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Lipids and proteins bioaccessibility in DHA-enriched fortified infant flours and formulas using a static *in vitro* 6-month-old infant digestion model

P262



Mathilde Cancalon, Marine Tournaux, Youna Hemery, Pauline Pinel, Mélina Robert, Valérie Micard, Erwann Durand, Bruno Baréa, Nathalie Barouh, Pierre Villeneuve and Claire Bourlieu Lacanal*

mathilde.cancalon@inrae.fr ; claire.bourlieu-lacanal@inrae.fr



CONTEXT

WHO recommendations

Exclusive breastfeeding for the first six months of life, followed by complementary breastfeeding up to the age of two years
WHO, 2021

Infant follow-on formulas (IFF)



Used as breast milk substitute (human milk is the most suitable nutrient source for infants)

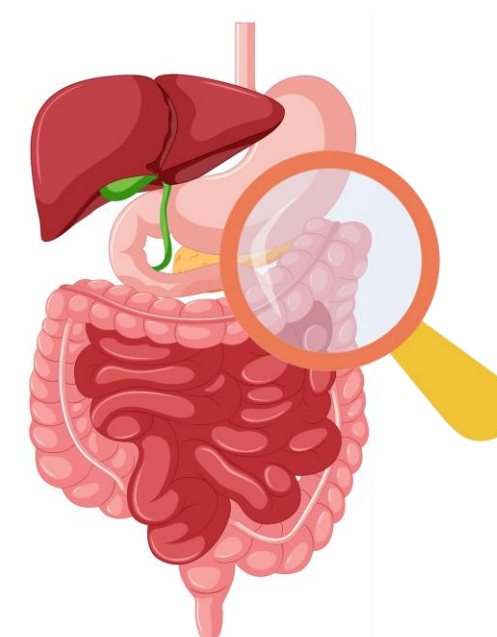
Infant flours (IF)



Used as a complementary food to start diversification from 6 months onwards

Digestive immaturity of 6-months infants

Characterized by limited secretory capacity and enzymatic activity affecting nutrients bioaccessibility



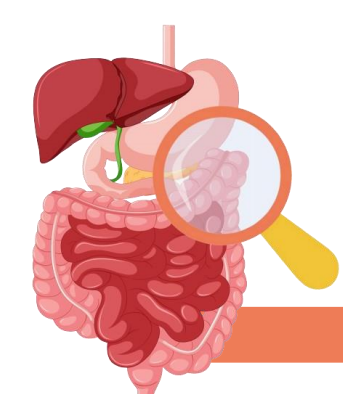
How does this immaturity impact the bioaccessibility of lipids and proteins in IFF and IF? and how can their composition be optimized to improve their nutritional properties?

AIM OF THE STUDY

Assess the bioaccessibility of lipids and proteins in optimized IF and IFF (IF_REF and IFF_REF) in terms of oxidative stability and nutritional composition in comparison with commercial products using a static *in vitro* 6-month-old infant digestion model



EXPERIMENTAL STRATEGY



1.

Overview of the nutritional values IF and IFF

Dataset of the nutritional values of 96 IF and 91 IFF

Cancalon et al., Data gov, 2023
Cancalon et al., Data in brief, 2023

2.

Formulation of optimized & DHA-ARA fortified IF and IFF

Selection of formulation and structural parameters that improve both oxidative stability and nutritional properties

Cancalon et al., Food Chem, 2023
Cancalon et al., EILST, 2023

3.

Selection of optimized IF and IFF

IF and IFF with improved nutritional and stability properties

4.

In vitro digestion of optimized IF and IFF

Evaluation of the nutrients bioaccessibility using an *in vitro* digestion model mimicking the digestive conditions of a 6-month-old infant

IFF_OP composition

Oil phase :
Dairy fat, rapeseed, sunflower, oleic sunflower oils and rich in ARA* & DHA*

Emulsifier :
Dairy phospholipid

Aqueous phase :
Lactose/Maltodextrine (50:50)
Whey protein/Casein (40:60)

* ARA: Arachidonic acid C20:4 ω6,
DHA: Docosahexaenoic acid C22:6 ω3

Static *in vitro* model adapted to the digestive conditions of a 6-month-old infant

