Fortification of Foods With Marine Omega-3 Fatty Acids: Food Technology Issues

Mukund V. Karwe, Ph.D.
Department of Food Science and Center for Advance Food Technology
Rutgers University

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Contributors/Collaborators

Dr. Chi-Tang Ho (flavor and natural products chemistry)
Dr. Geetha Ghai (biochemistry and nutraceuticals)
Dr. Bob Rosen (analytical chemistry)
Dr. Beverly Tepper (sensory evaluation)

Dr. Rafael Borneo (post-doc)
Ms. Ramapadmini Gadiraju (grad student)
Ms. Sughra Naqvi (technician)
What are Marine Omega-3 Fatty Acids?

- Long chain polyunsaturated fatty acids (LCPUFA): “good fat”
- Derived from α-linolenic acid (ALA or 18:3 Omega-3), an essential fatty acid
- Important for brain development, nerve development, alleviating CVD, autoimmune disorder, atherosclerosis
  : healthy living
EPA and DHA

Major examples of long chain Omega-3 fatty acids

EPA (20:5)
(Eicosapentaenoic Acid)

Lowers the level of cholesterol, cleans blood vessels, prevents stroke and irregularity of the heart

DHA (22:6)
(Docosahexaenoic Acid)

Maintains and improves human memory and learning behavior
Greenland Eskimo Study

• “Eskimo paradox”
  – traditional diet - high in fat and protein, low in fruit, fiber and leafy green vegetables
  – little evidence of heart disease and low blood cholesterol levels.

• Greater intake of seal, whale and fish (all contain high levels of DHA and EPA)
• Lower intake of omega-6 fatty acids
• Lead to interest in omega-3 fatty acids in fish oil for prevention of CVD

Fish is Brain Food

- Fish are high in DHA; brain has high DHA levels
- Studies of patients suffering from schizophrenia and ADHD show that increasing levels of DHA has beneficial impact on brain function
- Prior study with Eskimos showed benefits to the heart
- Have blood thinning effect similar to aspirin
- Possible protective roles in arthritis, hypertension, cancer and heart disease
Omega-3 and Omega-6 fatty acids

- Omega-6 are fatty acids derived from linoleic acid (LA, 18:2), also an essential fatty acid. They work with Omega-3 to promote health.

- Omega-3 and Omega-6 are precursors for the synthesis of eicosanoids – hormone-like compounds, regulators of immune and inflammatory responses.
Both compete for the same enzymes

LA

Omega-6

Linoleic acid (C 18:2)

Gamma-linolenic acid (C 18:3)

ω6-Desaturase

Dihomogamma-linolenic acid (C 20:3)

ω5-Desaturase

Arachidonic acid (C 20:4)

Elongase

C 22:4

Eicosapentaenoic acid (C 20:5)

Elongase

C 22:5

Docosahexaenoic acid (C 22:6)

Omega-3

Alpha-linolenic acid (C 18:3)

ω6-Desaturase

C 18:4

ALA

Omega-6:Omega-3 ratio is important!
Creating a Balance

Omega-6
Promote inflammation
Vegetable fats

Omega-3
Reduce inflammation
Marine animals

Imbalance in “western” diet → rising rate of inflammatory disease
Omega-3 and Omega-6 fatty acids in plant/seed oils

<table>
<thead>
<tr>
<th><strong>Linoleic Acid (Omega-6)</strong></th>
<th><strong>Linolenic Acid (Omega-3)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean (50-57%)</td>
<td>Flaxseed (35-56%)</td>
</tr>
<tr>
<td>Safflower (67-83%)</td>
<td>Soybean (5-10%)</td>
</tr>
<tr>
<td>Sunflower (48-74%)</td>
<td>Canola (6-14%)</td>
</tr>
<tr>
<td>Corn (34-62%)</td>
<td>Safflower Oil (0.1%)</td>
</tr>
<tr>
<td>Canola (16-25%)</td>
<td>Walnut (13%)</td>
</tr>
<tr>
<td>Sesame (35-50%)</td>
<td>Olive oil (0.2-1.5%)</td>
</tr>
</tbody>
</table>

