# USE of MARINE OILS in INFANT FORMULAE

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# Infant Formulae

- ***Infant formulae*** **refer to foods designed for satisfying the nutritional needs of nursing babies during their first 4-6 months, as the sole source of their nutrition.**

- ***Follow on formulae*** **refer to foods designed for nutrition of babies over 4 months, as their main source of liquids, as their diet gradually becomes more versatile.**

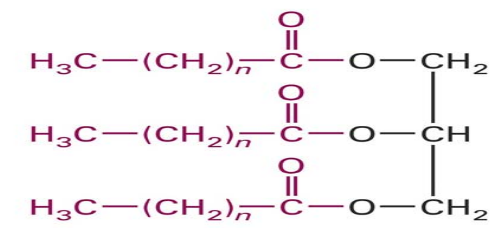
- **Even though presently there is consensus in medical circles on the superiority of mother's milk, more and more mothers are using these products, due to many diverse reasons. Therefore the respective market is quite huge (over 7 billion $ per annum in USA).**

# Human Milk Fat (HMF)

- Human milk contains 4.4 -4.5% fat
  ,
  which supplies 45-55% of the infant's total energy requirement,
- HMF consists of 98% triacylglycerols (TAG), 1% phospholipids and 0.5% sterols,
- It provides essential linoleic acid (LA) and alpha-linoleic acid (ALA) and longer chain (20- and 22-carbon) PUFA like arachidonic acid (AA) and docosahexaenoic

# Table 1. Fatty Acid Composition of Human Milk TAG´s and Fatty Acids at Sn-2 Position as Found by Different Researchers

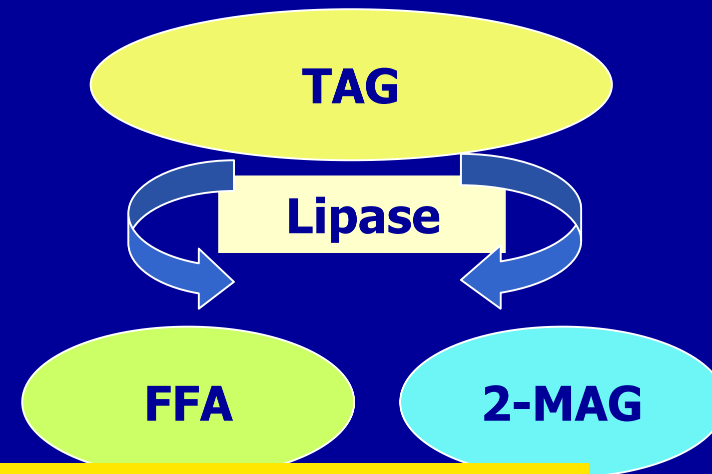
| Fatty acids | TAG | sn-2 |
|---|---|---|
| 8:0 | 0.29-0.36 | |
| 10:0 | 1.6-2.6 | 1.6 |
| 12:0 | 4.9-7.9 | 5.3-6.9 |
| 14:0 | 6.6-9.4 | 11.3-15.4 |
| 16:0 | 21.8-27.0 | 44.5-57.1 |
| 16:1n-7 | 2.2-3.6 | 1.6 |
| 18:0 | 5.8-8.0 | 1.2-4.9 |
| 18:1n-9 | 30.5-35.1 | 8.1-9.2 |
| 18:2n-6 | 10.3-14.1 | 3.7-7.1 |
| 18:3n-3 | 0.47-1.2 | |
| 20:3n-3 | 0.79 | |
| 20:4n-6 (AA) | 0.5-1.0 | |
| 20:5n-3 (EPA) | 0.07 | |
| 22:0 | 0.2 | |
| 22:1n-9 | 0.3 | |
| 22:4n-6 | 0.4 | |
| 22:6n-3 (DHA) | 0.26 | |



