

# Optimization of the oxidative stability and lipid profile of DHA-enriched infant follow-on formulas by the introduction of dairy lipids

**Mathilde CANCALON, Youna M. HEMERY, Nathalie BAROUH, Bruno BARÉA, Claire BERTON-CARABIN, Lucie BIRAULT, Erwann DURAND, Pierre VILLENEUVE and Claire BOURLIEU-LACANAL**

*mathilde.cancalon@cirad.fr*



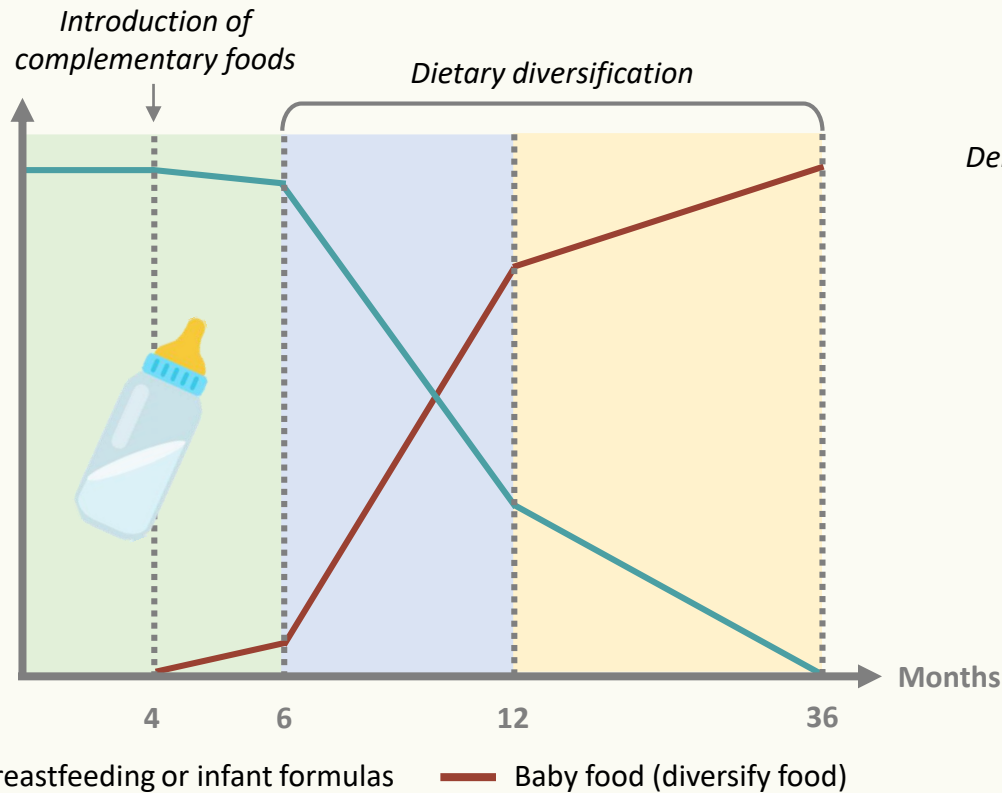
# CONTEXT



Significant growth and development

Specific nutritional requirements

## Development stages



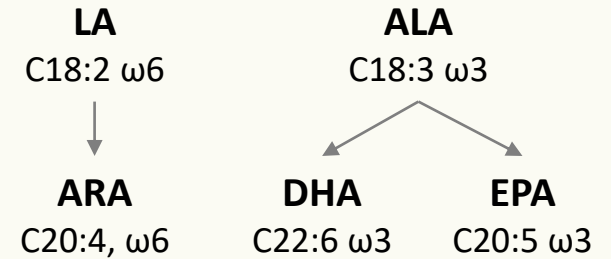
## Specific lipids requirements

### Dominant energy provider

(~45-55% of total energy)

*Delplanque, Gibson et al., J. Pediatr. Gastroenterol. Nutr., 2015*

### Essential fatty acids:



*Innis, Adv Nutr J., 2011*

*Agostoni, J. Pediatr. Gastroenterol. Nutr., 2008*

*WHO, Exclusive breastfeeding for six months best for babies everywhere, 2011*

*Martorell, Am. J. Hum. Bio, 2017*

# CONTEXT

Optimization of  
lipid profile of  
infant products

Specific  
nutritional  
requirements

Significant  
growth and  
development



## New European regulation - European regulation 2016/127/EC

Entry into force in 2020



### Revision of the limits for certain essential fatty acids:

- Increase of the lower limit in LA
- Increase of the upper limit for ALA
- Obligation to supplement formulas with 20 to 50 mg of DHA per 100 kcal (i.e. 0.5 to 1% of total fatty acids)

### What about ARA ?



Still not mandatory *despite its proven importance for child development*

A maximum limit 1% of the total fat content (*maximum of 60 mg per 100 kcal or 42 mg per 100 mL of formula*)

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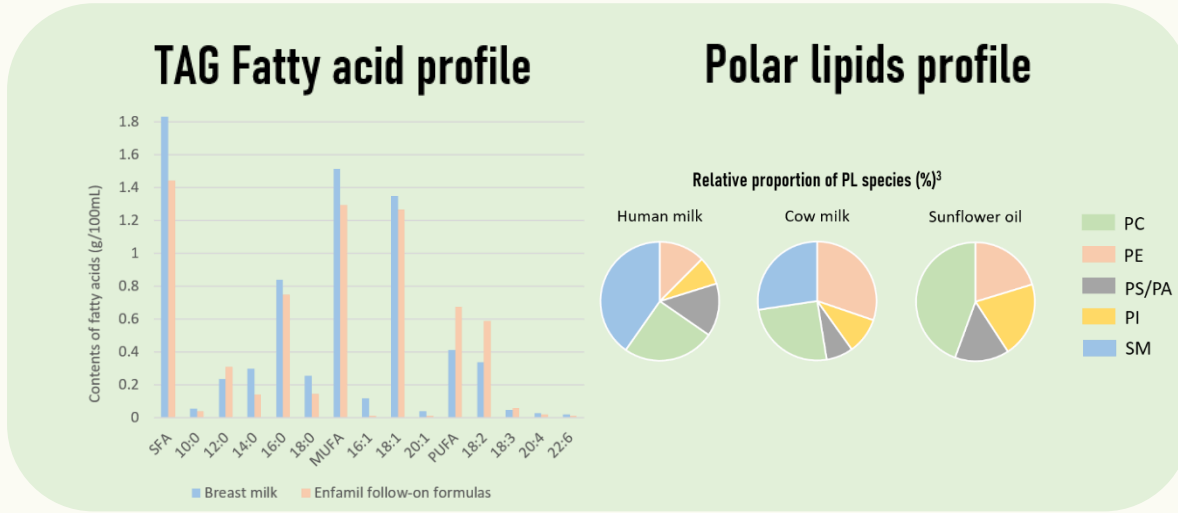
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Increase lipid oxidation sensitivity

# How to limit lipid oxidation in infant formulas playing on lipids ?

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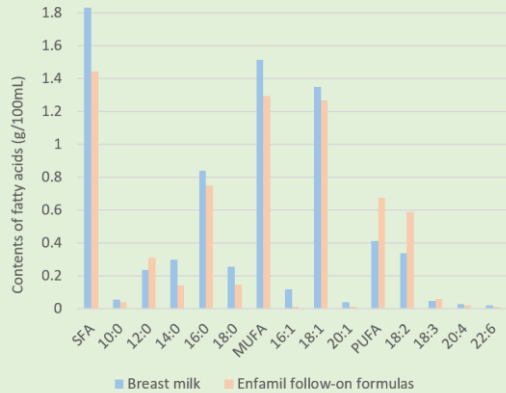


Bourlieu, C., Michalski, M. C., *Curr. Opin. Clin. Nutr. Metab. Care* 2015  
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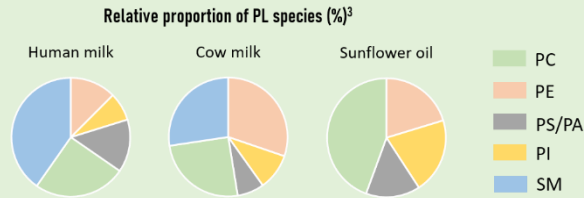


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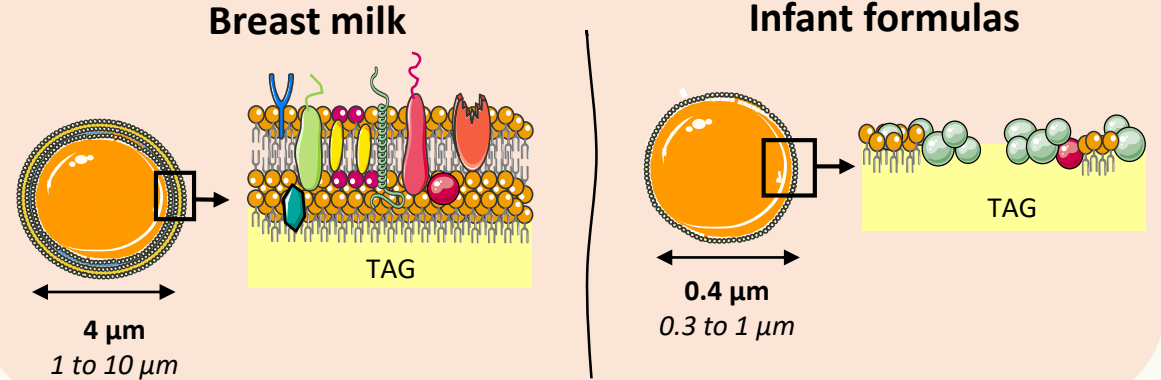
## TAG Fatty acid profile



## Polar lipids profile

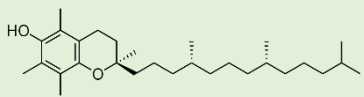


## Lipids structure

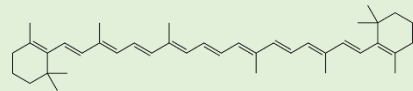


## Antioxidant composition, type and synergic effect

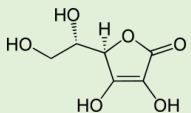
### Tocopherols



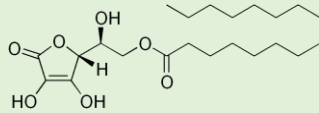
### Carotenoids



### Vitamin C



### L-ascorbyl palmitate



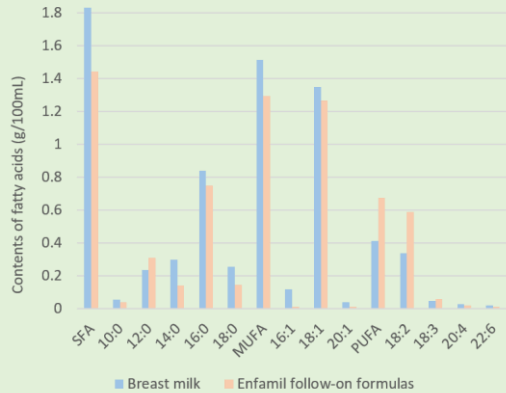
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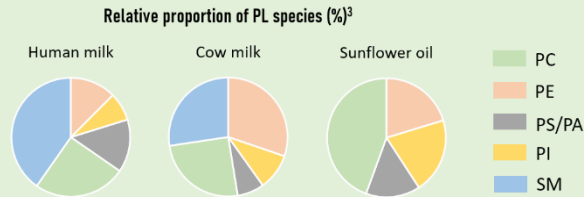


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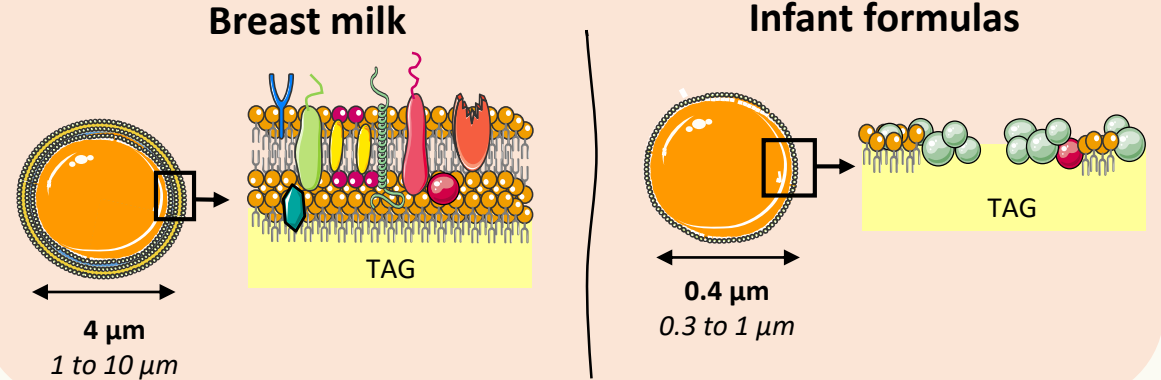
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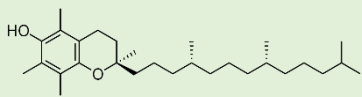


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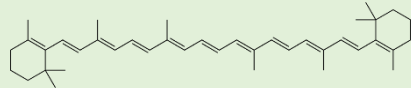


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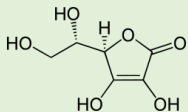
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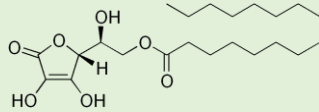
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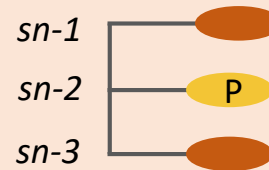
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And their combination ...