(Values in % of total fatty acids)
# Fatty Acid Composition of Vegetables and Fruits

<table>
<thead>
<tr>
<th>Vegetables/Fruits</th>
<th>LA (18:2\ ω_6)</th>
<th>ALA (18:3\ ω_3)</th>
<th>Ratio ((ω-6:ω-3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avocado</td>
<td>1.9</td>
<td>0.1</td>
<td>19</td>
</tr>
<tr>
<td>Bean, lima</td>
<td>0.5</td>
<td>0.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Bean, pinto</td>
<td>0.2</td>
<td>0.3</td>
<td>0.66</td>
</tr>
<tr>
<td>Broccoli</td>
<td>0.03</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>0</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>Kale</td>
<td>0.1</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Lettuce</td>
<td>0</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>Mustard</td>
<td>0</td>
<td>0.04</td>
<td>-</td>
</tr>
<tr>
<td>Peas</td>
<td>0.2</td>
<td>0.2</td>
<td>1</td>
</tr>
<tr>
<td>Raspberries</td>
<td>0.2</td>
<td>0.1</td>
<td>2</td>
</tr>
<tr>
<td>Soybean</td>
<td>0.4</td>
<td>2.1</td>
<td>0.19</td>
</tr>
<tr>
<td>Spinach</td>
<td>0.1</td>
<td>0.9</td>
<td>0.11</td>
</tr>
<tr>
<td>Strawberries</td>
<td>0.1</td>
<td>0.1</td>
<td>1</td>
</tr>
</tbody>
</table>
# Fatty Acid Composition of Nuts

<table>
<thead>
<tr>
<th>Nuts and Seeds</th>
<th>Linoleic Acid 18:2 ω6</th>
<th>Alpha-Linolenic Acid 18:3 ω3</th>
<th>Ratio (ω6:ω3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beechnuts</td>
<td>18.4</td>
<td>1.7</td>
<td>10.8</td>
</tr>
<tr>
<td>Butternut</td>
<td>34.0</td>
<td>8.7</td>
<td>3.9</td>
</tr>
<tr>
<td>Chia seeds</td>
<td>3.4</td>
<td>3.9</td>
<td>0.87</td>
</tr>
<tr>
<td>Hickory</td>
<td>20.9</td>
<td>1.0</td>
<td>20.9</td>
</tr>
<tr>
<td>Walnut, black</td>
<td>34.2</td>
<td>3.3</td>
<td>10.4</td>
</tr>
<tr>
<td>Walnut, English</td>
<td>32.3</td>
<td>6.8</td>
<td>4.8</td>
</tr>
</tbody>
</table>

# Fatty Acid Composition of Fats and Oils

<table>
<thead>
<tr>
<th>NAME OF FAT/OIL</th>
<th>Sat’d %</th>
<th>Linoleic Acid % (18:2 ω6)</th>
<th>α-LA % (18:3 ω3)</th>
<th>Mono-un Sat’d %</th>
<th>Ratio (ω 6:ω3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canola/Rapeseed oil</td>
<td>7</td>
<td>22</td>
<td>10</td>
<td>61</td>
<td>2.2</td>
</tr>
<tr>
<td>Flaxseed/linseed oil</td>
<td>10</td>
<td>17</td>
<td>55</td>
<td>18</td>
<td>0.31</td>
</tr>
<tr>
<td>Safflower Oil</td>
<td>10</td>
<td>76</td>
<td>Trace</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>12</td>
<td>71</td>
<td>1</td>
<td>16</td>
<td>71</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>13</td>
<td>57</td>
<td>1</td>
<td>29</td>
<td>57</td>
</tr>
<tr>
<td>Olive Oil</td>
<td>15</td>
<td>9</td>
<td>1</td>
<td>75</td>
<td>9</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>15</td>
<td>54</td>
<td>8</td>
<td>23</td>
<td>6.75</td>
</tr>
<tr>
<td>Lard</td>
<td>43</td>
<td>9</td>
<td>1</td>
<td>47</td>
<td>9</td>
</tr>
<tr>
<td>Beef Tallow</td>
<td>48</td>
<td>2</td>
<td>1</td>
<td>49</td>
<td>2</td>
</tr>
</tbody>
</table>
Intake Recommendations

