



# Mathilde CANCALON

Candidature au prix de thèse GERLI-SFN 2024

## // FORMATIONS

- 2020 - 2023** ● **Thèse de doctorat en sciences des aliments et nutrition**  
Université de Montpellier – Ecole doctorale GAIA  
Co-financement CIRAD/INRAE  
*Sujet : Étude des mécanismes de l'oxydation lipidique et des antioxydants dans des matrices alimentaires complexes en vue d'améliorer leur stabilité et la bioaccessibilité des lipides : Exemple des préparations pour nourrissons et farines infantiles enrichies en acides gras polyinsaturés à longues chaînes*
- 2017 - 2020** ● **Diplôme d'ingénieur en Agroalimentaire & Génie Biologique spécialisation Innovation et Nutrition Humaine**  
ENSCBP - Ecole Nationale Supérieure de Chimie, Biologie et de Physique, Bordeaux, France

## // EXPERIENCES

- 2023 - 2024** ● **Encadrement d'étudiants**  
Trois stagiaires (IUT, M1 et M2)  
*Encadrement et formation des étudiants, accompagnement dans les tâches de rédaction et de préparation de soutenance*
- Février 2024 - Janvier 2025** ● **Chercheuse post-doctorale**  
UMR STLO, INRAE, Rennes, France  
*Projet OBEINN - Oil bodies for innovative food products: From plant seed processing to digestive fate*  
Financement inter-carnot 3Bcar et QUALIMENT
- 2021 - 2023** ● **Vacataire d'enseignement**  
IUT Montpellier-Sète, Montpellier, France  
*Cours magistraux, travaux dirigés et pratiques sur la biochimie alimentaire et les techniques d'extractions utilisées dans le domaine de l'agroalimentaire*
- Avril - Septembre 2020** ● **Ingénieure Recherche & Développement - Stage de spécialisation**  
VeryFoody, Lyon, France  
*Développement de produits alimentaires, revalorisation de co-produits, caractérisation des valeurs nutritionnelles*
- Juillet - Novembre 2019** ● **Ingénieure en recherche clinique - Stage ingénieur**  
Unité Sport & Nutrition, université de Massey, Auckland, Nouvelle Zélande  
*Etude de la perception sensorielle d'une solution à réhydratation orale (ORS) à différents niveaux de déshydratation induit par l'exercice physique*
- Juin 2019** ● **Participation au concours national Ecotrophéla FRANCE**  
Avignon, France  
*Conception de Panna Vita, un dessert lacté enrichie en six composés d'intérêt nutritionnel (protéines, calcium, DHA, vitamine A, C et D) participants à un vieillissement en bonne santé*

## // CONTACT

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## // DIVERS

 Permis B, véhiculée

 Sports

Yoga, Aviron, Randonnée (trek en autonomie), Padel, Course à pied, escalade

 Voyage

Angleterre, Espagne, Fidji, Hollande, Italie, Île Cook, Malte, Nouvelle Zélande, Suisse, Etats Unis, Maroc, Portugal

 Association

Présidente de l'association Gala Cybèle (2018 à 2019)  
Organisation de la remise des diplômes des élèves de l'ENSCBP

Adhérente SFEL & GERLI

## // COMPETENCES

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### Langues

- Français - Langue natale
- Anglais - TOEIC 810

### Compétences professionnelles

- Chimie analytique
- Chimie des lipides et des antioxydants : *Niveaux d'oxydation, profils en acides gras, classes des lipides ...*
- Formulation de produits alimentaires fonctionnels
- Méthode d'analyses nutritionnelles : *Digestion in vitro, dosage de nutriments (lipides, protéines, vitamines et minéraux)*
- Analyses statistiques de données
- Gestion de projet et travail en équipe

## // PRINCIPALES PUBLICATIONS

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### Articles de recherche

M. Cancalon, Y. Hemery, N. Barouh, B. Baréa, C. Berton-Carabin, L. Birault, E. Durand, P. Villeneuve and C. Bourlieu-Lacanal (2023). **"Comparison of the effect of various sources of saturated fatty acids on infant follow-on formulas oxidative stability and nutritional profile"** Food Chemistry **429**: 136854.

M. Cancalon, N. Barouh, Y. Hemery, B. Baréa, E. Durand, C. Bourlieu-Lacanal and P. Villeneuve (2023). **"Stabilization of infant formulas against lipid oxidation: what are the key structural levers?"** European Journal of Lipid Science and Technology.