No Oral phase: short residence time of food in mouth

GASTRIC PHASE

60 min, 37°C, pH = 3
Meal/Digestive fluid ratio of 50:50
Pepsin: 295 U/mL, Lipase: 22 U/mL

INTESTINAL PHASE

120 min, 37°C, pH = 7
Meal/Digestive fluid ratio of 25:75
Lipase: 90 U/mL, Bovine bile: 3.1 mM

Bourlieu et al., Crit. Rev. Food Sci. Nutr., 2014
Minekus et al., Food Funct., 2014
Ménard et al., Food Chem., 2018

IF_OP composition



RESULTS

	IF_REF	IF_OP	IFF_REF	IFF_OP
Fatty acid profile (g/100 mL of IFF or IF)				
Total lipids	1.0	0.4	3.1	3.4
SFA	0.40 ± 0.05	0.13 ± 0.00	0.98 ± 0.05	0.92 ± 0.05
MUFA	0.23 ± 0.01	0.07 ± 0.00	1.24 ± 0.07	1.32 ± 0.07
PUFA	0.32 ± 0.01	0.18 ± 0.01	0.56 ± 0.03	0.58 ± 0.03
LA C18:2 ω6	0.30 ± 0.01	0.11 ± 0.00	0.46 ± 0.03	0.49 ± 0.03
ALA C18:3 ω3	0.03 ± 0.00	0.02 ± 0.00	0.06 ± 0.00	0.05 ± 0.00
ARA C20:4 ω6	-	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
DHA C22:6 ω3	-	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
ω6/ω3 ratio	10.9	2.5	5.7	8.1
Total tocopherols (ppm)	1105.8 ± 13.9	1033.6 ± 17.2	595.5 ± 18.8	366.3 ± 10.8
Droplet mode (μm)	-	-	0.47 ± 0.00	0.86 ± 0.08
Viscosity (mm/30 s)	112.7 ± 5.0	93.7 ± 10.0	-	-



Trypsin inhibitory activity in infant flours (mg/g of IF dry base)

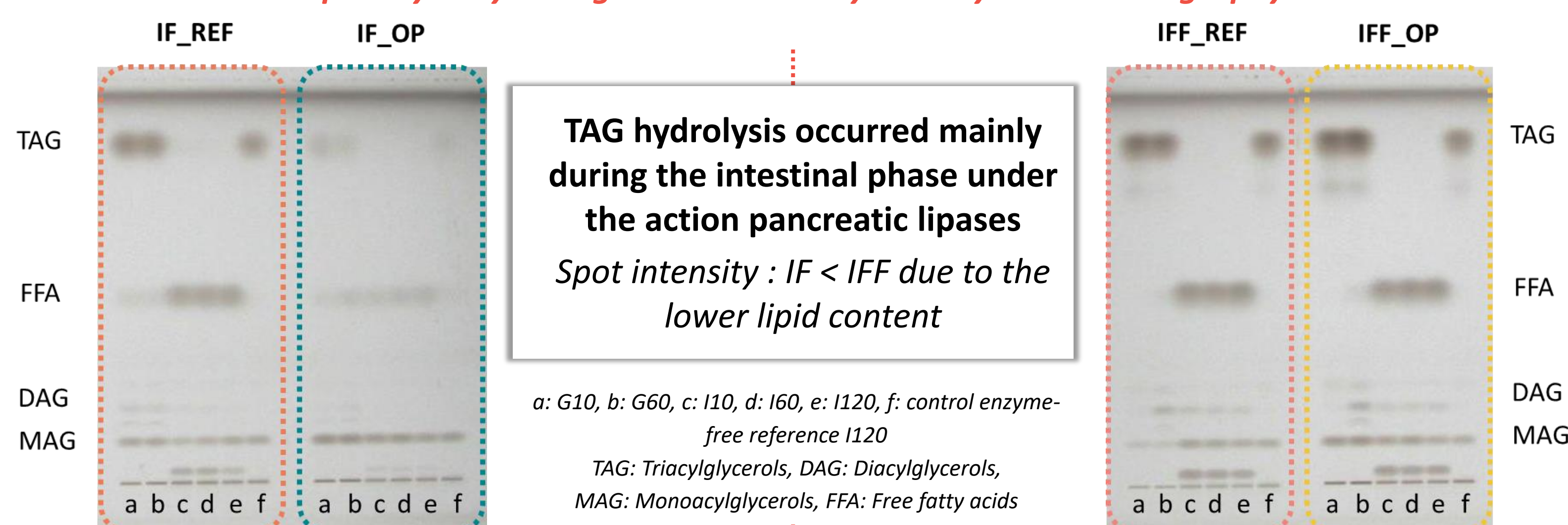
	Before cooking	After cooking
IF_REF	9.0 ± 0.1	N.D.*
IF_OP	55.2 ± 1.2	N.D.