• FDA – Do not exceed 2 g/p/d from conventional food and dietary supplement sources

• Simopolous et al. (2000):
  Omega-6: LA 4.44,
  Omega-3: ALA 2.22, EPA + DHA 0.66 (g/p/d)

## Omega-3 Fatty Acids in Fish

<table>
<thead>
<tr>
<th>Fish</th>
<th>EPA + DHA (%)</th>
<th>Daily amount needed for 3 g EPA + DHA (pounds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herring</td>
<td>2.72</td>
<td>0.24</td>
</tr>
<tr>
<td>Tuna</td>
<td>3.28</td>
<td>0.20</td>
</tr>
<tr>
<td>Salmon</td>
<td>2.86</td>
<td>0.23</td>
</tr>
<tr>
<td>Mackerel</td>
<td>1.75</td>
<td>0.38</td>
</tr>
<tr>
<td>Swordfish</td>
<td>0.79</td>
<td>0.84</td>
</tr>
<tr>
<td>Trout</td>
<td>0.59</td>
<td>1.12</td>
</tr>
<tr>
<td>Halibut</td>
<td>0.51</td>
<td>1.30</td>
</tr>
<tr>
<td>Cod</td>
<td>0.18</td>
<td>3.67</td>
</tr>
<tr>
<td>Sole</td>
<td>0.19</td>
<td>3.48</td>
</tr>
</tbody>
</table>

How to increase Omega-3 intake

• Eat more fatty fish

• Take a supplement

• Eat fortified foods
• Taking Omega-3 fatty acid capsules is like taking medicine pills
• Increasing fish consumption is challenging and may not be possible.
  – Difficult to eat amount of fish needed
  – Vegetarians
  – People with fish allergies
  – Those who don’t like fish
Food Fortification

• Food fortification is convenient and efficient
  – Allows one to eat assortment of foods fortified with Omega-3 fatty acids
Food fortification with Omega-3

Supplying Omega-3 fatty acids in the diet through fortified foods will meet the body’s metabolic needs better than a dietary supplement or pills. (Maki et al., 2003)

However, Omega-3 fatty acids are

• Prone to auto-oxidation – unstable with very short shelf life
• Sensitive to air, heat, light and humidity
• Generation of off-odors

Solutions to minimize or eliminate these problems lie in Food Technology
<table>
<thead>
<tr>
<th>Company</th>
<th>Product</th>
<th>Source</th>
<th>Quality/Ingredients</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omega Protein Houston</td>
<td>Omega Pure</td>
<td>Menhaden oil</td>
<td>12-18% EPA+7-12% DHA + vit E + TBHQ</td>
<td>Add oil to food (Only GRAS fish oil in the US)</td>
</tr>
<tr>
<td>Life Plus Co</td>
<td>EPA PLUS</td>
<td>Fish</td>
<td>DHA+ EPA</td>
<td>Dietary supplement capsules</td>
</tr>
<tr>
<td>Hypermot</td>
<td>Omega bar</td>
<td>Flaxseed</td>
<td>11 g ω-3/bar</td>
<td>Snack bar</td>
</tr>
<tr>
<td>Roche Vitamins, NJ</td>
<td>ROPUFA 30 (30% EPA+ DHA)</td>
<td>Fish oil</td>
<td>25% EPA, 12.5% DHA, C &amp; E, rosemary extract</td>
<td>Bulk, liquid</td>
</tr>
<tr>
<td>Wacker Biochem, MI</td>
<td>OMEGA DRY-1150</td>
<td>ω-3 w/i γ-cyclodextrin complex</td>
<td>6% omega-3, Stable at 100 °C for 30 min</td>
<td>Powder</td>
</tr>
<tr>
<td>Nutrinova, Germany</td>
<td>DHActive™</td>
<td>Micro Algae</td>
<td>43% DHA</td>
<td>Coating. oil</td>
</tr>
</tbody>
</table>
We have used