### Article de revue

M. Cancalon, N. Barouh, Y. Hemery, E. Durand, P. Guesnet, P. Villeneuve and C. Bourlieu-Lacanal (2022). **"Supplémentation des formules infantiles en acides docosahexaénoïque et arachidonique : effets sur le développement de l'enfant et difficultés associées à leur introduction"** Cahiers de Nutrition et de Diététique **57**(6): 370-383.

### Dataset et datapapers

M. Cancalon, Y. Hemery, N. Barouh, E. Durand, P. Villeneuve and C. Bourlieu-Lacanal (2023). **"Overview of the nutritional composition of infant flours commercialized worldwide in 2021"**, Recherche Data Gouv.

M. Cancalon, Y. Hemery, N. Barouh, R. Thomopoulos, E. Durand, P. Villeneuve and C. Bourlieu-Lacanal (2023). **"Panorama of the nutritional composition of follow-on infant formula commercialized worldwide in 2021"**, Recherche Data Gouv.

M. Cancalon, Y. Hemery, N. Barouh, R. Thomopoulos, B. Baréa, E. Durand, P. Villeneuve and C. B. Lacanal (2023). **"Dataset of the nutritional composition of follow-on infant formulas commercialized worldwide in 2021"** Data in Brief: 109649.

## // PRIX ET AUTRES VALORISATIONS

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### Trophée Minerva - Fondation F.inciativas - 2023

Lauréate de la catégorie Sciences de la vie et de l'environnement, Sciences des matériaux et Chimie

### European Travel grant, AOCS 2022

Lauréate du prix étudiant de l'American Oil Chemists Society (AOCS) Annual Meeting & Expo award

### Finale régionale (Occitanie) du concours de vulgarisation « Ma thèse en 180 secondes »

Concours national de médiation et vulgarisation scientifique

## Listes des valorisations des travaux de thèse

### 1. Articles

#### Articles de recherche

M. Cancalon, Y. Hemery, N. Barouh, B. Baréa, C. Berton-Carabin, L. Birault, E. Durand, P. Villeneuve and C. Bourlieu-Lacanal (2023). **"Comparison of the effect of various sources of saturated fatty acids on infant follow-on formulas oxidative stability and nutritional profile"** Food Chemistry **429**: 136854.

**Lien d'accès :** <https://doi.org/10.1016/j.foodchem.2023.136854>

M. Cancalon, N. Barouh, Y. Hemery, B. Baréa, E. Durand, C. Bourlieu-Lacanal and P. Villeneuve (2023). **"Stabilization of infant formulas against lipid oxidation: what are the key structural levers?"** European Journal of Lipid Science and Technology.

**Lien d'accès :** <https://doi.org/10.1002/ejlt.202300161>

M. Cancalon, C. Bourlieu-Lacanal, P. Villeneuve, N. Barouh, , B. Baréa, E. Durand, Y. M. Hemery. **"Influence of chemical forms and homogenization process on the stability of vitamin A in infant follow-on formulas"**, [under review]

M. Cancalon, Y. M. Hemery, N. Dormoy, N. Barouh, B. Baréa, E. Durand, R. Barbar, C. Antoine-Assor, M. Lebrun, L. Lhomond, A. Reau, P. Villeneuve and C. Bourlieu-Lacanal. **"Optimization of the lipid composition and oxidative stability of infant flours"**, [en preparation pour soumission]

M. Cancalon, M. Tournaux, Y. M. Hemery, P. Pinel, M. Robert, J. Claudel, N. Barouh, B. Baréa, E. Durand, P. Villeneuve and C. Bourlieu-Lacanal. **"Bioaccessibility of lipids and proteins in infant follow-on formulas and flours using a static *in vitro* digestion model for 6-month-old infants"**, [en preparation pour soumission]

#### Article de revue

M. Cancalon, N. Barouh, Y. Hemery, E. Durand, P. Guesnet, P. Villeneuve and C. Bourlieu-Lacanal (2022). **"Supplémentation des formules infantiles en acides docosahexaénoïque et arachidonique : effets sur le développement de l'enfant et difficultés associées à leur introduction"** Cahiers de Nutrition et de Diététique **57**(6): 370-383.

**Lien d'accès :** <https://doi.org/https://doi.org/10.1016/j.cnd.2022.04.007>

#### Datasets

M. Cancalon, Y. Hemery, N. Barouh, E. Durand, P. Villeneuve and C. Bourlieu-Lacanal (2023). **"Overview of the nutritional composition of infant flours commercialized worldwide in 2021"**, Recherche Data Gouv.