Triacylglycerol

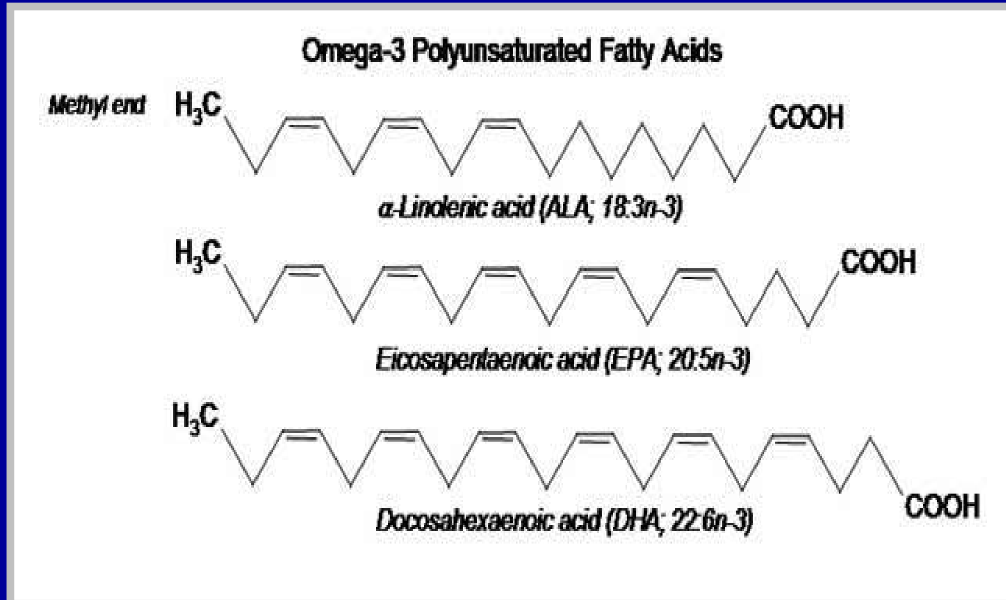
# Absorption characteristics of HMF

- During digestion process, human milk fat is hydrolyzed to produce free fatty acids (FFA) and sn-2-monoacylglycerols (2-MAG) that are absorbed in the small intestine.

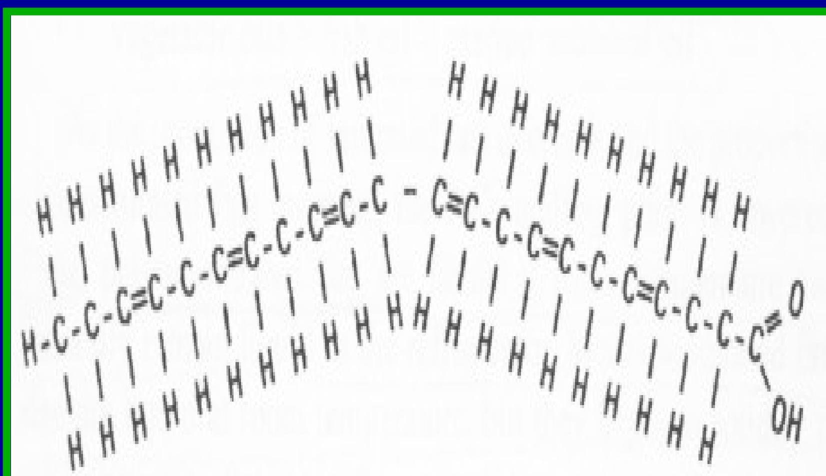



- Free palmitic acid released from sn-1,3 positions can form insoluble calcium-fatty acid complexes, also referred to as "calcium soaps" which cannot be absorbed in the small intestine and are excreted.
- This causes infants to have lower fatty acid and calcium absorption levels and higher loss of dietary energy. Also stool hardness, constipation and even cases of bowel

