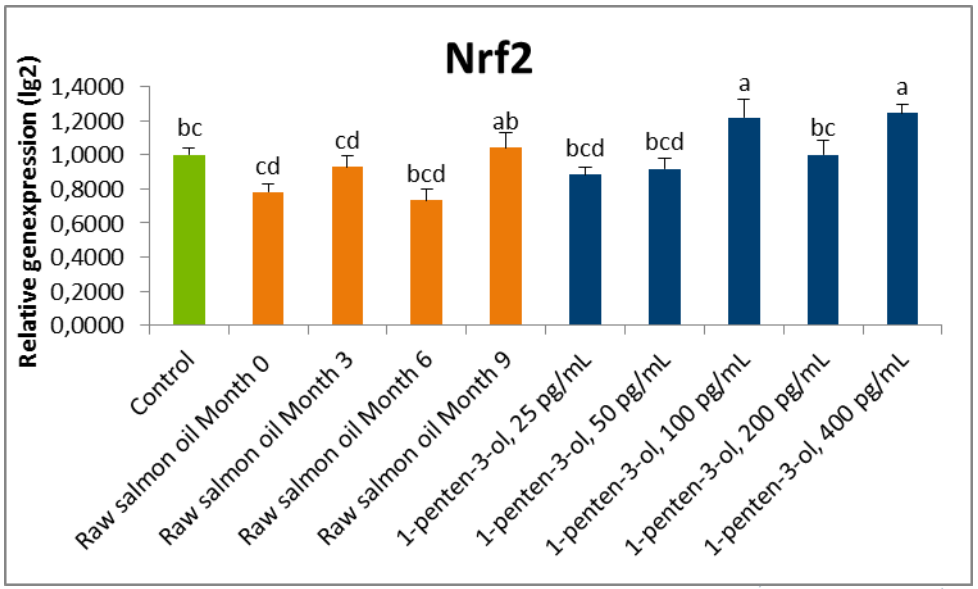
- Tone-Kari Østbye
- Elin-Merete Nicolaisen
- Frank Lundbye
- Inger Ø Kristiansen
- Merete Rusås Jensen

Nofima Lipid Platform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Relative genexpression (lg2)
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bcd
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bc
a
Control
Raw salmon oil Month 0
Raw salmon oil Month 3
Raw salmon oil Month 6
Raw salmon oil Month 9
1-penten-3-ol, 25 pg/mL
1-penten-3-ol, 50 pg/mL
1-penten-3-ol, 100 pg/mL
1-penten-3-ol, 200 pg/mL
1-penten-3-ol, 400 pg/mL