**Lien d'accès :** <https://doi.org/10.57745/TUSCZF>

M. Cancalon, Y. Hemery, N. Barouh, R. Thomopoulos, E. Durand, P. Villeneuve and C. Bourlieu-Lacanal (2023). **"Panorama of the nutritional composition of follow-on infant formula commercialized worldwide in 2021"**, Recherche Data Gouv.

**Lien d'accès :** <https://doi.org/10.57745/CLER60>

### Datapapers

M. Cancalon, Y. Hemery, N. Barouh, R. Thomopoulos, B. Baréa, E. Durand, P. Villeneuve and C. B. Lacanal (2023). "**Dataset of the nutritional composition of follow-on infant formulas commercialized worldwide in 2021**" Data in Brief: 109649.

**Lien d'accès :** <https://doi.org/10.1016/j.dib.2023.109649>

M. Cancalon, Y. Hemery, N. Barouh, B. Baréa, E. Durand, P. Villeneuve and C. Bourlieu-Lacanal. "**Dataset of the nutritional composition of infant flours commercialized worldwide in 2021**" Data in Brief, en préparation pour soumission

## 2. Congrès, colloques et webinaires

### Conférences orales

M. Cancalon, N. Barouh, Y. Hemery, B. Baréa, E. Durand, P. Villeneuve et C. Bourlieu-Lacanal. "**Vitamin A stability in follow-on infant formulas depending on the chemical form and the homogenization process**" - 19<sup>th</sup> Euro Fed Lipid Congress and Expo (EFL) - Septembre 2023 - Poznan, Pologne

**Lien d'accès :** <https://hal.science/hal-04548267>

M. Cancalon, Y. Hemery, N. Barouh, B. Baréa, E. Durand, P. Villeneuve et C. Bourlieu-Lacanal. "**Réintroduction de lipides laitiers dans les formules infantiles : impact sur la stabilité oxydative et sur le profil nutritionnel**" - Journée CNIEL par la Société Française pour l'étude des lipides (SFEL) - Septembre 2022 : « Les lipides laitiers : Dans les 1000<sup>1ers</sup> jours et au-delà... » - Paris, France

**Lien d'accès :** <https://hal.science/hal-04145354v1>

M. Cancalon, Y. Hemery, N. Barouh, B. Baréa, C. Berton-Carabin, L. Birault, E. Durand, P. Villeneuve et C. Bourlieu-Lacanal. "**Optimization of the oxidative stability and lipid profile of DHA-enriched infant follow-on formulas by the introduction of dairy lipids**" - Journée Chevreul par la Société Française pour l'étude des lipides (SFEL) - Janvier 2023 : « 80 ans de la SFEL » - Paris, France

**Lien d'accès :** <https://hal.science/hal-04145552v1>

M. Cancalon, Y. Hemery, N. Barouh, B. Baréa, C. Berton-Carabin, L. Birault, E. Durand, P. Villeneuve et C. Bourlieu-Lacanal. "**Effect of various saturated fatty acids sources on oxidative stability and nutritional profile of DHA-enriched infant follow-on formulas**" - 15<sup>th</sup> Congress of International Society for the Study of Fatty Acids and Lipids (ISSFAL) - Juillet 2023 - Nantes, France

**Lien d'accès :** <https://hal.science/hal-04149608v1>

M. Cancalon, N. Barouh, Y. Hemery, B. Baréa, E. Durand, P. Villeneuve et C. Bourlieu-Lacanal. "**Oxidation kinetics of model systems representative of follow-on formulas**" - Journée scientifique « Montpellier Saint Pée sur Nivelle » - Décembre 2021 - Montpellier, France

M. Cancalon, N. Barouh, Y. Hemery, B. Baréa, E. Durand, P. Villeneuve et C. Bourlieu-Lacanal. "**Does replacing palm oil in follow-on formulas with other sources of saturated fat provide a better nutritional profile without affecting oxidative stability ?**" - Webinaire mensuel de la Société Française pour l'étude des lipides (SFEL) - Octobre 2021 : « Webinaire jeune chercheur » - En ligne

M. Cancalon, N. Barouh, Y. Hemery, B. Baréa, E. Durand, P. Villeneuve et C. Bourlieu-Lacanal.



**"Étude des mécanismes de l'oxydation lipidique et des antioxydants dans des matrices alimentaires complexes en vue d'améliorer leur stabilité et la bioaccessibilité des lipides : Exemple des préparations pour nourrissons et farines infantiles enrichies en acides gras polyinsaturés à longues chaînes"** - Webinaire mensuel de la Société Française pour l'étude des lipides (SFEL) - Janvier 2024 – En ligne