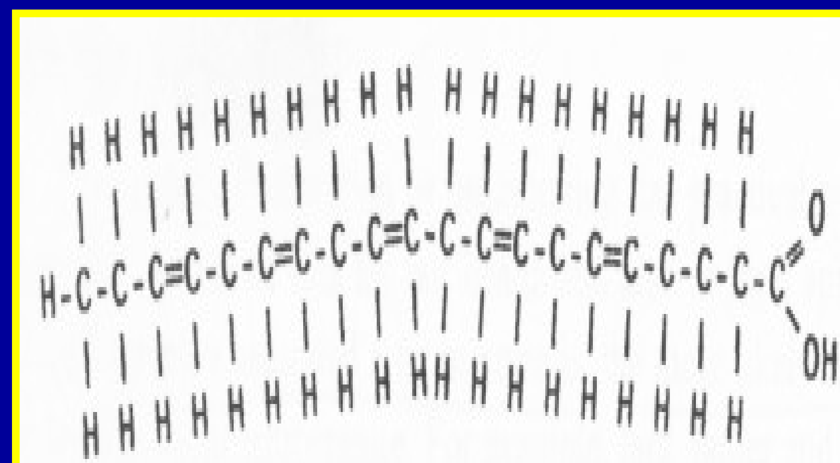
The location of palmitic acid in the triacylglycerol molecules is hypothesized as greatly influencing the absorption and deposition of HMF in the infant's body.




DHA (C22:6)



EPA (C20:5)



# Sources of Omega-3 PUFA

- Synthesised mainly by uni- and multicellular phytoplanktons and algae.
- Fish fed on algae and marine mammals eating these fish become rich in omega-3 PUFA.
- Best Sources:

The flesh of fatty fish such as herring, mackerel, menhaden, salmon, anchovy, tuna; the liver of white lean fish such as cod and halibut; the blubber of marine mammals such as seals and whales.

# Justifications for omega3-PUFA supplementation of Infant Formulae

- Omega-3 Polyunsaturated fatty acids (n-3PUFA) have been theorised to have significance for brain development and cognitive function (learning abiity) as well as for vision, since they are found to accumulate in brain and eye of the fetus, especially during the last trimester of pregnancy.

- Babies cannot synthesize these fatty acids, but they are found in the fat of human milk. Blood levels of these fatty acids are typically higher in breast-fed infants than in infants fed formulas not containing these fatty acids. Infants fed with formulae lacking LC-PUFA have also been reported to have lower red cell

# Health Benefits of Omega-3 Fatty Acids

| Afflictions Associated with a Deficiency of Omega-3 Fatty Acids | | |
|---|---|---|
| Acne | Cardiovascular Disorder | Macular Degeneration |
| Aging | Chrones Disease | Menopause |
| Aids | Depression | Mental Illness |
| Allergies | Diabetes | Multiple Sclerosis |
| Alzheimers | Dyslexia | Obesity |
| Angina | Dysmenorrhea | Pregnancy |
| Arthritis | Eczema | Postate Cancer |
| Asthma | Fatty Liver | Psoriasis |
| Atherosclerosis | Heart Disease | Raynaud's Disease |
| Atopy Dermatitis | High Blood Pressure | Rheumatoid Arthriris |
| Attention Deficit Disorder | Hyperactivity | Schizophrenia |
| Autism | Immune Deficiencies | Stroke |
| Autoimmune Disorder | Kidney Disease | Ulcerative Colitis |
| Breast Cancer | Ligament Injuries | Vision Disorder |
| Cancer | Learning disorders | |
| Cystic Fibrosis | Lupus | |

# Health Benefits of Specific Omega-3 Fatty Acids

**EPA**

Benefits the cardiovascular system and inflammatory conditions by:

Suppressing AA for anti-inflammatory effect

Decreasing triglycerides, increasing HDLs

Preventing arrhythmias/ stabilizing heart rhythm

Producing anti-thrombotic effects

**DHA:**

Predominant structural f.a. in the grey matter of the brain and the eye's retinal tissues.

Plays an important role in the mental development and visual acuity of infants especially the premature ones.

Maintain membrane fluidity and function, regulate cellular receptors

Facilitate normal growth and cognitive development

Participate in development, composition and function of the central nervous system.

