Long-chain Omega-3 Oils: Sources, Ingredient quality and Methods of Analysis

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FOOD & NUTRITION, OCEANS & ATMOSPHERE
www.csiro.au
LC Omega-3 Oils: Coverage today

- **Sources of LC Omega-3** - Resource issue; Future sources, an Australian perspective; algal oils, krill oil, & including new land plant LC-Omega-3

- **Supplements** - Selected Australian products, including Fish, Calamari & Krill oils

- **Analytical methods & issues** - Observations & feedback with emphasis on LC Omega-3 oils in Australia & NZ

- **Summary**
Essential Fatty Acid Families

**ω6 family**

- 18:2ω6: Linoleic (LA)
  - Corn Oil
  - Safflower Oil
  - Sunflower Oil
- 20:4ω6: Arachidonic (AA)
  - Meat, Eggs, Brains
  - Thrombotic
  - Inflammatory

**ω3 family**

- 18:3ω3: α-Linolenic (ALA)
  - Flaxseed Oil
  - Canola Oil
  - Soybean Oil
- 20:5ω3: Eicosapentaenoic (EPA)
- 22:6ω3: Docosahexaenoic (DHA)
  - Microalgae
  - Seafood
  - Anti-thrombotic
  - Anti-inflammatory

**LC Omega-3 Oils:** ≥C20, two or more double bonds
Global Fisheries - are there enough fish anyway - ?

“We estimate that large predatory fish biomass today is only about 10% of pre-industrial levels.”

Loved to death: our fish stocks in crisis
National Fisheries:

- Australian fisheries - take home message is that Australian fisheries are now generally deemed to be sustainable

**Imports** (70% of national seafood consumption)

- Message is not always as clear
- Often available at lower cost, e.g. Tilapia, Nile perch, Basa, NZ Hoki (MSC ratified)
  - Some of these imported lower cost species contain low contents of *LC Omega-3*
Other Sources of **LC Omega-3**

**Microalgae:** several University-Industry consortia

- **Phototrophs** (open ponds)
- **Heterotrophs** (fermenters)

- Recent move in algal biofuels R&D towards *HTP* rather than forming *biodiesel (FAME)*

- **LC Omega-3** directed activities
Other Sources of LC Omega-3

**Krill**: new collaboration of Aker-IMAS at Utas is due to start (ARC-Linkage)

- Resource monitoring & catch limits overseen by CCAMLR (Hobart HQ)
- MSC certified fishery (Aker)
- AAD, IMAS - Stephen Nicol (2014), Assoc Prof:

“The Antarctic krill fishery has been a source of controversy for several years, mainly because of perception that it is not sustainable. However, in several studies and research reports many experts have found the opposite to be true – that the Antarctic krill fishery is actually one of the most sustainably managed in the world.”
CSIRO Food & Nutrition: LC Omega-3 Oils

- LC Omega-3 oils essential for human & marine fish health
- Global fish catches static or declining
- Microalgae biosynthesize the LC omega-3 oils that fish consume & store. Fish do not make EPA+DHA

**CSIRO-wide project**
Goal: Isolate omega-3 genes from microalgae & transfer them to crop plants to sustainably produce LC omega-3 oils
2010
Partnership between CSIRO, Nuseed & GRDC

AAOCS - OMEGA-3 SYMPOSIUM - SEPTEMBER 9-11, 2015
www.csiro.au
Timeline of DHA biosynthesis in oilseeds

Summary - Surinder Singh to update

- Achieved fish oil-like levels of DHA in oil seed.
  1 Ha of canola at 12% = DHA from 10,000 fish
- High omega-3 / omega-6 ratio:
  11:1 including LA & ALA, 60:1 with new LC Omega-3 only.
- Application trials underway / planned

Petrie et al. PLOS One 2013; Inform 2013
Investigation of Existing & New Sources & Product Quality needs Adequate Analytical Methods
Use of a range of protocols - all steps

- Extraction
- Methylation
- Other derivitization
- GC column
- GC program
- GC drop-off
- Use of - standard methods, standards and/or a ‘standard reference material’
- Use of GC standards - all long-chain omega-3 FA are needed
- GC-MS verification or NOT (previously dual GC columns)
- Reporting - (i) % of TFA vs (ii) mg/100 g (need Int. Std.)
- Methods & data scrutiny / technical & scientific expertise
Reported results - Non-validated

• Long-chain fatty alcohols derived from Wax Esters in orange roughy co-elute with EPA & DHA on some polar GC columns

• NUTTAB 2006 - Land plants do not contain EPA & DHA

• NUTTAB 2006 - Meat products - high levels of LC Omega-3 needed further validation

All of the above case studies were from accredited labs
# Analytical Report - Review Process

<table>
<thead>
<tr>
<th></th>
<th>Fish A</th>
<th>Fish B</th>
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<tbody>
<tr>
<td>18:2</td>
<td>11.1</td>
<td>4.7</td>
</tr>
<tr>
<td>18:2 trans</td>
<td>&lt; 0.1</td>
<td>4.3</td>
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<tr>
<td>18:3</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>18:3w3</td>
<td>2.6</td>
<td>2.3</td>
</tr>
<tr>
<td>20:0</td>
<td>0.9</td>
<td>0.5</td>
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<tr>
<td>20:1</td>
<td>1.1</td>
<td>0.5</td>
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<tr>
<td>20:2</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>20:3 (20:4)</td>
<td>0.9</td>
<td>2.8</td>
</tr>
<tr>
<td>22:4 (22:0)</td>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>24:0</td>
<td>1.8</td>
<td>0.8</td>
</tr>
<tr>
<td>20:5w3</td>
<td>4.9</td>
<td>2.0</td>
</tr>
<tr>
<td>22:6w3</td>
<td>5.5</td>
<td>6.3</td>
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Other PUFA - not reported: 22:5w3 (DPA), 22:5w6, 22:4w6, 20:3w6, ....
Marine samples