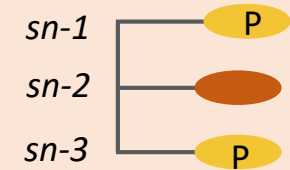
## Fatty acid redistribution and TAG structure

### Breast milk



About 70% of C16:0 is internally esterified (*sn-2*)

### Infant formulas

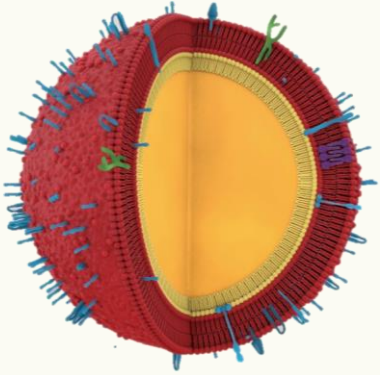


C16:0 esterified mainly at external positions (*sn-1,3*)

Bourlieu, C., Michalski, M. C., *Curr. Opin. Clin. Nutr. Metab. Care* 2015  
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# INTEREST OF DAIRY LIPIDS

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**Milk fat globule**

*Mead Johnson Nutrition*

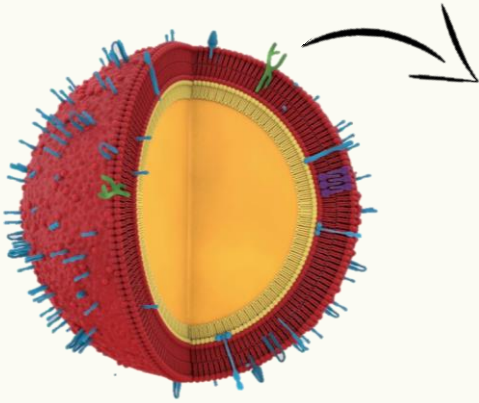
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- Biomimetic lipid structure of breast milk

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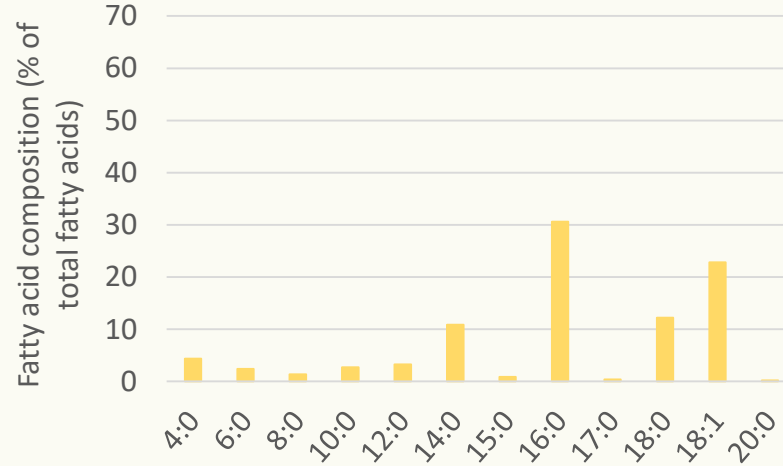


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## Fatty acid profile of bovine milk fat

*Lindmark Mansson et al. 2008*



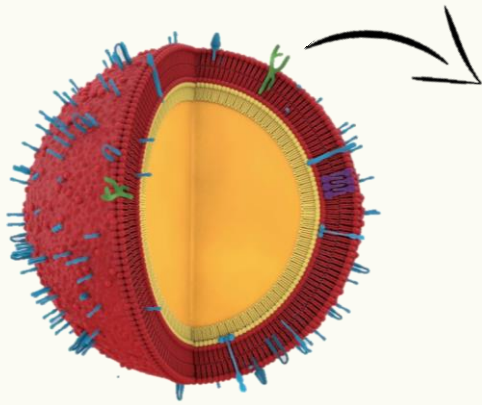
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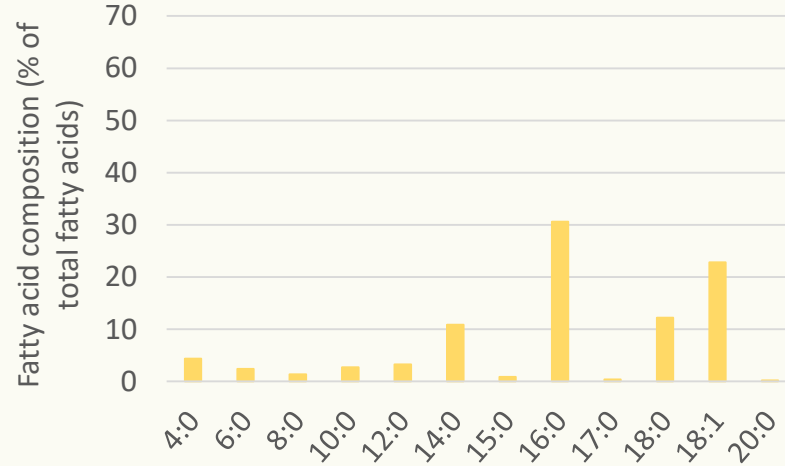


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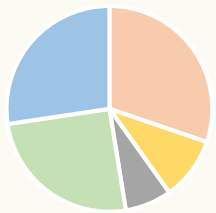
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## Relative proportion of PL species (%)

*Changhoon et al., Foos Sci Anim Resour, 2022*

Bovine milk



- PC
- PE
- PS/PA
- PI
- SM

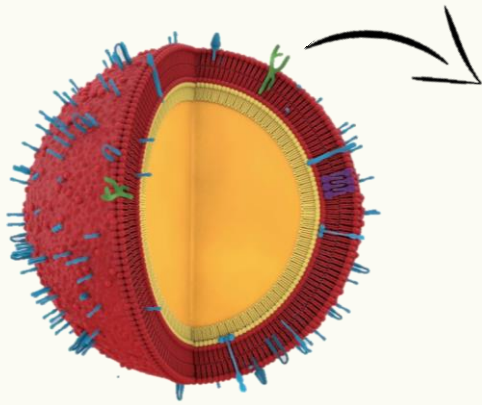
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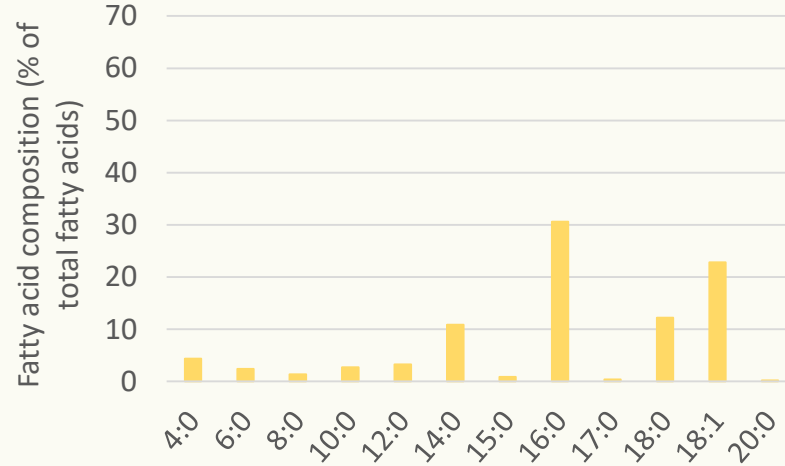


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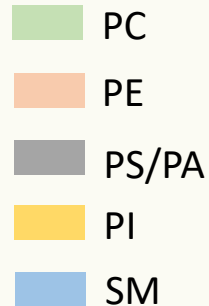
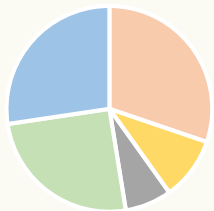
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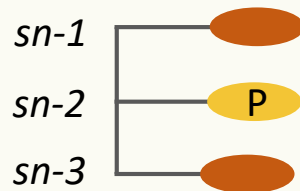
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## Fatty acids regiodistribution



Palmitic acid (C16:0) mainly internally esterified (*sn*-2)

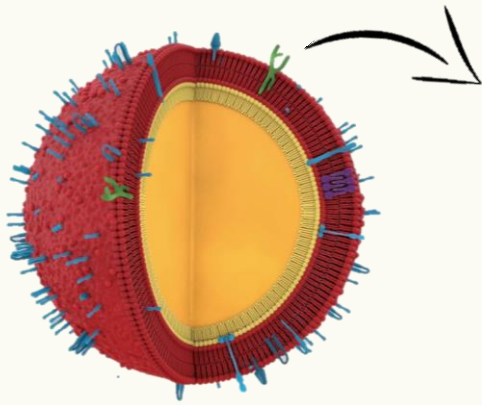
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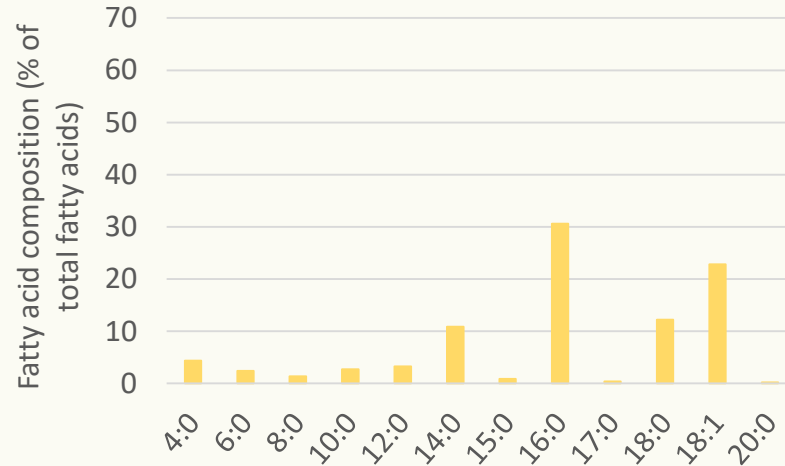


Milk fat globule

Mead Johnson Nutrition

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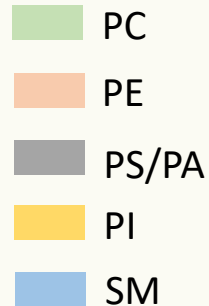
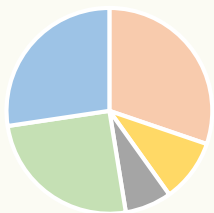
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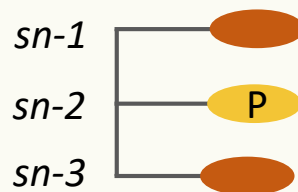
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- Improves lipid digestion and metabolism
- Protection effect against lipid oxidation

Innis, Adv Nutr, 2011

Bourlieu et al., Food chem, 2018

# AIM OF THE STUDY



**Structure and formulation  
optimization of DHA-enriched  
IFF in order to improve their  
lipid profile and oxidative  
stability**

*Canalon et al. [Under Review] 2023*



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## EXPERIMENTAL STRATEGY

1.

Setting an overview of marketed follow-on formulas

2.