*N.D.: not detected

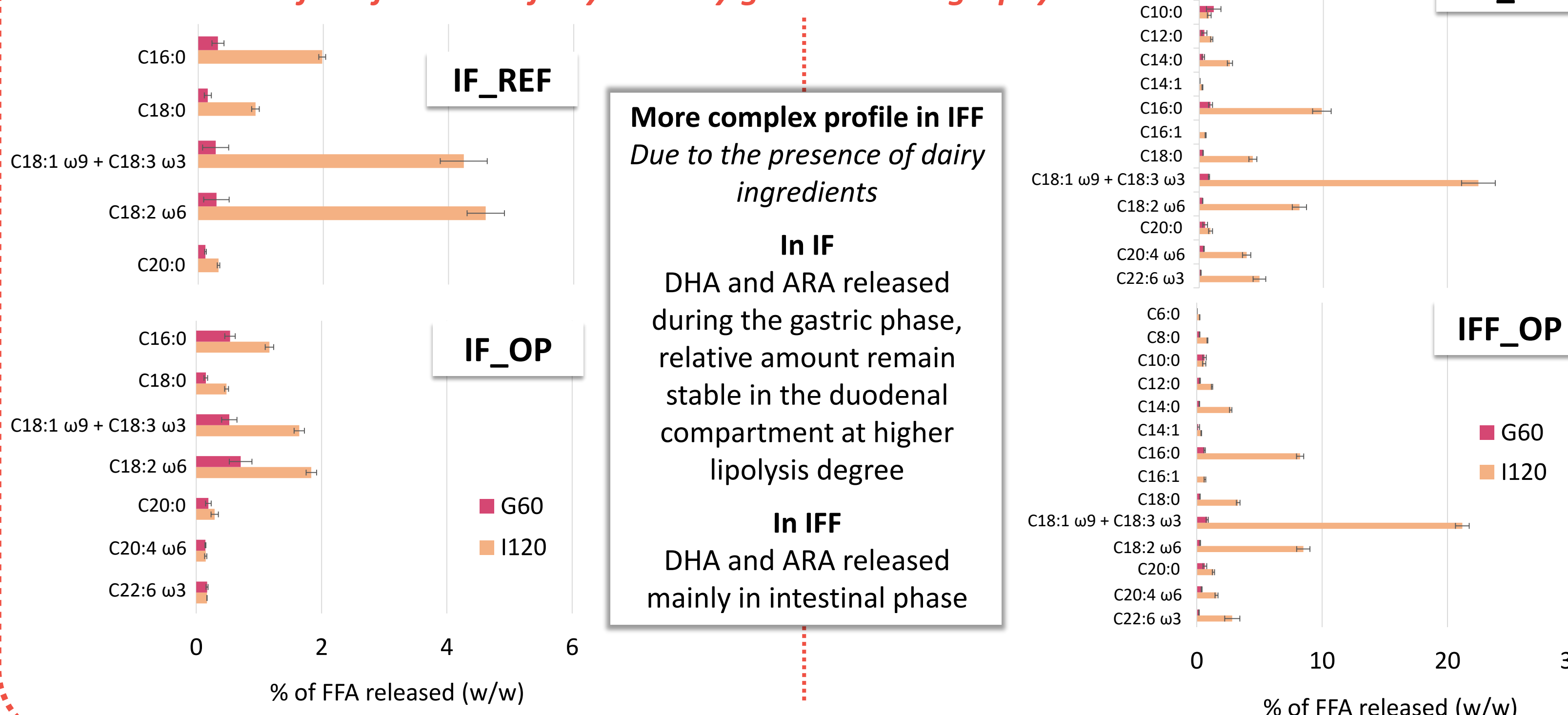
Inhibition of antitrypsin factors during cooking