- all low in **EPA** & **DHA**
- very high 24:1 & 20:3
- most FA after 18:1 mis-identified

Most FA identifications incorrect
Case Studies - Selected Methylation Issues

1. Starting oils (2x) and product (1x)
   - FAME prepared
   - Starting oils (2x): (i) % FA & (ii) absolute FA ok
   - Product oil (1x): (i) % FA ok, but (ii) absolute FA data very low *
   - Re-methylated product: (i) % FA & (ii) absolute FA data were then ok

2. Aquaculture trial
   - Multiple replicates of fish tissues
   - (i) % FA & (ii) % lipid data ok, (iii) total FA mg/g data - low & therefore inconsistent with (i) & (ii).
   - Methylations repeated with more care, mg/g FA data were then OK

*Care is required with FA methylation step, including where possible cross checking yield(s) when absolute (FA content) data is being prepared
NZ Fish Oils Case Study - Oil Quality

• NZ paper *Nature Scientific Reports* (Albert et al., Jan 2015)
  
  Two issues reported:

  (i) **EPA+DHA did not meet label claim**, markedly so for many (69%) products
  (ii) **Oils highly oxidized** (high PV, AnV) - health implications raised, including in media. Although regards health implications, the NZ study authors stated:

  "One relatively short study has compared the effects of oxidized and unoxidized fish oil in humans (32), observing no evidence of acute oxidative stability."

• Considerable negative media

• O3C, GOED, AAOCS, NZ Oils, TGA followed up; brief summary:

  ➢ Methods issues raised by these groups & by a large range of scientists
  ➢ O3C contacted main manufacturers; retesting occurred (see over)
  ➢ GOED & TGA have overseen further analyses of Aust & NZ products

  - recent update: very different results

• Industry very concerned; considerable feedback to O3C
NZ Fish Oils - Oil Quality

- 3 of 32 FO supplements contained quantities of EPA & DHA ≥ than label.
- Most products tested (69%) containing <67% EPA & DHA.
- Vast majority exceeded recommended levels of oxidation markers. 83% products exceeded PV threshold, 25% exceeded AV thresholds, & 50% exceeded recommended Totox levels.
- Only 8% met international recommendations, not exceeding any of these indices.
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NZ Fish Oils - Oil Quality

**Previous studies**
- 2014 *Nutrients*, Bengtson Nash et al.
- 2014 *Nutrients*, Nichols et al.
- Several others

**Recent analyses since the NZ fish oils study**
- Aust & NZ manufacturers - retesting
- GOED - 4 independent labs commissioned, 47 NZ samples
- TGA - 15 Aust & NZ samples

For all publications/sets of analyses above
- Aust & NZ oils generally - *met LC Omega-3 claims & were NOT oxidized*
- Next steps - under discussion
Fish, Squid & Krill Oil Capsules

mg of EPA & DHA per Capsule

Enriched products

18 / 12 Oils

Other Oils

Nichols et al. Nutrients 2014 - (FO & KO results, SO unpublished data)
**TGA follow up analyses**

(Personal communication, August 2015)

- In response to NZ paper & media attention, **TGA** surveyed similar fish oil products on the Australian market.

- 15 products tested.
  - **8 products**: ‘fish oil’ captured under **TGA** Compositional Guideline for ‘fish oil - natural’
  - **7 products**: captured under the **BP** (*British Pharmacopoeia*) monograph on ‘Concentrated Omega-3 triglycerides - fish’.

- Products analysed using **BP** methods for testing fish oils.

- **Testing for oxidation gave satisfactory results for all products in relation to PV.** 4 products gave high results for **ANV** which can be attributed to the presence of excipient fragrances or flavourings (aldehydes) which interfere with the test.

- **All 15 products gave acceptable results for content of omega-3 fatty acids**, that is they were all above the legislated (or official) lower limit of...
Summary

* **TGA follow up analyses**

  - Retesting by the industry, products gave satisfactory results.
  - Testing for oxidation gave satisfactory results for all products in relation to PV.
  - All 15 products gave acceptable results for content of omega-3 fatty acids.

* **GOED**

  - Similar findings to TGA; results being finalized.

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**Overall**  
(for all publications, previous & now these new analyses)

- Aust & NZ oils - *met LC Omega-3 claims & were NOT oxidized*
- Next steps - feedback welcome (and to O3C), responses under discussion.
Positive News re LC Omega-3

Joint Omega-3 Symposium O3C-AAOCS, Newcastle, November 2013: Published in Nutrients Special Issue (2014). Book also published in late 2014.

“Recent Advances in Omega-3: Health Benefits, Sources, Products and Bioavailability”.

http://www.mdpi.com/journal/nutrients/special_issues/omega-3_conference

➢ 12 papers in the Special Issue. Australian / NZ emphasis

➢ Similar plans & opportunities for this joint Symposium
Summary & Future Directions

FA analyses – care is needed by analysts
- Examples of issues shown today that may occur in the analytical processes used for LC Omega-3 profiling
- Cross checking FA data is needed where ‘anomalies’ occur
- Consideration of use of standard methods, reference materials, etc

Areas not covered today
- Institutional review process, Journal review process, Journal editorial process, Media review & PR processes

Australian & NZ fish oil supplements
- Generally *DO meet Omega-3 Claims & are NOT highly oxidized*
Thank you

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