Formulation of model systems representative of marketed follow-on formulas

*Canalon et al. [Under Review] 2023*

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**Comparison of oxidation stability of model systems**  
*Fatty acids profile: iso SFA, MUFA, PUFA,  $\omega 6$ ,  $\omega 3$*   
*Vitamins: normalization of vitamins A & E contents*

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### Formulation and structure levers

Substitution of palm oil by other saturated fats  
*Red palm oil, coconut oil or dairy fat*



Canalon et al. [Under Review] 2023

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Substitution of palm oil by other saturated fats  
*Red palm oil, coconut oil or dairy fat*



Substitution of soy lecithin by dairy polar lipids



*Canalon et al. [Under Review] 2023*

# RESULTS – 1. Overview of infant formulas

## Overview of follow-on formulas (N=91)

### Selection criteria

- Type of products - *follow-on formulas only*
- « Classic » formulas- *no hypoallergenic, AR formulas, etc.*
- Widely consumed products

→ 91 follow-on formulas from 16 brands



<sup>1</sup>Average values extracted from the follow-on formulas overview

<sup>2</sup>Mäkelä, Linderborg et al., Eur. J. Nutr, 2013

<sup>3</sup>Commission Delegated Regulation (EU) 2016/127, September 25, 2015

For 100 mL of preparation

	Overview <sup>1</sup>	Breast milk <sup>2</sup>	EU Regulation <sup>3</sup>	
			Min	Max
<b>Energy (kcal)</b>	67.2 ± 1.3	67	60	70
<b>Fat (g)</b>	3.3 ± 0.2	3.5	2.64	4.2
SFA (g)	1.1 ± 0.4	1.65	-	-
MUFA (g)	1.5 ± 0.4	1.54	-	-
PUFA (g)	0.6 ± 0.1	0.35	-	-
LA (mg)	502.2 ± 0.1	310	300	840
ALA (mg)	53.7 ± 12.6	28	30	70
ARA (mg)	9.4 ± 5.6	18	-	42
DHA (mg)	14.8 ± 4.0	7 to 42	12	35
<b>Carbohydrates (g)</b>	7.8 ± 0.4	7.5	5.4	9.8
<b>Proteins (g)</b>	1.4 ± 0.2	1	1.08	1.75
Caseins (g)	0.8 ± 0.3	0.4	-	-
<b>Vitamin A (µg)</b>	61.5 ± 6.4	-	42	79.8
<b>Vitamin D (µg)</b>	1.4 ± 0.3	-	1.2	2.1
<b>Vitamin E (mg)</b>	1.4 ± 0.4	-	0.36	3.5
<b>Vitamin C (mg)</b>	9.7 ± 2.3	-	2.4	21
<b>Iron (mg)</b>	0.976 ± 0.155	0.05	0.36	1.4
<b>Copper (mg)</b>	0.052 ± 0.006	-	0.036	0.070

Data Paper : Cancalon et al. [Under Review] 2023

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### Ingredient list analysis

14%

Oil phase  
includes dairy  
lipids

70%

Soy lecithin as  
emulsifier

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# RESULTS – 2. Model systems formulation

## Objective

<b>SFA (g)</b>	33.3
<b>MUFA (g)</b>	45.5
<b>PUFA (g)</b>	18.2
<b>LA (g)</b>	15.2
<b>ALA (g)</b>	1.6
<b>ARA (mg)</b>	284.8
<b>DHA (mg)</b>	448.5

# RESULTS – 2. Model systems formulation

Objective		Palm oil based oil mixture
SFA (g)	33.3	33.3
MUFA (g)	45.5	48.5
PUFA (g)	18.2	18.2
LA (g)	15.2	15.2
ALA (g)	1.6	1.6
ARA (mg)	284.8	289.2
DHA (mg)	448.5	468.4



## Palm oil based oil mixture

*Reference system*

- 67.8% **Palm oil**
- 9.1% Sunflower oil
- 3.1% Oleic sunflower oil
- 18.4% Rapeseed oil
- 1.0% DHA enriched oil
- 0.6% ARA enriched oil



# RESULTS – 2. Model systems formulation

Objective		Palm oil based oil mixture	Red palm oil based oil mixture
SFA (g)	33.3	33.3	33.3
MUFA (g)	45.5	48.5	48.5
PUFA (g)	18.2	18.2	18.2
LA (g)	15.2	15.2	15.2
ALA (g)	1.6	1.6	1.6
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## Red palm oil based oil mixture

- 71.0% **Red palm oil**
- 12.0% Sunflower oil
- 15.4% Rapeseed oil
- 1.0% DHA enriched oil
- 0.6% ARA enriched oil

## Declination of reference system depending of the saturated fat source



Palm oil or red palm oil

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SFA (g)	33.3	33.3	33.3	33.3
MUFA (g)	45.5	48.5	48.5	48.5
PUFA (g)	18.2	18.2	18.2	18.2
LA (g)	15.2	15.2	15.2	15.2
ALA (g)	1.6	1.6	1.6	1.6
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- 1.0% DHA enriched oil
- 0.6% ARA enriched oil

## Coconut oil based oil mixture

- 30.6% **Coconut oil**
- 14.4% Sunflower oil
- 34.9% Oleic sunflower oil
- 18.6% Rapeseed oil
- 1.0% DHA enriched oil
- 0.6% ARA enriched oil

## Declination of reference system depending of the saturated fat source



Palm oil or red palm oil



Coconut oil

# RESULTS – 2. Model systems formulation

Objective		Palm oil based oil mixture	Red palm oil based oil mixture	Coconut oil based oil mixture	Dairy fat based oil mixture
SFA (g)	33.3	33.3	33.3	33.3	33.3
MUFA (g)	45.5	48.5	48.5	48.5	48.5
PUFA (g)	18.2	18.2	18.2	18.2	18.2
LA (g)	15.2	15.2	15.2	15.2	15.2
ALA (g)	1.6	1.6	1.6	1.6	1.6
ARA (mg)	284.8	289.2	289.2	289.2	289.2
DHA (mg)	448.5	468.4	468.4	468.4	468.4

## Declination of reference system depending of the saturated fat source



Palm oil or red palm oil



Coconut oil



Olein of dairy fat



### Palm oil based oil mixture

*Reference system*

- 67.8% **Palm oil**
- 9.1% Sunflower oil
- 3.1% Oleic sunflower oil
- 18.4% Rapeseed oil
- 1.0% DHA enriched oil
- 0.6% ARA enriched oil

### Red palm oil based oil mixture

- 71.0% **Red palm oil**
- 12.0% Sunflower oil
- 15.4% Rapeseed oil
- 1.0% DHA enriched oil
- 0.6% ARA enriched oil

### Coconut oil based oil mixture

- 30.6% **Coconut oil**
- 14.4% Sunflower oil
- 34.9% Oleic sunflower oil
- 18.6% Rapeseed oil
- 1.0% DHA enriched oil
- 0.6% ARA enriched oil

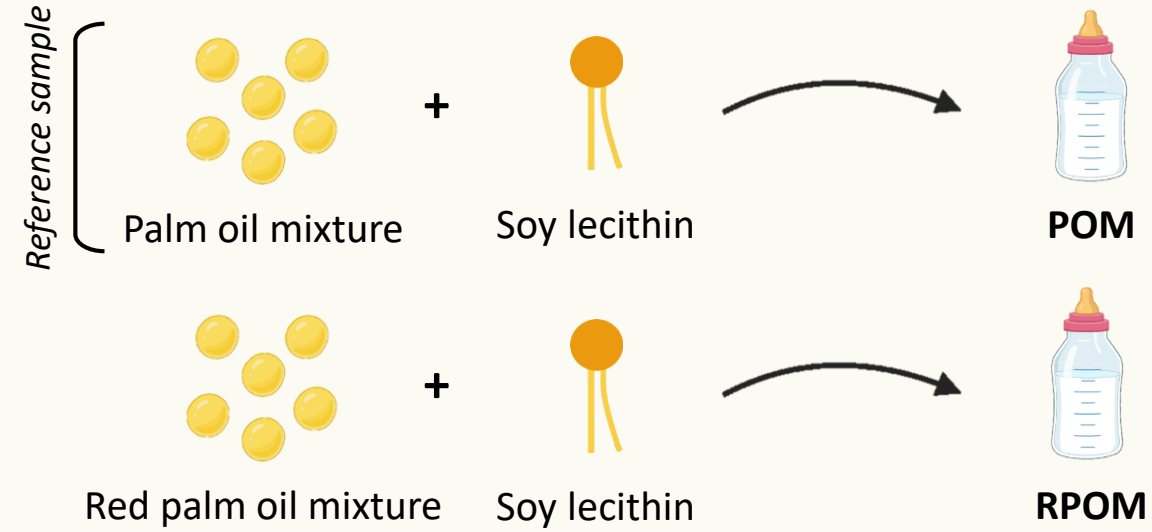
### Dairy fat based oil mixture

- 55.3% **Dairy fat**
- 19.4% Sunflower oil
- 11.1% Oleic sunflower oil
- 12.6% Rapeseed oil
- 1.0% DHA enriched oil
- 0.6% ARA enriched oil