1. ROPUFA ’10’ Omega-3 Food Powder (DSM)
2. ROPUFA ’75’ n-3 EE (DSM)
3. OmegaDry Powder (Wacker)
ROPUFA ’10’ Omega-3 Food Powder

- Spray Dry Product (5% moisture)
- Refined fish oil dispersed in a cornstarch-coated matrix of fish gelatin
- Minimum 30% ROPUFA Omega-3 Fish Oil (29% crude fat)
- Tocopherols, rosemary extract and sodium ascorbate
- Min 7% LCPUFA (7.85-7.91%)
- Sensitive to air, light, heat and humidity
- Claim: Can store unopened for 12 mos, below 15°C
ROPUFA ’75’ n-3 EE

- Refined ethyl esters of fish oil
- 75% Omega-3 PUFAs, predominantly ethyl esters of EPA and DHA
- Rosemary extract, ascorbyl palmitate, tocopherols, and citric acid
- Sensitive to air, heat, light and humidity
- Claim: Can store unopened for 18 mos, below 15°C
OmegaDry Powder

- Commercially available - Wacker, Inc. Ann Arbor, MI
- Due to instability of fish oils, encapsulated source of EPA and DHA used
- Refined menahden oil stabilized with natural tocopherols and/or TBHQ, $\gamma$-cycloextrin inclusion complex
- Consists of 6% EPA and DHA
- Claim: OmegaDry is stable for 10 hrs at 100$^\circ$C
- The OmegaDry powder was subjected to DSC and the glass transition was observed.
Baking Technology

Advanced technology helps to solve food fortification issues

• Jet Impingement
• Multi-mode
  – Microwave and jet impingement
Jet-impingement oven

- Higher moisture retention in baked products can enhance the quality of processed food. (Walker, 1987)
- Lower thermal conductivity and moisture diffusivity of crust leads to higher moisture retention in product
- Greater levels of EPA and DHA retained
- Retention of Omega-3 fatty acids was higher when hot air jet impingement was used to make a baked fish product
  - Might be due to antioxidant action of water retained, thus causing more Omega-3 retention. (Borquez et al, 1999)


Encapsulation should survive the processing conditions.

Exothermic

Original

Rescan

Temperature
Adding
Omega-3 Fatty Acids
to
Sugar Cookies
Omega-3 in Sugar Cookies

• EPA and DHA encapsulated in gammacyclodextrin (OmegaDry)

• Exploring effects of antioxidants (mixed tocopherols, ascorbic acid, rosemary extract) on stability of EPA and DHA
Cookies without OmegaDry

Cookies with OmegaDry
EPA and DHA content in fortified dough and cookies with added antioxidants

<table>
<thead>
<tr>
<th>% of antioxidant blend in cookies</th>
<th>Avg EPA &amp; DHA – conventional oven (mg)</th>
<th>Avg EPA &amp; DHA - jet impingement oven (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>23.02 ± 3.9</td>
<td>33.5 ± 4.2</td>
</tr>
<tr>
<td>0.5</td>
<td>25.02 ± 5.8</td>
<td>32.73 ± 3.6</td>
</tr>
<tr>
<td>1</td>
<td>30.19 ± 4.7</td>
<td>36.43 ± 5.2</td>
</tr>
</tbody>
</table>
Omega-3 in Sugar Cookies

- Fortified cookies had lower amounts of EPA and DHA compared to those based on the claims by the manufacturer of encapsulated product – 36 mg vs 70 mg
- Presence of antioxidants did not significantly enhance the retention of EPA and DHA
Adding Omega-3 Fatty Acids to Bread
Omega-3 Bread