LIPIDS BIOACCESSIBILITY

Lipids hydrolysis degree evaluated by thin layer chromatography

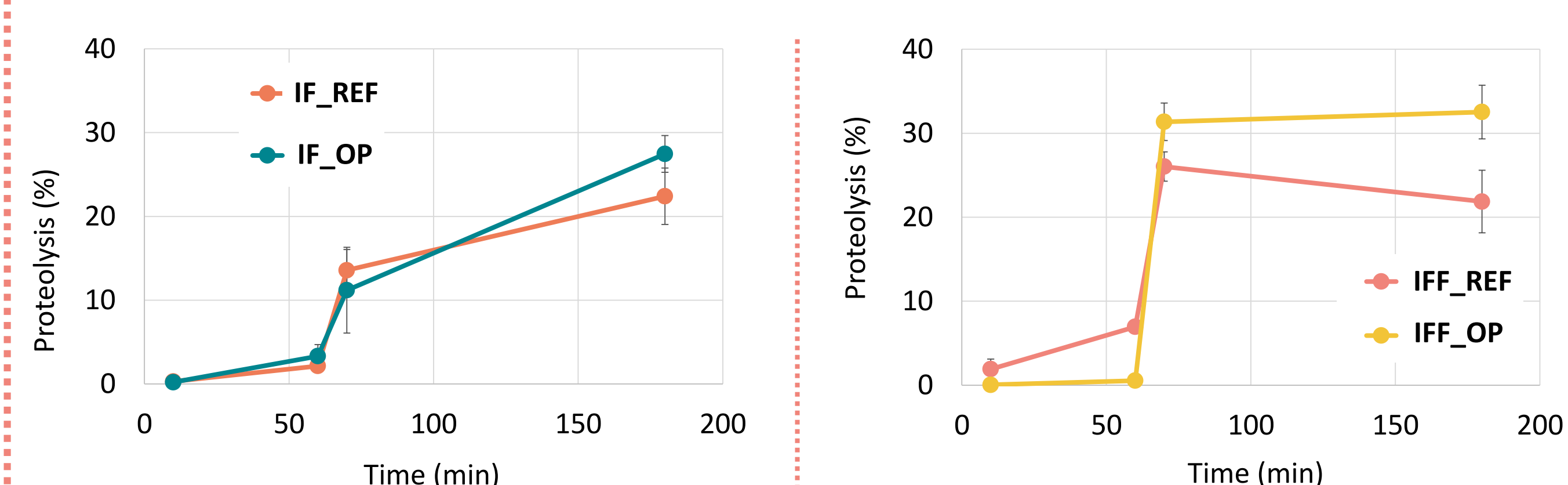


Profile of released fatty acids by gaz chromatography



PROTEINS BIOACCESSIBILITY

Protein hydrolysis degree evaluated by released NH₂ out of total using ninhydrin assay



Moderate levels of intestinal proteolysis (less than 40% released NH₂ out of total)

Influenced by large droplet mode in IFF_OP and the presence of anti-nutritional factors in IF_OP

TOCOPHEROLS BIOACCESSIBILITY

Percentage of bioaccessible tocopherols after 120 minutes digestion

IF_OP	IFF_OP	Low tocopherols bioaccessibility	IFF_REF	IFF_OP
20.7 ± 0.7	N.D.	Low in comparison with the study of Reoul, Richelle et al. (IAFC, 2006)	15.4 ± 3.6	7.1 ± 3.1

CONCLUSIONS

The optimized IFF and IF subjected to *in vitro* digestion present overall good FA and LC-PUFA (including DHA) bioaccessibility. However a limited proteolysis was observed (NH₂ released < 40% out of total) and 7-20 % tocopherols bioaccessibility. This approach should be investigated further but has to be bear in mind to have global multicriteria optimization approaches when optimizing infant foods.