# 3. Challenge test

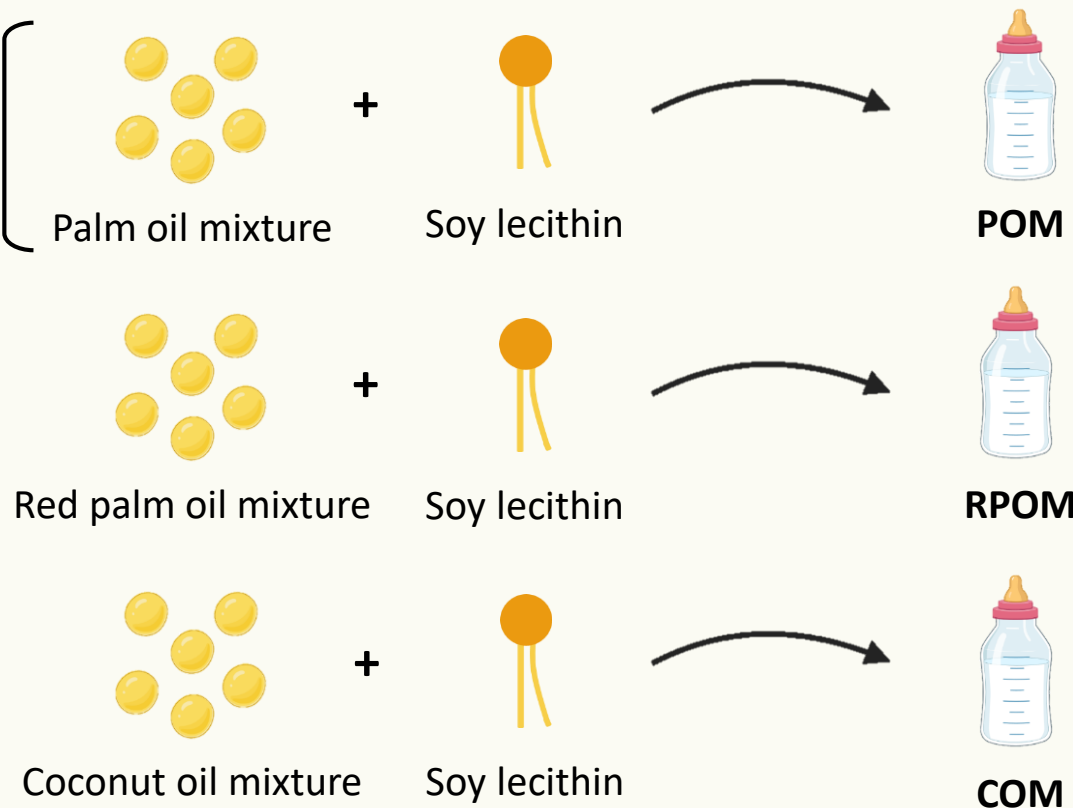


# 3. Challenge test



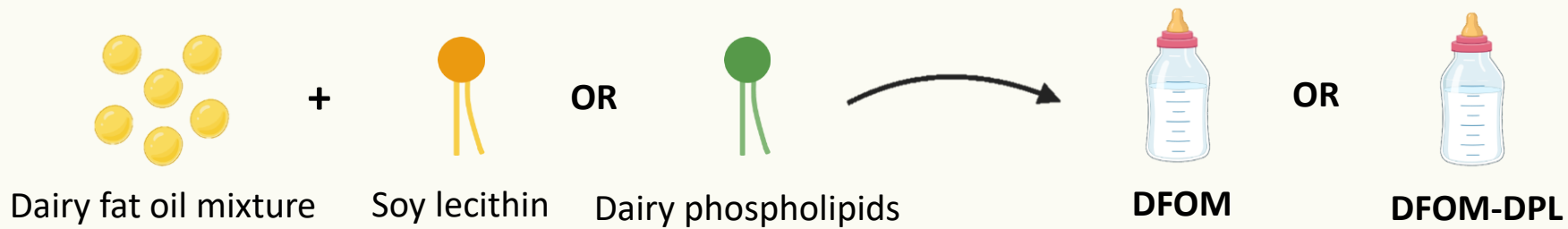
# 3. Challenge test

Reference sample



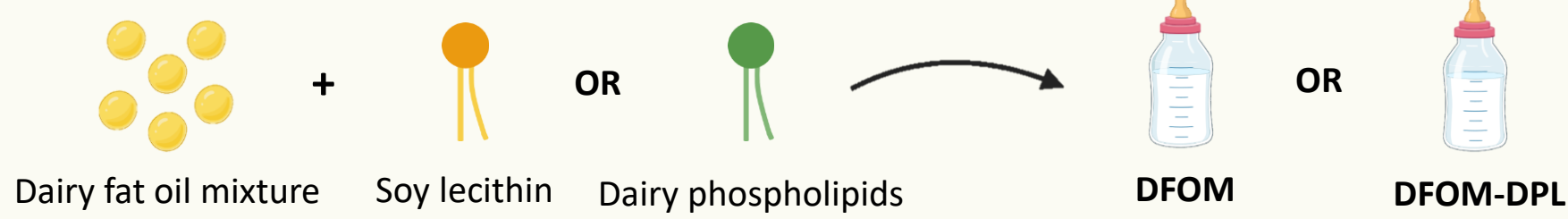
# 3. Challenge test

Reference sample

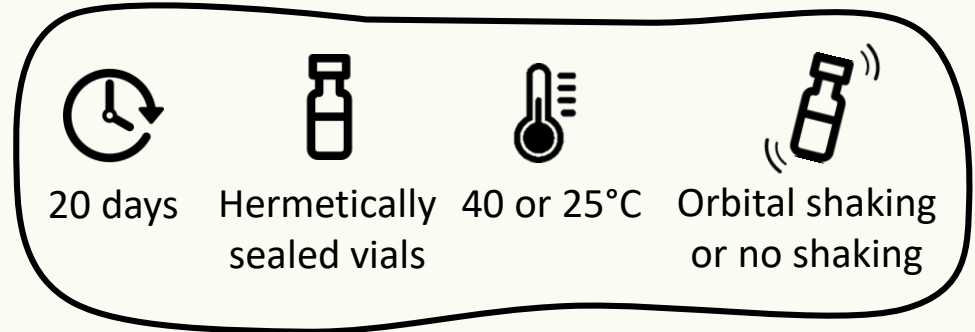


# 3. Challenge test

Reference sample

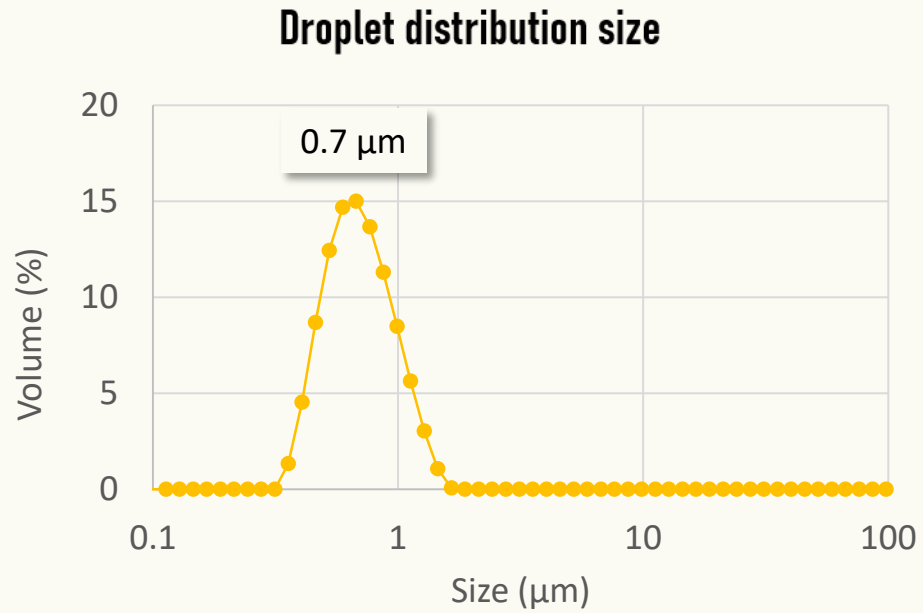


- Primary oxidation compounds - PV
- Secondary oxidation compounds - Tbars
- Vitamins A & E degradation
- Fatty acid profile evolution





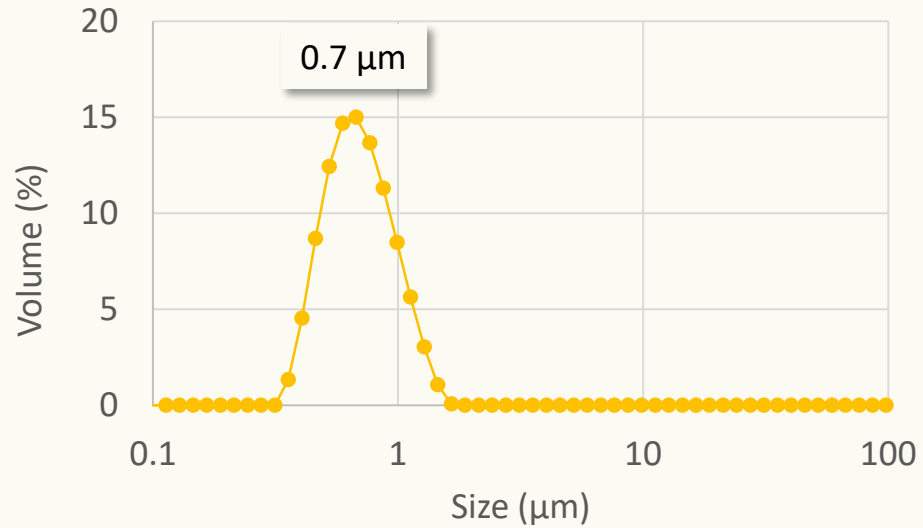
# RESULTS – 3. Challenge test - Physical state



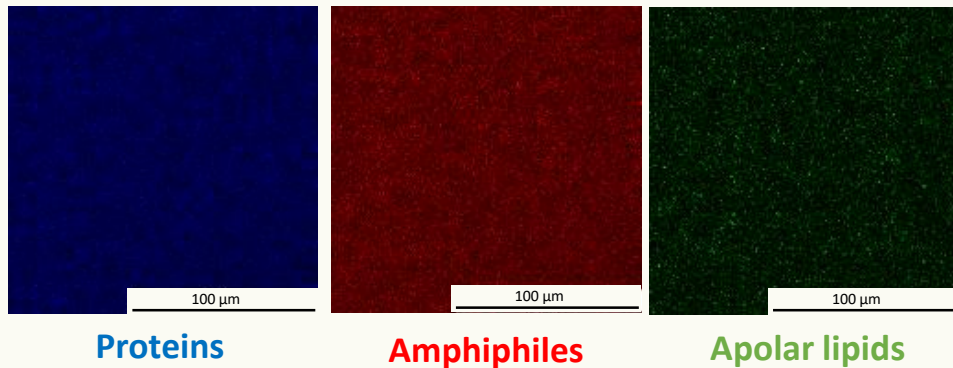
Monomodal droplet size distribution centered on 0.7 µm

# RESULTS – 3. Challenge test - Physical state

### Droplet distribution size



### Confocal microscopy

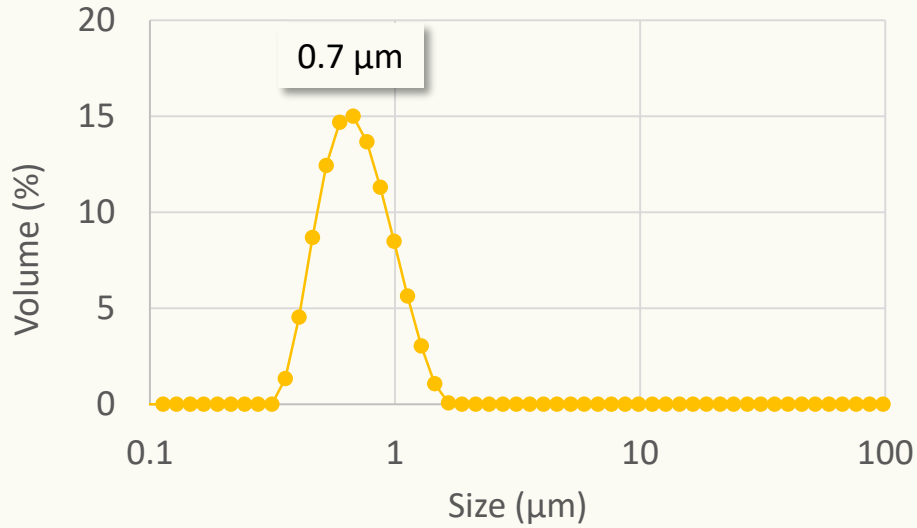


Homogeneity of structure of all the model IFF with core of apolar lipids stabilized by proteins and amphiphilic compounds

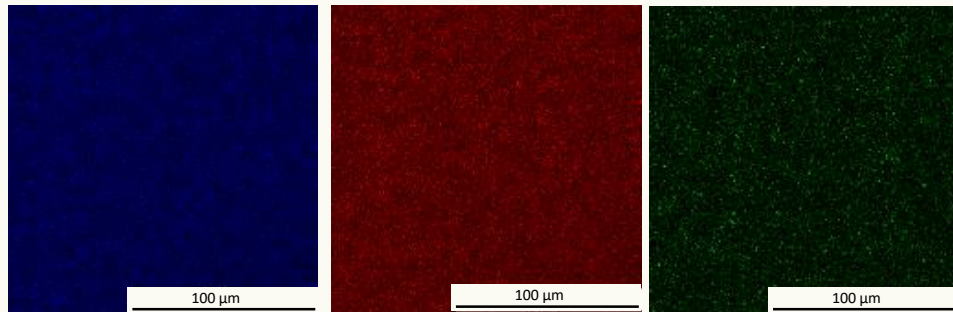
# RESULTS – 3. Challenge test - Physical state

## Crystallization behavior

### Droplet distribution size



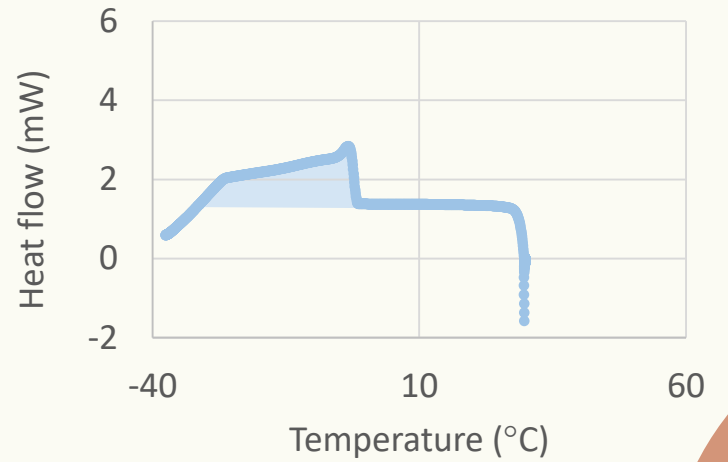
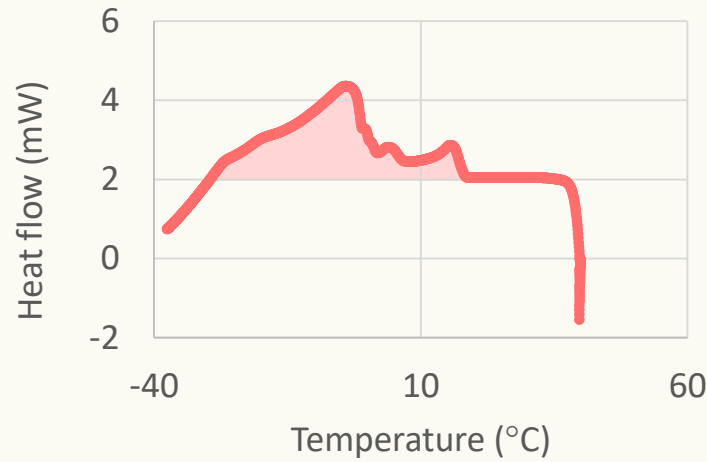
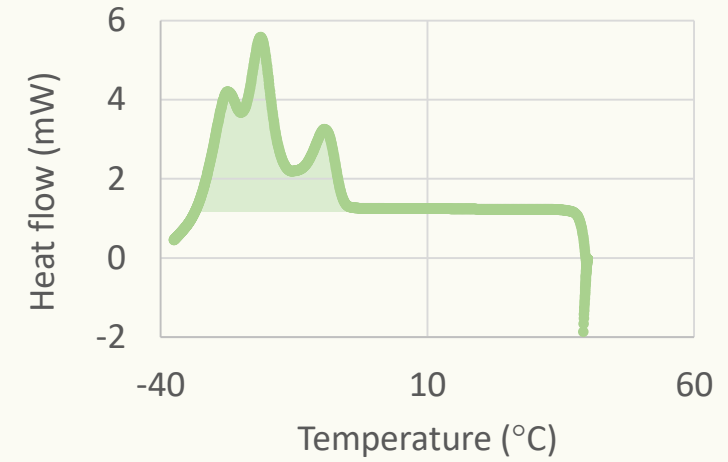
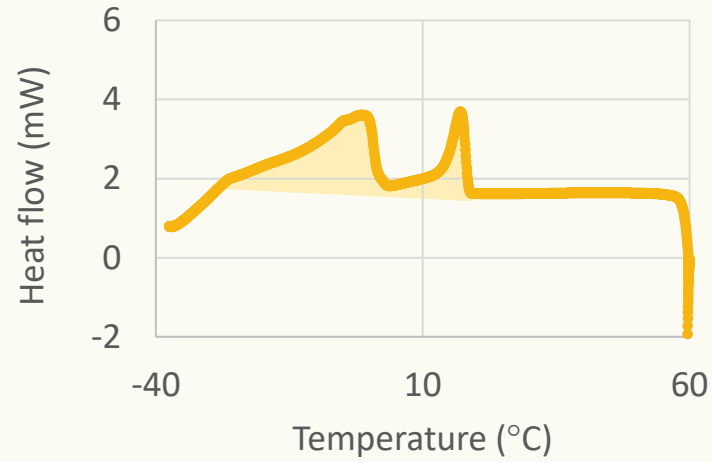
### Confocal microscopy