- High moisture product
- Lower internal temperature than cookies
- Brown crust and white inside (for white bread)
- OmegaDry powder added to dough
Using Conventional Oven

Without OmegaDry powder

- Orange/brown color
- Slightly thicker/heavier appearance
- Fish smell apparent when baking

With OmegaDry powder
Hybrid Microwave + Jet Impingement oven

- Addition of OmegaDry powder

Without omega-3 powder  
With omega-3 powder (10%)  
With omega-3 powder (7%)  
10 mg of EPA+DHA in 10 g of bread
Adding Omega-3 Fatty Acids to Sandwich Cookies
Sandwich Cookies

- Convenient, easy to eat, store or carry, ready to eat
- Minimally processed (no heat)
- Easy to manufacture/simple technology
Prototype Development

• Filling formulas developed
• Tested with sensory panel
• Feedback
  – Too sweet
  – Some sensed metallic aftertaste
  – Throat burning sensation
  – Fish oil flavor detected
## Final Formulation

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar 10X (Domino)</td>
<td>43.06%</td>
</tr>
<tr>
<td>Super Envision (Domino)</td>
<td>0.43%</td>
</tr>
<tr>
<td>ROPUFA (DSM)</td>
<td>25.83%</td>
</tr>
<tr>
<td>Vegetable Shortening (Crisco)</td>
<td>17.94%</td>
</tr>
<tr>
<td>Corn Syrup</td>
<td>6.09%</td>
</tr>
<tr>
<td>Gelatin Solution (5 g/55 ml)</td>
<td>3.44%</td>
</tr>
<tr>
<td>Lemon extract (McCormick)</td>
<td>2.69%</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.27%</td>
</tr>
<tr>
<td>Salt</td>
<td>0.22%</td>
</tr>
<tr>
<td>Color (yellow)</td>
<td>0.03%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.00%</strong></td>
</tr>
</tbody>
</table>
Omega-3 in Sandwich Cookies

• Final Formulation
  – Delivers 400 mg DHA + EPA per cookie (20 g)
  – No detection of fish oil smell or off-odor
  – No aftertaste/throat burning sensation
ROPUFA/Cookie Chromatograms

ROPUFA

Cookie
Stability Study

• Three Lots of Production (Replicates)

• Two Storage Temperatures (22°C, 37°C)

• Two Packaging Conditions (Vacuum, Regular)
Stability Study

Loss of DHA in Sandwich Cookies on Storage

DHA (mg/g)

Days

0 3 7 14 28

Reg 22
Reg 37
Vac 22
Vac 37
Conclusions

• Food fortification – possible alternative. Changes in formulations and processes are needed.
• Use of high moisture content systems, like muffins, might help in better retention of EPA & DHA
• Advanced technology has allowed for better cooking, higher moisture retention and greater retention of compounds
Future Work

• Ensure no harmful products are formed during degradation of EPA and DHA
• Examine different encapsulation materials
• Study effect of different antioxidants during processing to stabilize EPA and DHA
Methodology - Bread

• Used conventional oven and hybrid oven for testing
• Chef’s method to prepare bread
• Develop procedure to bake bread without omega-3 fatty acids
  – Conventional oven – variables: time, temperature
  – Take time/temperature measurements using T-thermocouple placed in center of bread
  – Use hybrid oven – variables: time, temperature, jet velocity, and microwave level
  – Take time/temperature measurements, find optimal baking conditions
Methodology - Bread

• Add omega-3 fatty acids to bread
  – Powder form added as dough is mixed
  – 10% by weight
• Analyze for EPA and DHA retention
  – Soxhlet extraction method using hexane
  – Rotovapor to remove excess solvent
  – Dry under N$_2$
  – Form FAMEs with BF$_3$ and methanol
  – Add water and hexane, then remove upper layer (hexane) for GC analysis
Methodology – Sandwich Cookies