- Since there is extensive scientific literature suggesting that their regular consumption and/or dietary supplementation may lead to many other health benefits, increasing their intake seems a wise strategy for the long term prevention of many chronic illnesses.

# Codex* Recommendations for (LC-PUFA) Supplemented Formulae

| | Linoleic acid | α-linolenic acid | linoleic/ α-linolenic acid | EPA | DHA |
|---|---|---|---|---|---|
| ESPGHAN* FISPGHAN** IEG*** | Min: 300 mg/100 kcal Max: 1200mg/100 kcal | Min: 50mg/100 kcal Max: No value | 5-15/1 | EPA< DHA | <0.5% of total fat |

*The European Society for Pediatric and Gastroenterology, Hepatology and Nutrition

**Federation of International Societies on Pediatric Gastroenterology, Hepatology and Nutrition

***International Expert Group for consultation on IF compositional requirements and optional igredient levels

*Codex Committee on Nutrition and Foods for Special Dietary Uses

# Legal Status for Omega-3PUFA Supplementation

EU Ministerial Decree implementing Directive 96/4/EEC amending Directive 91/321 EEC on infant formulae and follow-on formulae:

- Omega-3 LC-PUFA< 1% of total fat content
- Omega-6 LC-PUFA <2% of total fat content
- EPA content< DHA content

The adequate intakes (AI) for:

- AA: 0.50%
- DHA: 0.35%

# The present situation: (LC-PUFA) supplemented formulae

- In Europe, US, and Canada, some commercially available infant formulae for term and preterm infants already contain LC-PUFA (i.e. DHA and AA).
- The ranges of LC-PUFA concentration levels in these products are:

For DHA:

8-19 mg/100

for AA:

22-34 mg/100

# STRATEGIES for SUPPLEMENTATION of INFANT FORMULAE WITH OMEGA-3 FATTY ACIDS

Recent developments in food technology (i.e. encapsulation) allow fortification of foods with marine omega-3PUFA, without the undesirable fish odor/taste. This is possible by adding to the composition of infant formulae either:

- I. Omega-3 PUFA concentrates of marine oils

or

- II. HMF´s containing omega-3 PUFA, tailor-

# Strategy I: Adding omega-3 PUFA concentrates of oils to formulae

**Example1: United States Patent #5397591 :**

**"Infant formula and baby food containing DHA obtained from dinoflagellates"**

Infant formula and baby food compositions are presented which contain single cell edible oil recovered from dinoflagellates and which lack unpleasant tastes and fishy odors. This single cell edible oil comprises at least 70% triglycerides which contain about 20-35% (DHA) but lacks (EPA).

To produce this single cell oil, the dinoflagellates are cultivated in fermentors and induced to produce the single cell oil which is subsequently recovered by extraction with solvents.

## Example 2: DHA-rich SCO(Single cell oil)

- An other DHA-rich SCO refers to a mixture of an oil extracted from the microalgal species *Crypthecodinium cohnii* and high oleic sunflower oil (HOSO).
- The resulting mixed oil contains 40-45% of product weight as DHA and is marketed as an enrichment ingredient for infant formula as well as for other functional foods.

# II. Production of HMF'S (Human Milk Fat Substitutes)

- Structured lipids resembling HMF TAG's are being produced, mainly by interesterification of tripalmitin or palm stearin with selected vegetable oils and/or fatty acids(i.e.oleic acid etc.) catalyzed by 1,3-specific lipases:

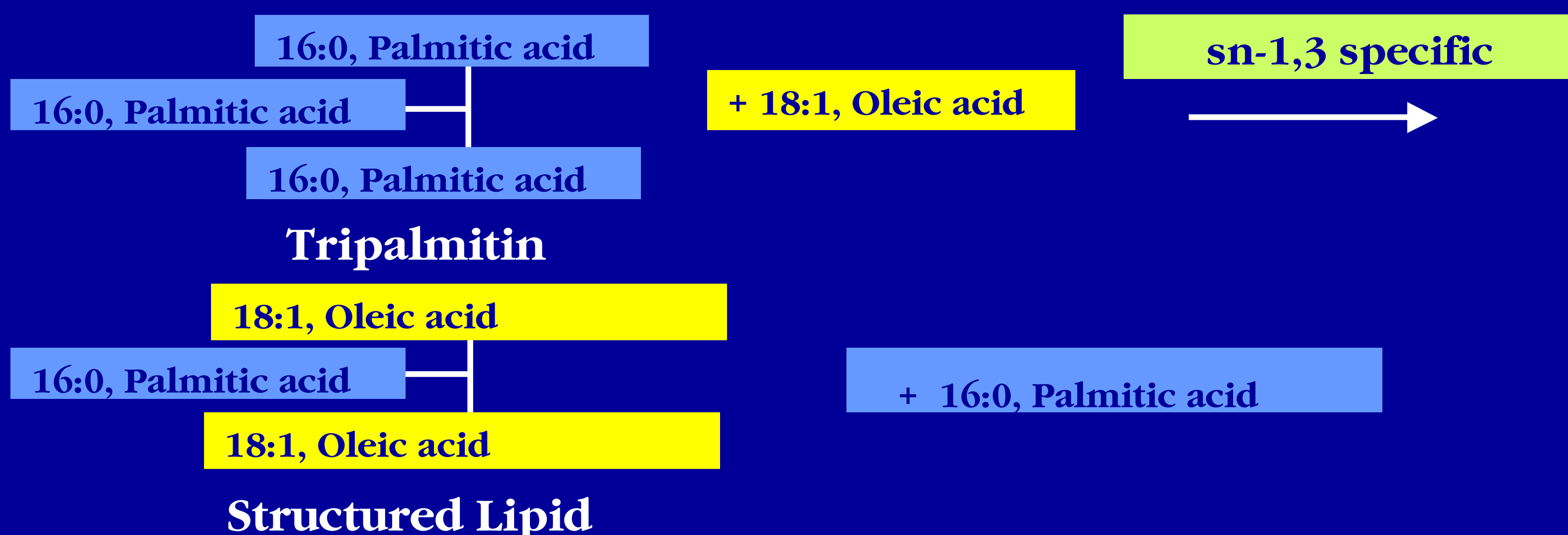



**Figure 1.** Schematic presentation of enzymatic interesterification of tripalmitin with oleic acid using sn-

# Human milk fat substitutes (HMF'S)

**BETAPOL®** is an example for an already commercialised HMF product, developed by Loders Croklaan (Unilever) Company, through lipase-catalyzed reactions, which is being offered to the market for its inclusion in infant formulas.

It is basically a mixture of 1,3-dioleoyl 2-palmitoyl TAG(OPO) and 1,2-dipalmitoyl 3-oleoyl TAG(PPO), quite rich in palmitic acid (30-45%), 60% of which is at the sn-2 position and thus mimics the fatty acid positioning and absorption characteristics

# Table 2 : Selected Research Studies On Production Of HMF'S Using Marine Oils And and omega-3PUFA

| Substrate | Enzyme | Resulting SL | | Researcher |
|---|---|---|---|---|
| Butter | Sn-1,3 specific lipase | 43.52% oleic, 22.50% palmitic, 10.76% linoleic acid, 0.31% EPA and 0.13% DHA | 34.96% palmitic, 31.48% oleic and 11.74 % myrictic acids at the sn-2 position | Christensen, T.C. and Holmer, G., 1993 |
| Tripalmitin, fish oil and oleic acid | Lipozyme® IM | OPO with >72% yield containing 94% palmitic acid in the sn-2 position | 2-MG from fish oil were produced by alcoholysis at 84 %yield and | Schmid et al., 1998 |
| Tripalmitin and omega-3 PUFA | Lipozyme® TL IM | HMFS containing omega-3 polyunsaturated fatty acids (PUFA) | Inc. of omega-3 fatty acids into tripalmitin: 42% | Yang, T.K., Fruekilde, M.B., and Xu, X., 2003 |

# Our Study: Production of HMF TAGS Containing Omega-3 FA

**Research Aims:**

- To synthesize a HMFS enriched with EPA and DHA through enzymatic interesterification reactions,
- To investigate the effects of "substrate molar ratio", "reaction temperature" and "reaction time" on the incorporation of EPA+DHA and oleic acid to this HMFS product during its synthesis,
- To optimize the reaction conditions by response surface methodology (RSM).