#### Posters

M. Cancalon, N. Barouh, Y. Hemery, B. Baréa, E. Durand, P. Villeneuve et C. Bourlieu-Lacanal. **"Lipid oxidation kinetics of model systems representative of follow-on formulas"** - 2022 American Oil Chemists Society (AOCS) Annual Meeting & Expo - Mai 2022 - Atlanta, Etats-Unis

**Lien d'accès :** <https://theses.hal.science/QUALISUD/hal-04173417v1>

M. Cancalon, Y. Hemery, R. Barbar, N. Barouh, B. Baréa, L. Lhomond, A. Reau, E. Durand, V. Micard, P. Villeneuve et C. Bourlieu-Lacanal. **"Optimization of the oxidative stability and lipid profile of omega-3 enriched infant flours"** - 19<sup>th</sup> Euro Fed Lipid Congress and Expo (EFL) - Septembre 2023 - Poznan, Pologne

**Lien d'accès :** <https://hal.science/hal-04548115>

M. Cancalon, Y. Hemery, N. Barouh, B. Baréa, E. Durand, P. Villeneuve et C. Bourlieu-Lacanal. **"Nutritional values of infant formulas and flours and their adequacy with the requirements of infants aged 6 to 12 months"** - 2021 American Oil Chemists Society (AOCS) Annual Meeting & Expo - Mai 2021 - Congrès en ligne

**Lien d'accès :** <https://hal.science/hal-03252226v1>

M. Cancalon, Y. Hemery, N. Barouh, B. Baréa, E. Durand, P. Villeneuve et C. Bourlieu-Lacanal. **"Oxidative stability and nutritional profile of omega-3 enriched flours optimized for infant nutrition"** - 15<sup>th</sup> Congress of International Society for the Study of Fatty Acids and Lipids (ISSFAL) - Juillet 2023 - Nantes, France

**Lien d'accès :** <https://hal.science/hal-04149612v1>

M. Cancalon, Y. Hemery, N. Barouh, B. Baréa, V. Micard, E. Durand, P. Villeneuve et C. Bourlieu-Lacanal. **"Adéquation des valeurs nutritionnelles des préparations pour nourrissons et farines infantiles avec les recommandations et besoins des enfants de 6 à 12 mois"** - Journées Francophones de Nutrition (JFN) 2023 - Décembre 2023 - Marseille, France

**Lien d'accès :** <https://hal.science/hal-04548203>

M. Cancalon, M. Tournaux, Y. Hemery, P. Pinel, M. Robert, V. Micard, E. Durand, B. Baréa, N. Barouh, P. Villeneuve et C. Bourlieu-Lacanal. **"Lipids and proteins bioaccessibility in DHA-enriched fortified infant flours and formulas using a static in vitro 6-month-old infant digestion model"** - 8<sup>th</sup> International Conference on Food Digestion (ICFD) 2024 - April 2024 – Porto, Portugal

**Lien d'accès :** <https://hal.science/hal-04548227>

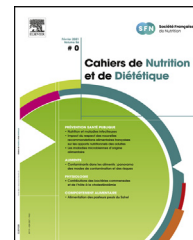


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## ALIMENTS

# Supplémentation des formules infantiles en acides docosahexaénoïque et arachidonique : effets sur le développement de l'enfant et difficultés associées à leur introduction



*Supplementation of infant formula with docosahexaenoic and arachidonic acids: Effects on child development and difficulties associated with their introduction*

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Youna Hemery<sup>b,d</sup>, Erwann Durand<sup>a,b</sup>,  
Philippe Guesnet<sup>e</sup>, Pierre Villeneuve<sup>a,b</sup>,  
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Disponible sur Internet le 27 juin 2022

### MOTS CLÉS

Formule infantile ;  
Lipides ;  
DHA ;  
ARA ;  
Stabilité oxydative

**Résumé** La période postnatale est caractérisée par une croissance et un développement rapide. Les lipides, et plus particulièrement les acides gras polyinsaturés à longues chaînes (AGPI-LC), tels que les acides docosahexaénoïque (DHA) et arachidonique (ARA), jouent un rôle essentiel durant cette période. L'objectif principal des formules infantiles est d'être biomimétique de « l'étalon d'or » : le lait maternel, aussi bien en termes de composition que de structure, pour ainsi offrir la meilleure alternative possible à l'allaitement lorsqu'il a échoué et/ou n'est pas possible. Dans ce contexte, la réglementation européenne a évolué et impose

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désormais la supplémentation des formules en DHA. Elles sont également fortifiées en ARA, autre AGPI indispensable pour le développement de l'enfant, bien qu'aucune obligation ne soit établie quant à sa teneur minimale. Leur important degré d'insaturation rend ces acides gras particulièrement sensibles à l'oxydation lipidique, complexifiant ainsi leur introduction dans les formules infantiles. L'objectif de cette revue est donc de faire un état de l'art sur les différents paramètres clés permettant de mieux maîtriser la supplémentation des formules infantiles en AGPI-LC.