Proteins

Amphiphiles

Apolar lipids



Depending on the used SFA source the thermogram profiles varied strongly

# RESULTS – 3. Challenge test - Indicators of oxidation

## Statistical analysis of dataset

*Using Rstudio software*

Low oxidation level in the presence of AOX despite the addition of DHA and ARA

No significant evolution of the fatty acid profile and secondary oxidation compounds

Vitamin A undergoes a strong degradation according to complex mechanisms  
*No strong correlation with PV and tocopherols evolutions*

*30 to 75%  
degradation*



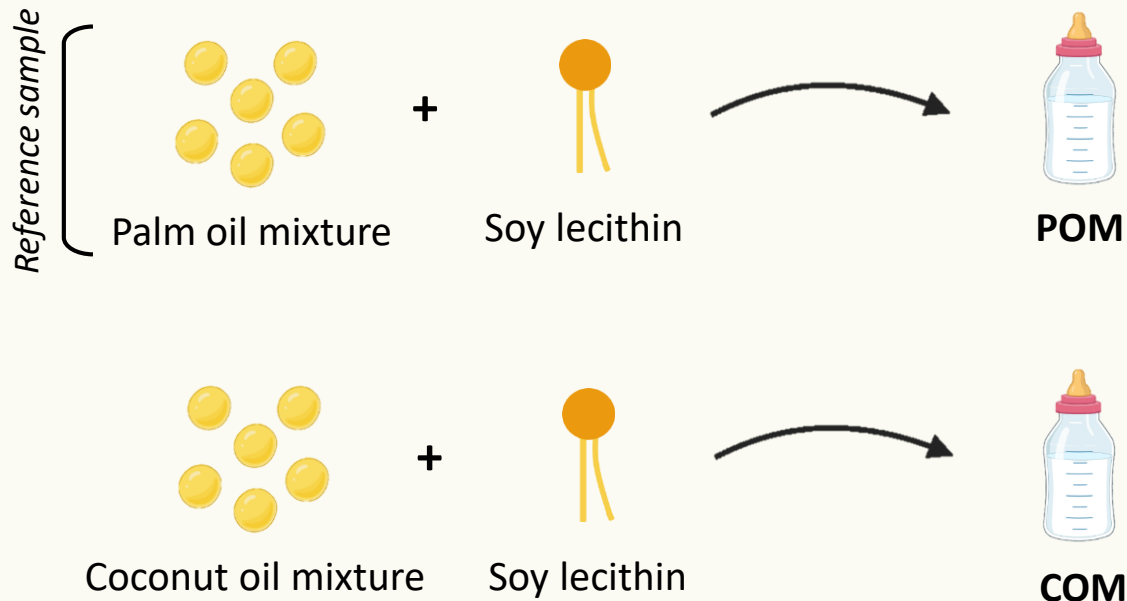
## Descriptive indicators

- **Peroxide value evolution** - *Primary oxidation compounds*
- **Tocopherols degradation**

*Jiang et al., LWT, 2021*

# RESULTS – 3. Challenge test - SFA sources modulates the oxidation kinetics

## Comparison of palm and coconut oils



### Nutritional benefits

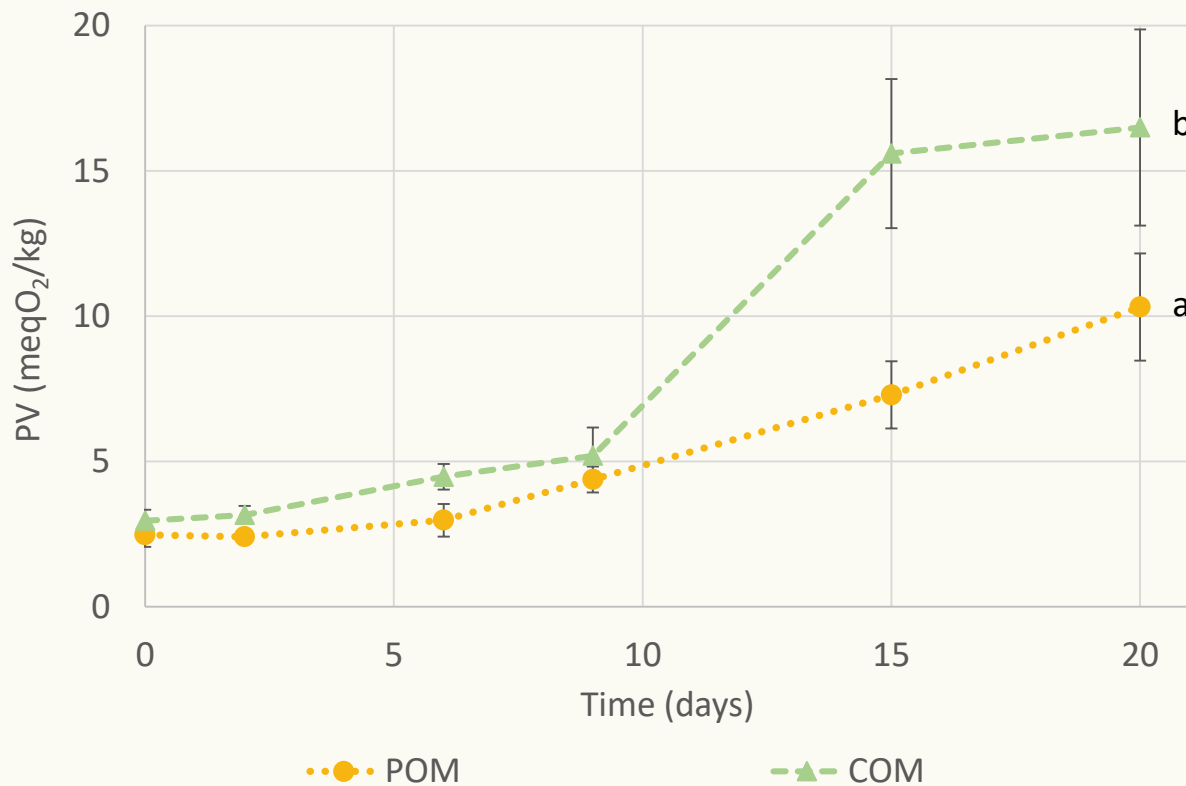
**Provides medium chain fatty acids (C12:0)**

*Improves lipid digestion and metabolism*

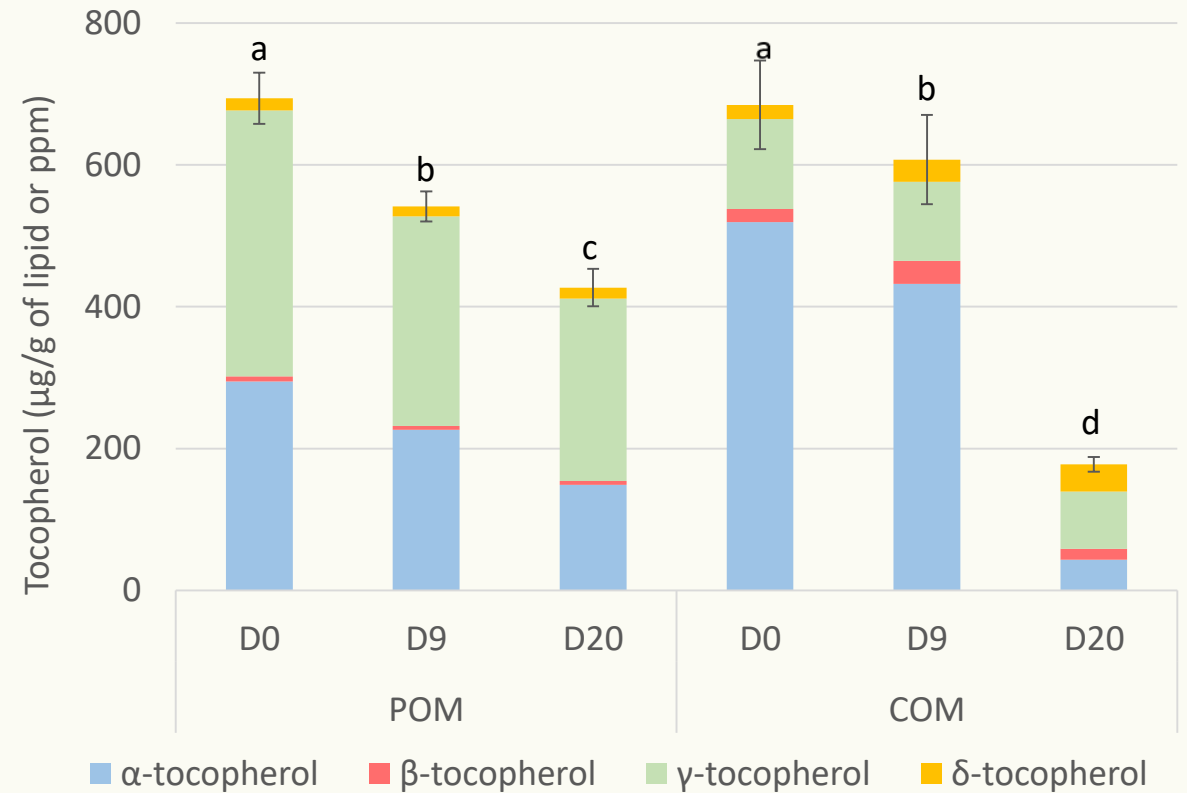
# RESULTS – 3. Challenge test - SFA sources modulates the oxidation kinetics