***Ref:*** *Şahin, N., Akoh, C.C., Karaali, A., 2006: "Human Milk Fat Substitutes Containing Omega-3 Fatty Acids", Journal of Agricultural and Food Chemistry, 54 (10), 3717-3722.*

# Our Study:
## HMF TAGS containing omega-3 fatty acids

**Materials:**

- Substrate: Tripalmitin, hazelnut oil, and omega-3 f.a. concentrate we obtained from menhaden oil which contained 24.4% EPA and 30.1% DHA,
- Enzyme: Lipozyme® RM IM

**Methods:**

- Preparation of reaction blend:

  a. Omega-3 fatty acids in the menhaden oil were concentrated by 'urea crystallization' using the method developed by Wanasundara and Shahidi, 1999.

  b. A blend of hazelnut oil fatty acids with this menhaden omega-3 fatty acids concentrate was prepared with a ratio of 60:40 (w/w) and was used in the interesterification reactions.
- Response surface methodology (RSM) was used to model and optimize the reaction conditions

# Concentrate obtained from menhaden oil

| Fatty acid | (mol %) |
|---|---|
| C16:0 | 1.5 |
| C16:1n7 | 2.4 |
| C16:2n4 | 3.1 |
| C16:3n4 | 11.2 |
| C18:1n9 | 2.2 |
| C18:2n6 | 1.2 |
| C18:4n3 | 22.6 |
| C20:5n3 (EPA) | 24.4 |
| C22:5n3 | 1.4 |
| C22:6n3 | 30.1 |

# 'Targeted' product: HMFS enriched with Omega-3 Fatty Acids

- The targeted product was aimed to contain EPA+DHA and oleic acid at 5% and 40%, respectively at sn-1,3 positions of TAG
- sn-2 position being occupied by palmitic acid at 70%.