• Analyze using GLC for DHA and EPA retention
  – Add standard
  – Evaporate solvent under N₂
  – Make FAMEs using BF₃ and Methanol
  – Heat, then add hexane, NaCl saturated Soln.
  – Transfer upper phase (hexane)
  – Re-extract lower phase with hexane, transfer and combine hexane phases
  – Concentrate and inject into GC
Methodology – Sandwich cookies

- Test with sensory panel against placebo
- Repeat until acceptable formulation found
- Run stability study
  - 3 Lots (replicates)
  - 2 Storage temperatures (22°C, 37°C)
  - 2 Packaging conditions (Vacuum, Regular)
Prototype Development

• Second Filling Formula
  – 180-210 mg EPA+DHA per cookie

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powdered Sugar</td>
<td>60.4</td>
</tr>
<tr>
<td>Glycerine</td>
<td>12.7</td>
</tr>
<tr>
<td>High Fructose Corn Syrup</td>
<td>5.6</td>
</tr>
<tr>
<td>Shortening</td>
<td>7.2</td>
</tr>
<tr>
<td>Salt</td>
<td>0.3</td>
</tr>
<tr>
<td>Titanium Dioxide</td>
<td>0.2</td>
</tr>
<tr>
<td>Vanillin</td>
<td>0.1</td>
</tr>
<tr>
<td>Roche PUFA</td>
<td>13.6</td>
</tr>
</tbody>
</table>

• Feedback
  – Very sweet
  – Strong aftertaste, throat burning sensation
  – No fish oil flavor detected
Prototype Development

• Third filling formula
  – No glycerin formula
  – 200 mg EPA + DHA
  – Flavors: Vanilla, Chocolate and Orange

• Feedback
  – No detection of fish oil flavor
  – No aftertaste/throat burning sensation
  – Vanilla flavor: not acceptable
  – Chocolate: too overpowering
  – Orange: acceptable and preferred
Prototype Development

• Preliminary Evaluation (5 Panelists)
  – No detection of fish oil flavor
  – No aftertaste/throat burning sensation
  – Vanilla flavor: not acceptable
  – Chocolate: too overpowering
  – Orange: acceptable and preferred
Prototype Development

Feedback:
- Too sweet
- No difference between placebo and ω-3 containing cookies
- Some sensed metallic after taste

• Second Filling Formula
  – 180-210 mg EPA+DHA per cookie

• Feedback
  – Very sweet
  – Strong aftertaste, throat burning sensation
  – No fish oil flavor detected
Methodology – Sugar Cookies

- Cookies prepared through AACC method, 10-50D (1984)
- OmegaDry added at different concentrations
- Cookies – 8 cm thick, 4.75 cm diameter
- Processing Conditions:
  - Conventional oven – 200°C, 10 min
  - Jet impingement oven – 149°C, 9 min, 175 Pa
- Temperature measured at center of cookie with T-thermocouple
Methodology – Sugar Cookies

• Comparisons of cookies baked in conventional and jet-impingement ovens
  – Average temperature of cookie
  – Surface color of cookie
  – Center temperature of cookie

• Moisture analysis – 130°C

• Fat analysis:
  – Soxhlet extraction (AOAC method: 963.15)
  – Saponification
  – Methylation
  – Extraction
  – Concentration
  – GC Analysis
Methodology – Sugar Cookies

• Further ensure stability, antioxidant mix was used (Chang et al. 1991: US Patent No. 5077069)
  – Tocopherols (0.10%)
  – Ascorbic acid (0.02%)
  – Citric acid (0.02%)
  – Deoiled soybean phospholipids (0.20%)

• Used in 3 different concentrations (all percentages by weight of shortening)
  – 1X
  – 5X
  – 10X
Omega-3 in Sugar Cookie

- EPA & DHA retained in presence of antioxidants
  - 25-40 mg/g of cookie
  - 39 mg/g of dough - initially
- Cookies baked in either oven retained 70-90% EPA & DHA (similar to cookies with no antioxidants added)
- Presence of antioxidants did not significantly enhance the retention of EPA and DHA