## Comparison of palm and coconut oils

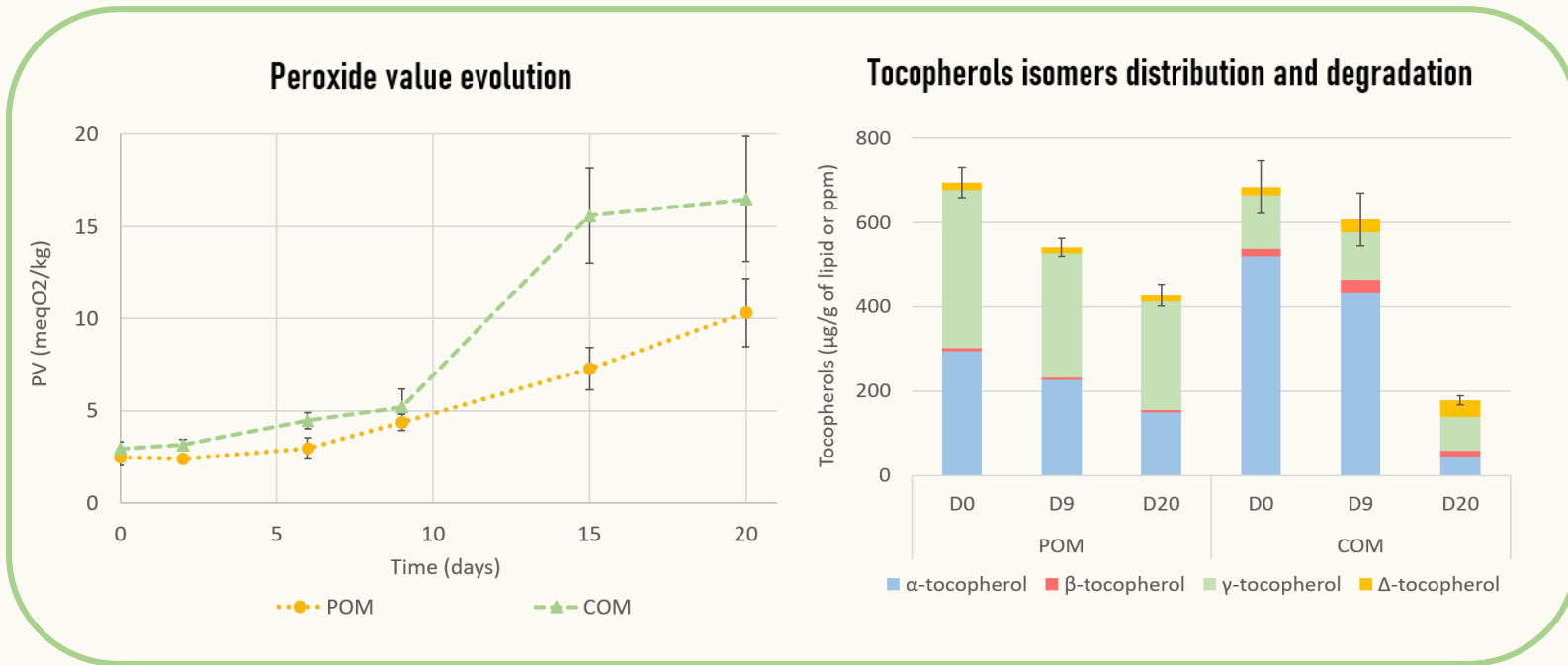
### Peroxide value evolution



### Tocopherols isomers distribution and degradation



# RESULTS – 3. Challenge test - SFA sources modulates the oxidation kinetics

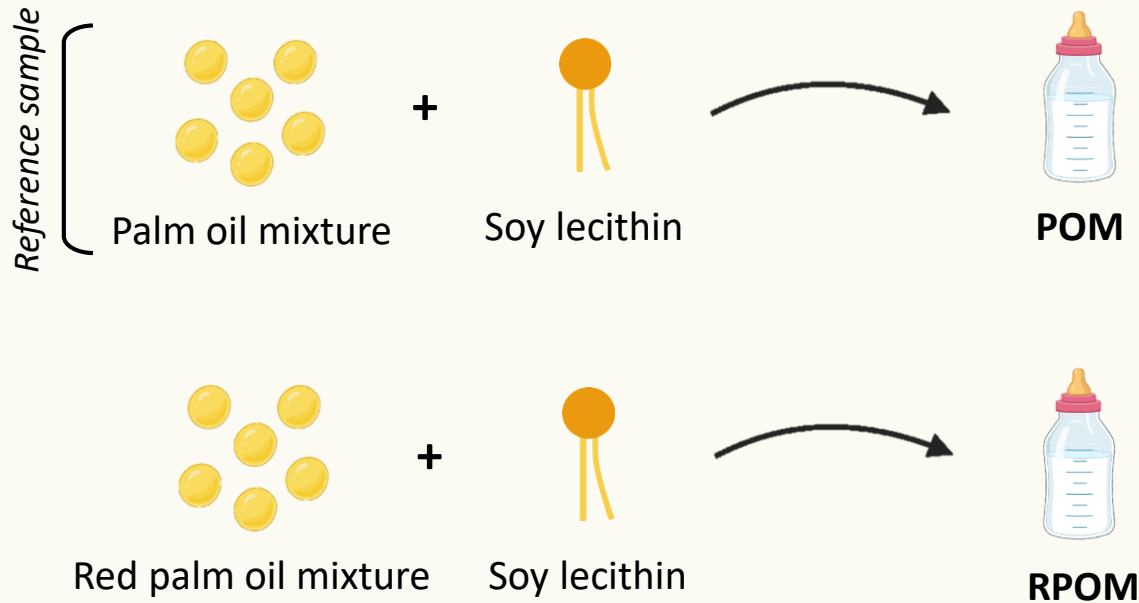


## Comparison of palm and coconut oils

- Coconut oil is more adapted to the infant's nutritional needs
- The substitution of palm oil by coconut oil increases the oxidative sensitivity
- Tocopherols act as sacrificial agents – Isomer content impact the oxidation kinetics

# RESULTS – 3. Challenge test - SFA sources modulates the oxidation kinetics

## Comparison of palm and red palm oils



### Nutritional benefits

**High amount of provitamin A carotenoids ( $\alpha$ - and  $\beta$ -carotenoids) and tocotrienols**

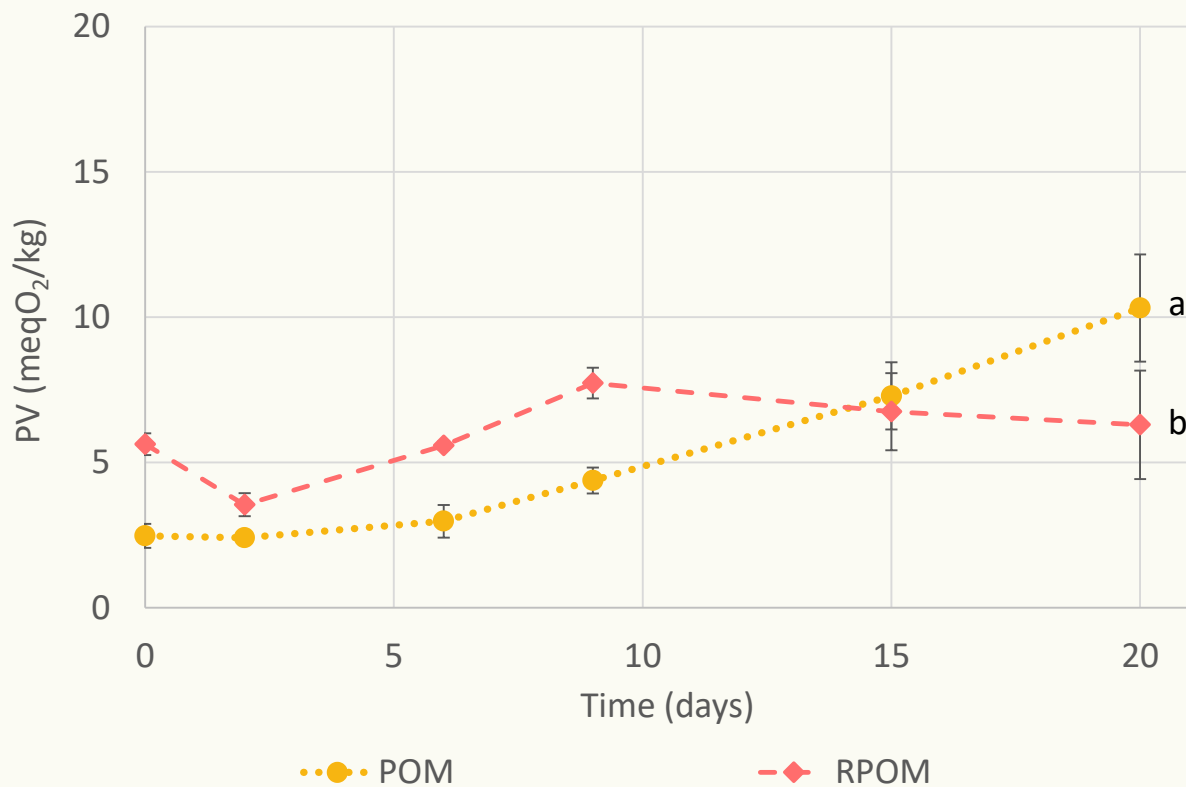
*Effective strategy to fight against vitamin A deficiency disease especially for infant and lactating women in developing countries*



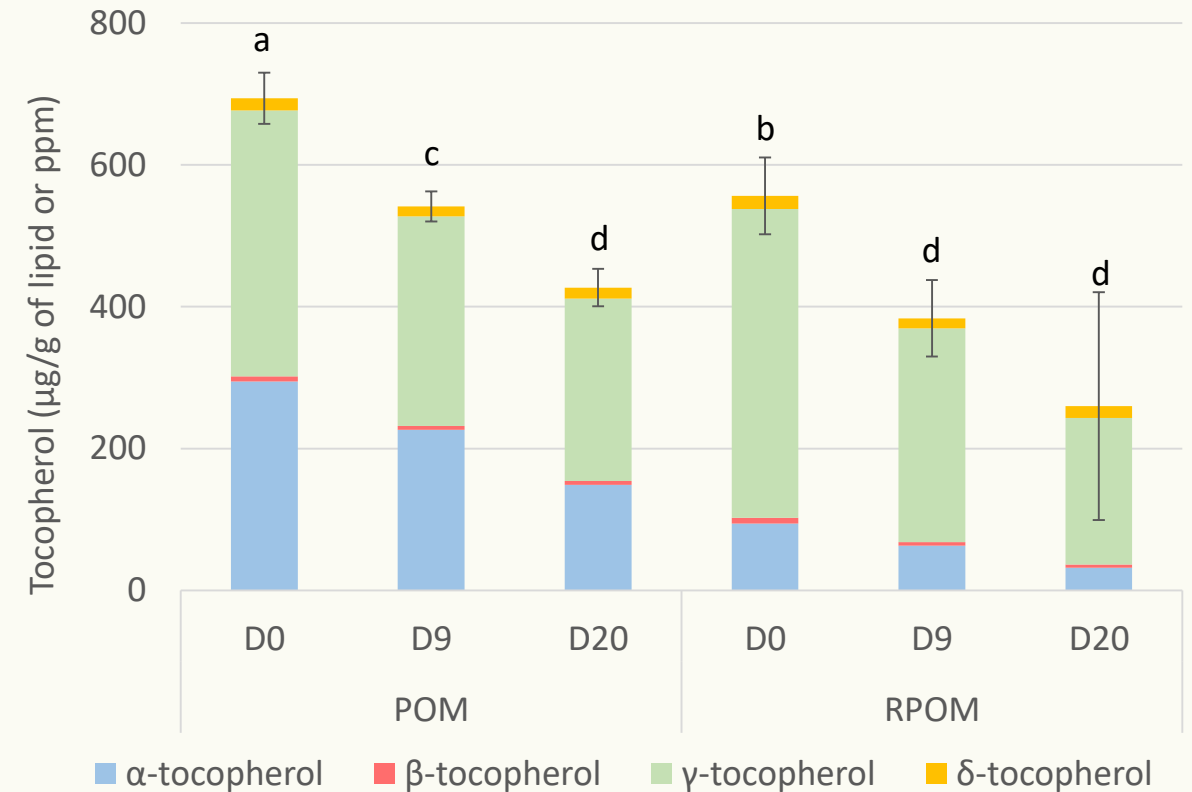
# RESULTS – 3. Challenge test - SFA sources modulates the oxidation kinetics