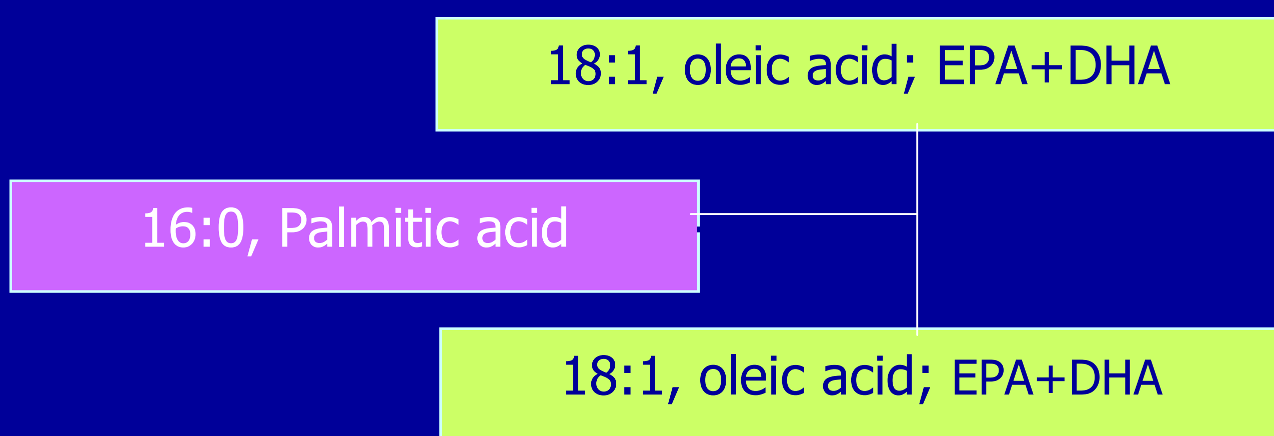



**Figure 2: Targeted HMFS Product Structure**

# Experimental design for RSM study

**Selected Factors and Ranges:**

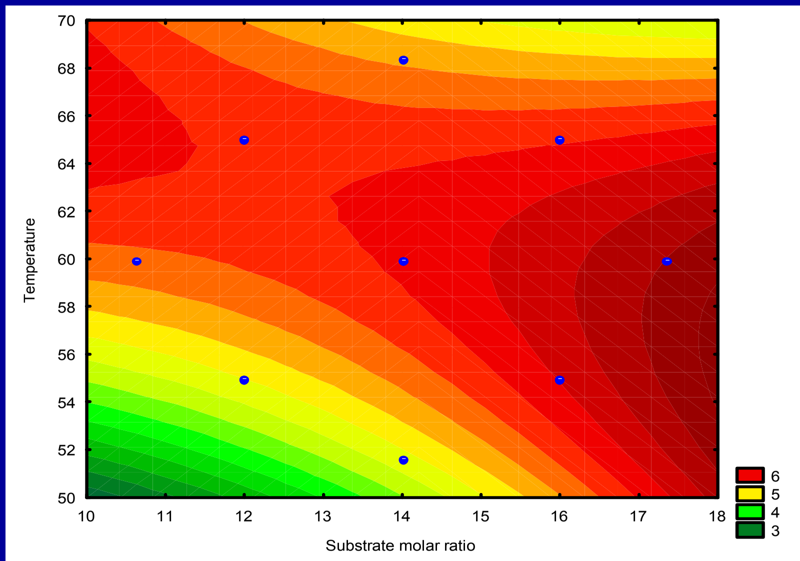
- **Substrate molar ratio ($S_r$, total FA/tripalmitin), [12-16mol/mol],**
- **Reaction temperature ($T_e$, ºC ) [55-65ºC],**
- **Reaction time ($T_i$, hour) [12-24 h]**

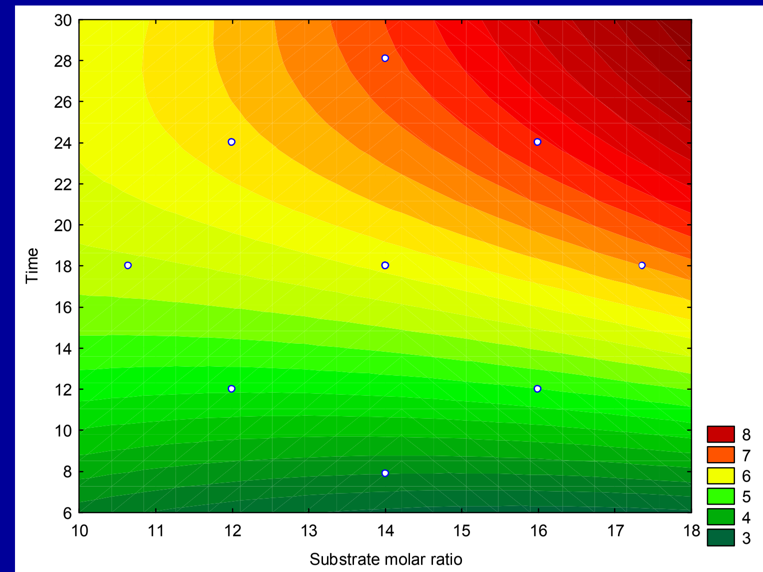
# Table 4. Observed Responses in CCD* Experiments

| | Independent variables | | | Responses | |
|---|---|---|---|---|---|
| No | $S_r$ (mol/mol) | $T_e$ ($^oC$) | $T_i$ (h) | DHA+EPA inc., mol % | Oleic Acid inc., mol % |
| 1 | 12 | 55 | 12 | 3.3 | 31.1 |
| 2 | 16 | 55 | 12 | 4.2 | 33.1 |
| 3 | 12 | 65 | 12 | 4.7 | 34.6 |
| 4 | 16 | 65 | 12 | 4.7 | 36.8 |
| 5 | 12 | 55 | 24 | 5.3 | 36.2 |
| 6 | 16 | 55 | 24 | 7.4 | 41.5 |
| 7 | 12 | 65 | 24 | 6.1 | 38.2 |
| 8 | 16 | 65 | 24 | 6.6 | 41 |
| 9 | 10.64 | 60 | 18 | 5.8 | 34.8 |
| 10 | 17.36 | 60 | 18 | 6.3 | 38.3 |
| 11 | 14 | 51.6 | 18 | 4.9 | 35.9 |
| 12 | 14 | 68.4 | 18 | 5.1 | 39 |
| 13 | 14 | 60 | 7.9 | 3.7 | 31.3 |
| 14 | 14 | 60 | 28.1 | 6.9 | 40 |
| 15 | 14 | 60 | 18 | 5.9 | 38.6 |
| 16 | 14 | 60 | 18 | 5.6 | 37.4 |
| 17 | 14 | 60 | 18 | 5.9 | 37.7 |

*CCD: Central Composite design

# DHA+EPA incorporation



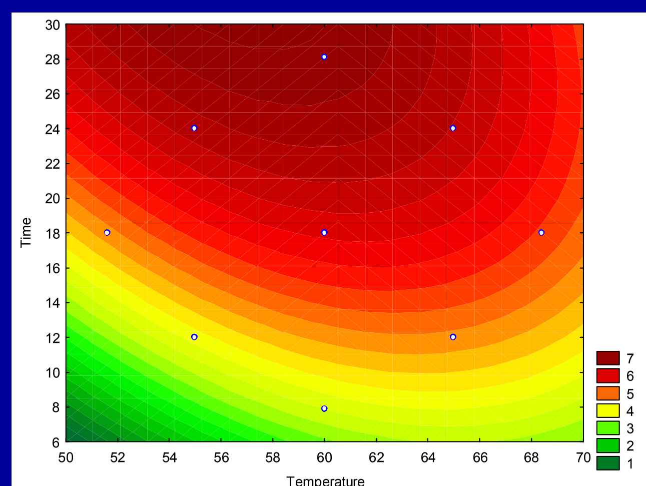




**Figure 3. (A) DHA+EPA inc., $T_e$-$S_r$, (B) DHA+EPA inc., $T_i$-$S_r$, and (C) DHA+EPA inc., $T_e$-$T_i$**

# Optimum conditions

- **The optimal conditions:**

❖ **Sr (Substrate molar ratio): 12.4 mol/mol,**

❖ **Te (Temperature): 55ºC**

❖ **Ti (Time): 24 h**

# Verification of the model

**Enzymatic interesterification reactions were repeated with the optimum conditions obtained by RSM.**

**Table 5. Fatty acid composition of HMFS produced under optimal conditions**

| FA | FA (mol %) | FA at sn-2 (mol %) |
|---|---|---|
| 16:0 | 45.5 | 76.6* |
| 18:1 | 37.5 | 14.9 |
| 18:2 | 4.4 | 2.0 |
| EPA+DHA | 6.2 | < 1.0 |

***The sn-2 position of SL was occupied mostly by palmitic acid(>76%).**

# Conclusions

- **The specific distribution of fatty acids in HMF makes it important and valuable for infant nutrition and growth**

- **Structured lipids which contain palmitic acid predominantly at the sn-2 position, and which are further enriched with DHA, EPA, and other omega-3 PUFA from marine sources can *preferrably* be used in infant formulae to mimic the physical and chemical structure of human milk fat, as well as to provide the health benefits associated with these acids.**

# THANK YOU for YOUR ATTENTION