## Comparison of palm and red palm oils

Peroxide value evolution

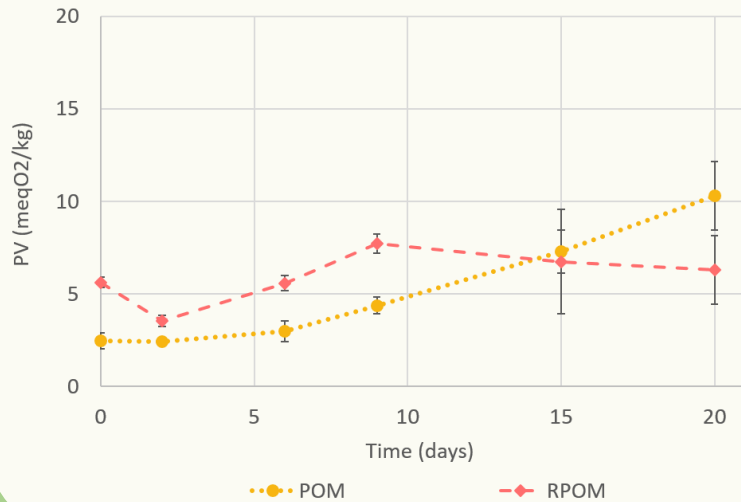


Tocopherols isomers distribution and degradation

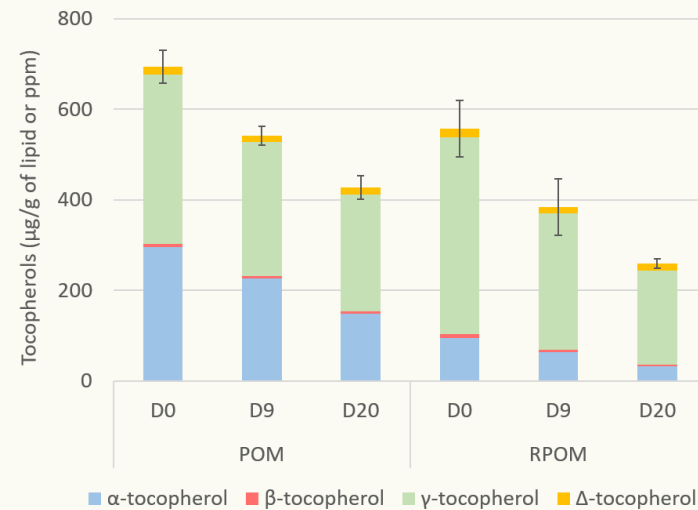


# RESULTS – 3. Challenge test - SFA sources modulates the oxidation kinetics

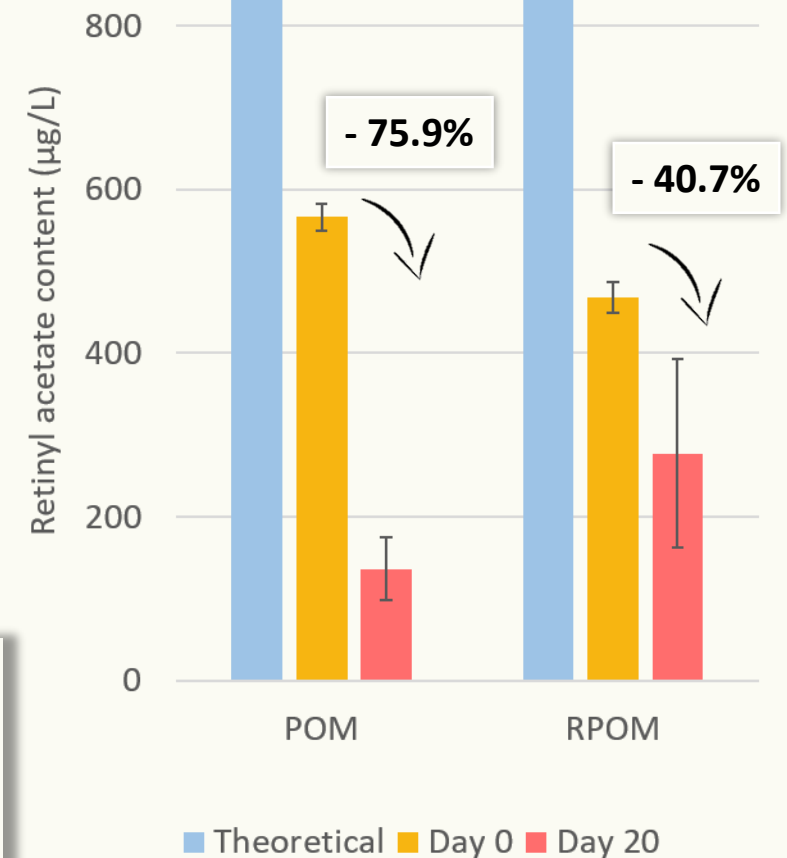
Peroxide value evolution



Tocopherols isomers distribution and degradation



Retinyl acetate degradation



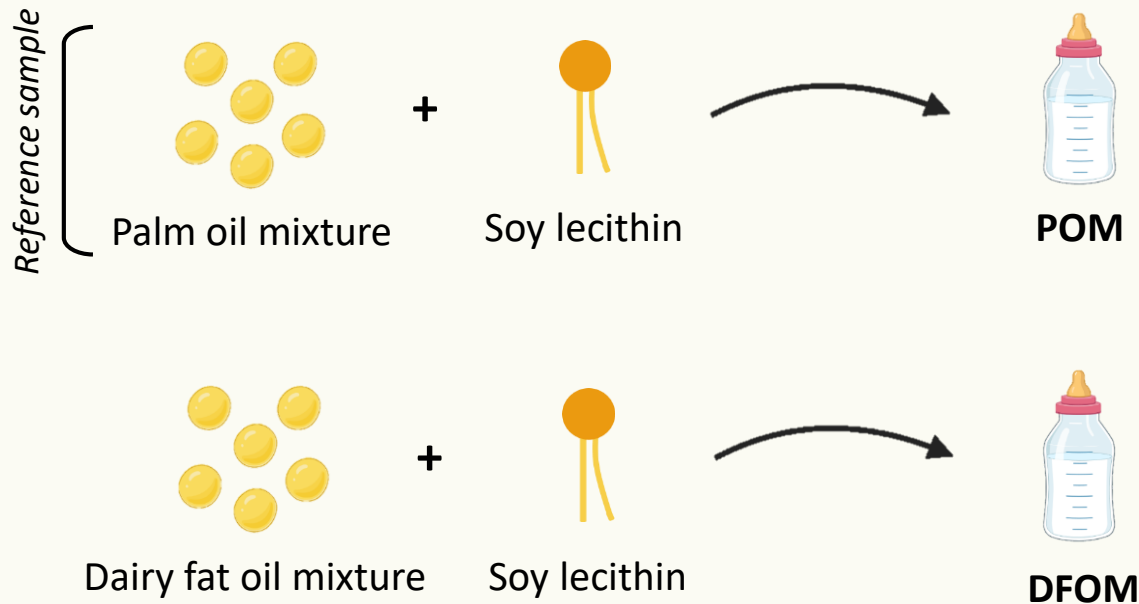
## Comparison of palm and red palm oils

- The substitution of palm oil by red palm oil improve the oxidative resistance  
*Due to its carotenoids and tocotrienols content*

*Regeneration of tocopherols*

# RESULTS – 3. Challenge test - SFA sources modulates the oxidation kinetics

## Comparison of palm oil and dairy fat (olein fraction)



### Nutritional benefits

#### More diversified fatty acid profile

*Provides short and medium saturated chains*

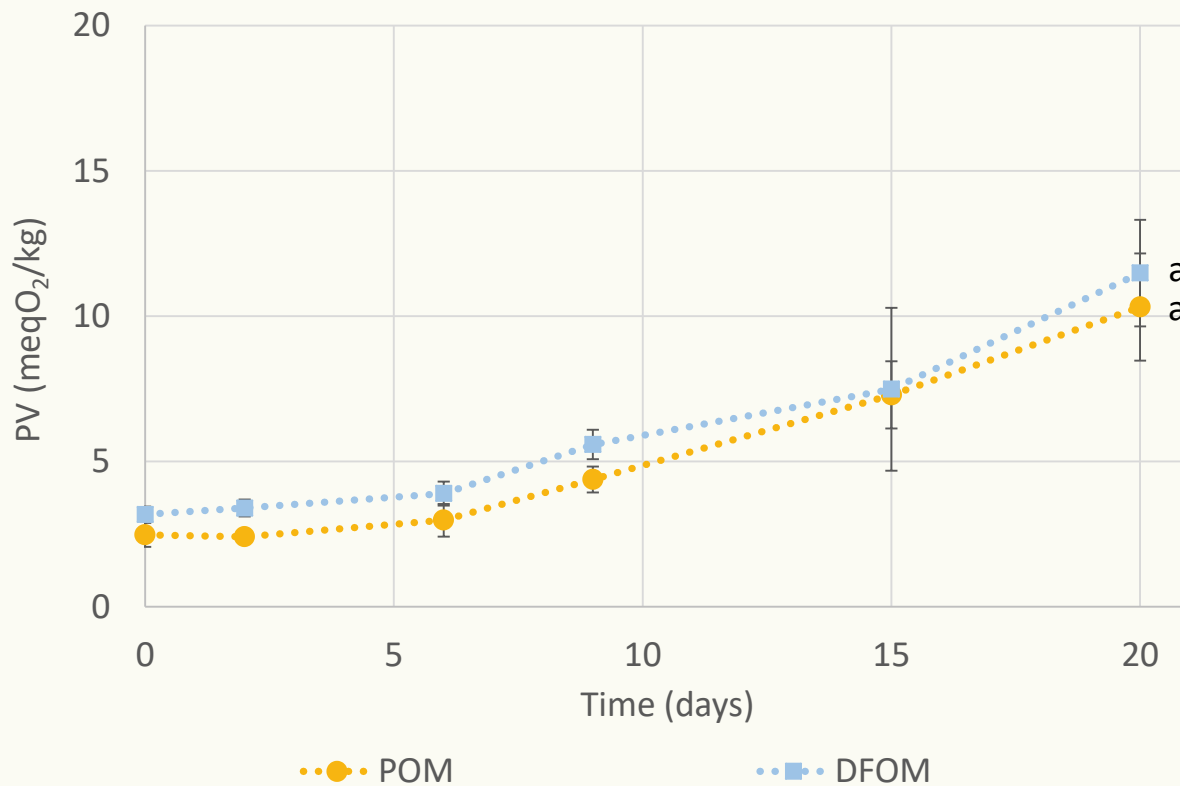
#### Particular organization of fatty acids on the TAG backbone

*Better lipidic absorption by avoiding the formation of insoluble soaps*

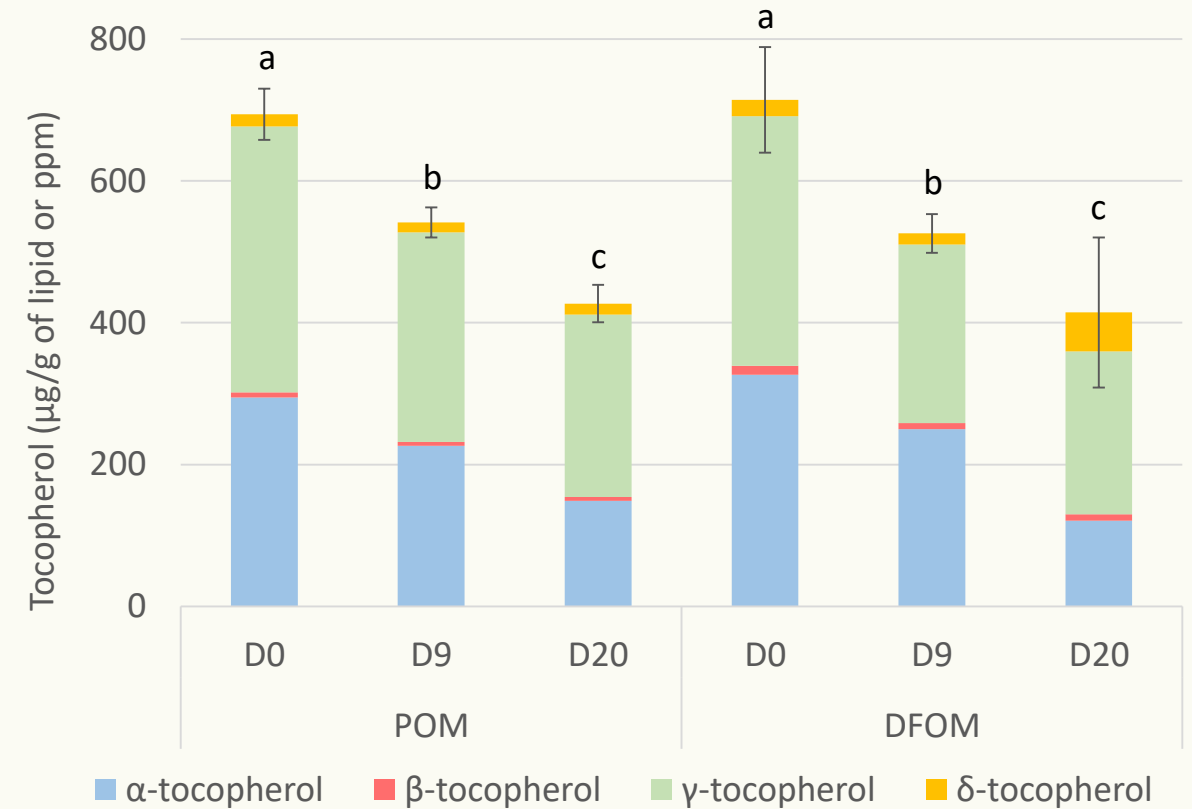
# RESULTS – 3. Challenge test - SFA sources modulates the oxidation kinetics

## Comparison of palm oil and dairy fat (olein fraction)

### Peroxide value evolution

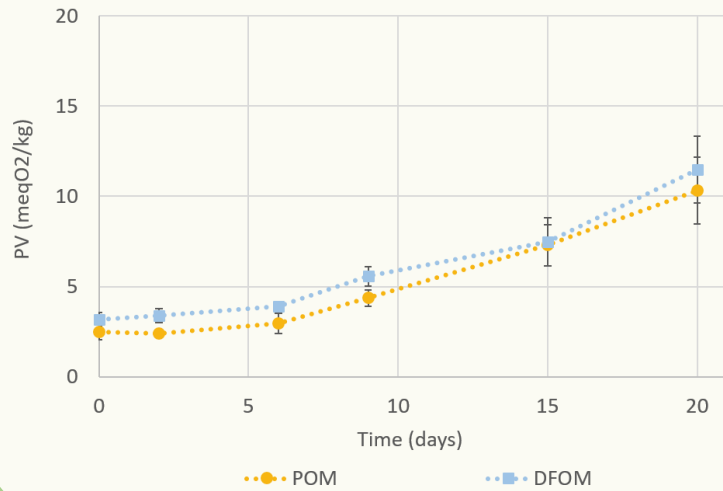


### Tocopherols isomers distribution and degradation

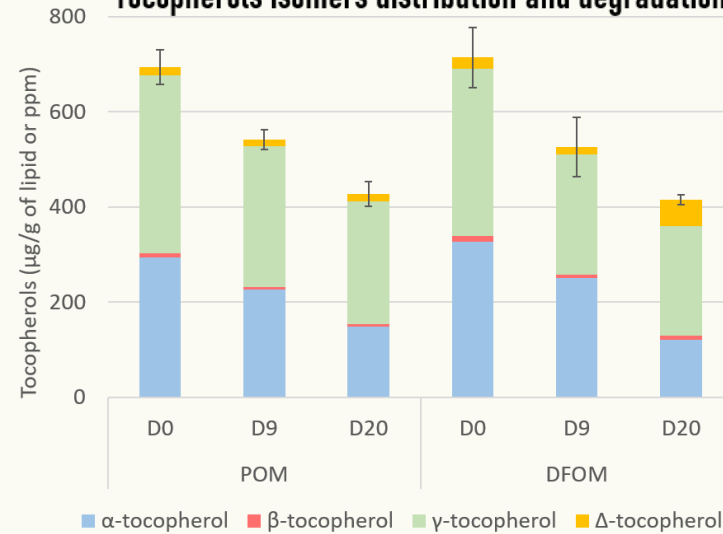


# RESULTS – 3. Challenge test - SFA sources modulates the oxidation kinetics

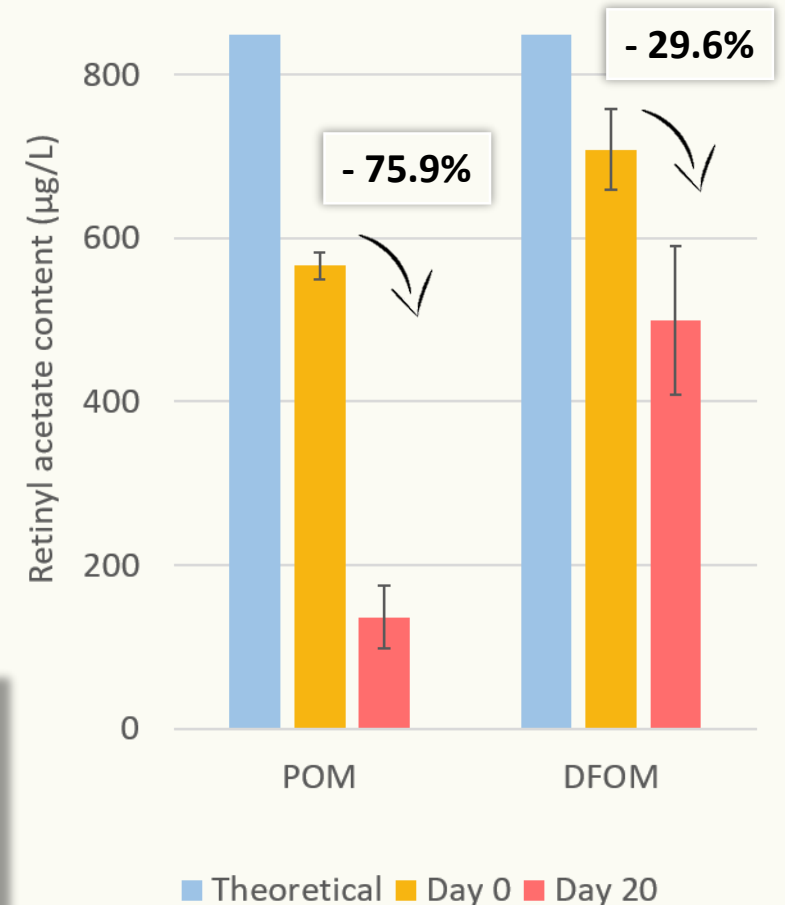
Peroxide value evolution



Tocopherols isomers distribution and degradation



Retinyl acetate degradation



## Comparison of palm oil and dairy fat

- Dairy fat is more adapted to the infant's nutritional needs
- The substitution of palm oil by dairy fat results in a similar oxidative stability resistance

# RESULTS – 3. Challenge test - Optimization of infant formulas by introduction of dairy lipids

## Comparison of soy lecithin and dairy phospholipids

Reference sample



Palm oil mixture

+



Soy lecithin



POM



Dairy fat oil mixture

+



Soy lecithin



DFOM



Dairy fat oil mixture

+



Dairy phospholipids



DFOM-DPL

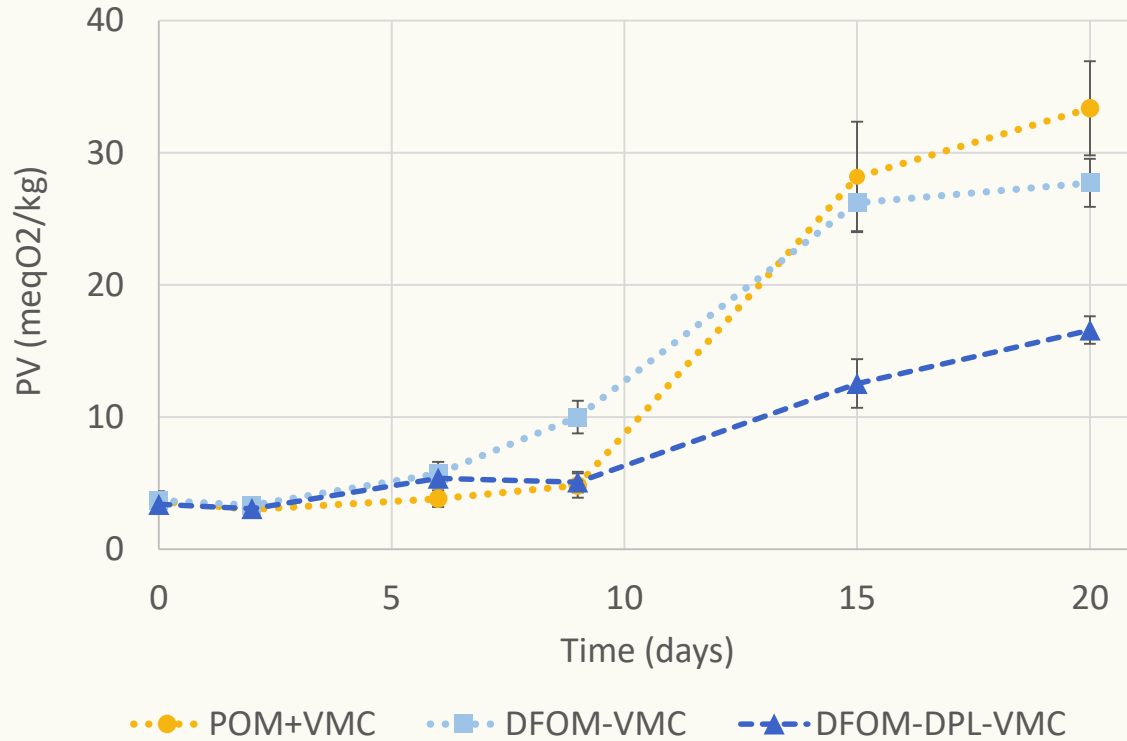
### Nutritional benefits

**Provides different PL species like SM  
and very long saturated chains**

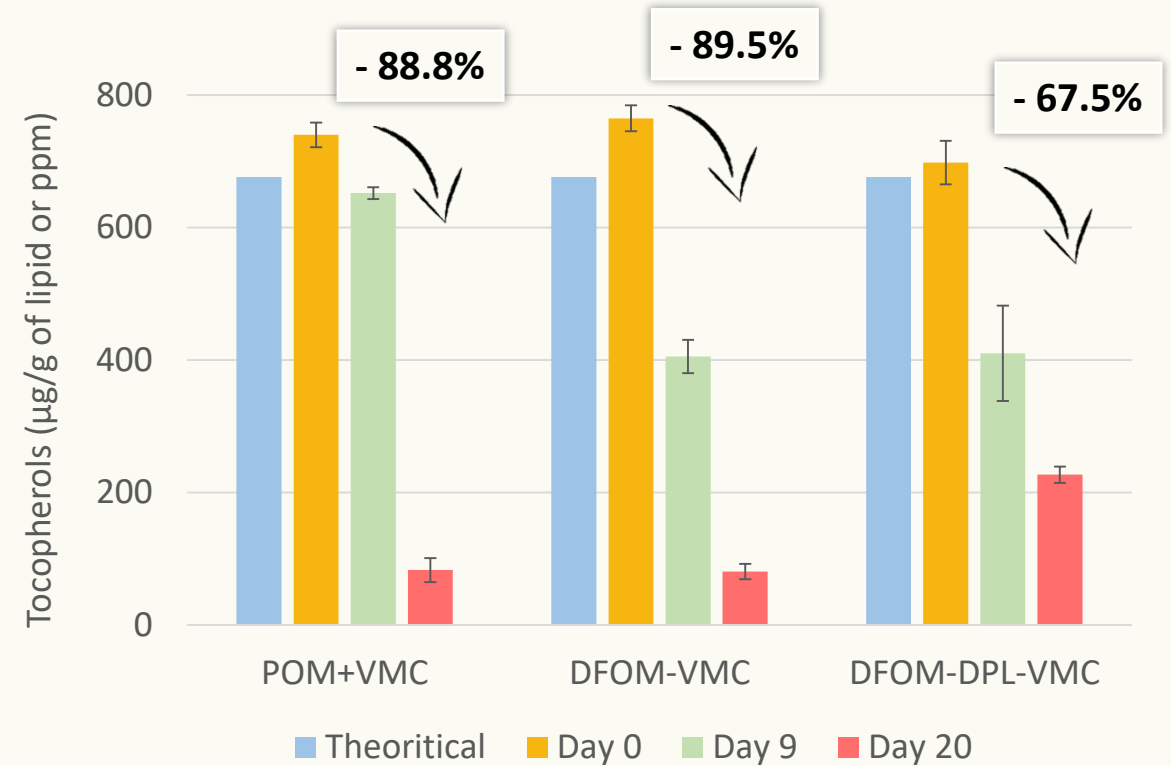
Formation of rigid domains at the interface

# RESULTS – 3. Challenge test - Optimization of infant formulas by introduction of dairy lipids

## Peroxide value evolution



## Tocopherols degradation

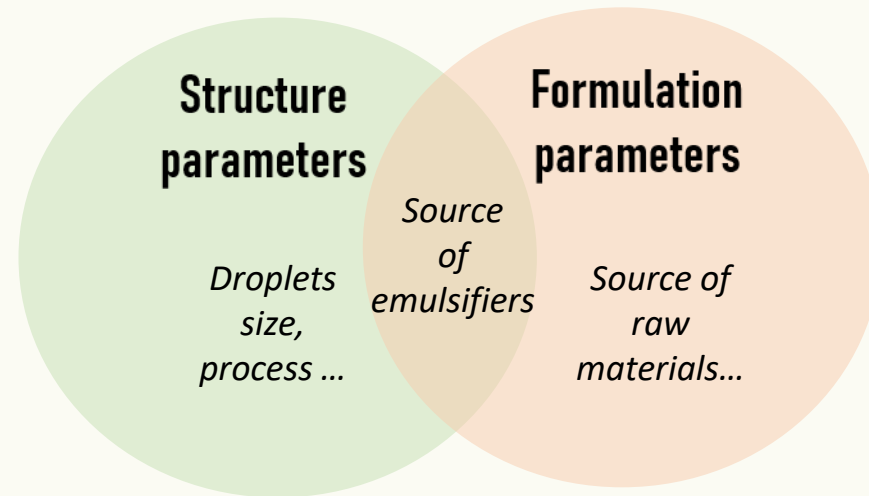


**The combined use of dairy fat and dairy phospholipids improves the oxidative resistance**

# CONCLUSION



**Follow-on formulas**  
*Aim: Biomimetic of breast milk*



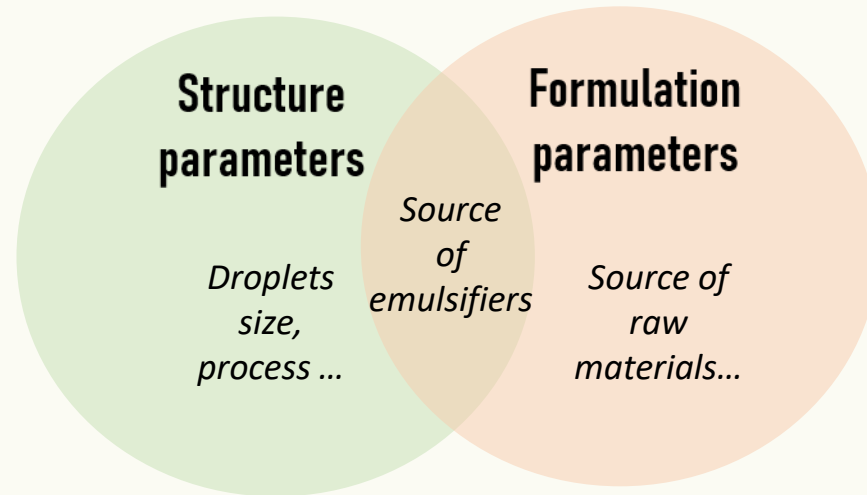
**Inclusion of carotenoids and dairy lipids in infant formulas would improve both nutritional profile and oxidative stability**



# CONCLUSION



**Follow-on formulas**  
*Aim: Biomimetic of breast milk*



**Inclusion of carotenoids and dairy lipids in infant formulas would improve both nutritional profile and oxidative stability**



**Balanced with other formulation or technological solutions**

**Proteins source**  
*Addition of dairy proteins*

**Pro-oxidant metals**  
*Iron fortification*

**Packaging**  
*Inert atm  
AOX packaging*

*Zou et al., Food Chem, 2015  
Jia et al. Cyta-J Food, 2019  
Wang et al., Food Sci Nutr, 2020  
Jo et al., Food Packag, 2020*

# RAW MATERIALS SUPPLIERS



# TEAM MEMBERS



**Claire Bourlieu-Lacanal**



**Pierre Villeneuve**



**Mathilde Cancalon**



**Youna Hemery**



**Erwann Durand**



**Nathalie Barouh**



[mathilde.cancalon@cirad.fr](mailto:mathilde.cancalon@cirad.fr)

Journées Chevreul – 80 YEARS OF SFEL

# THANK YOU FOR YOUR ATTENTION

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*Any questions ?*

**INRAE**

 **cirad**

  
Institut de Recherche  
pour le Développement  
FRANCE

*mathilde.cancalon@cirad.fr*