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## KEYWORDS

Infant formulas;  
Lipids;  
DHA;  
ARA;  
Oxidative stability

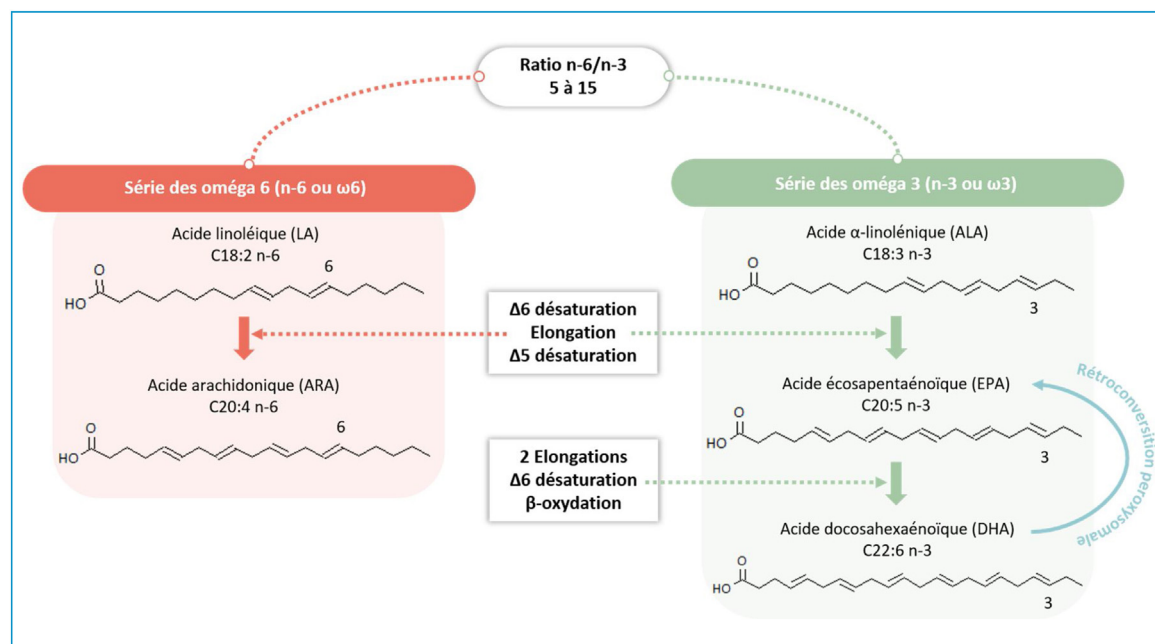
**Summary** The postnatal period is characterized by rapid growth and development. Lipids, especially long-chain polyunsaturated fatty acids (LC-PUFA) such as docosahexaenoic (DHA) and arachidonic (ARA) acids, play an essential role during this period. The main objective of infant formulas is to be biomimetic of the "golden standard" *i.e.* breast milk, both in terms of composition and structure, thus offering the best possible alternative to breastfeeding when it has failed and/or is not possible. In this context, European regulations have evolved and now require DHA supplementation of formulas. Formulas are also fortified with ARA, another PUFA essential for child development, although there is no requirement for a minimum content. Their high degree of unsaturation makes these fatty acids particularly sensitive to lipid oxidation, making their inclusion in infant formulas more difficult. The objective of this review is therefore to provide a state of the art on the various key parameters that allow better control of LC-PUFA supplementation in infant formulas.

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## Introduction

Les besoins nutritionnels durant la période néonatale puis l'enfance sont très importants et spécifiques et servent à supporter le développement et la croissance rapide caractéristique de ces périodes physiologiques particulières. Les lipides notamment jouent un rôle essentiel, car en plus d'être le vecteur principal d'énergie avec 45 à 55 % des calories fournies via leur  $\beta$ -oxydation, ils sont fortement impliqués dans le développement cérébral, visuel, intestinal et immunitaire de l'enfant. Parmi les différentes classes de lipides, certains sont qualifiés d'indispensables (ou d'essentiels) du fait de l'incapacité de l'organisme humain à les synthétiser. C'est notamment le cas des précurseurs métaboliques et chefs de file des familles non interconvertibles que sont les oméga 6 (n-6 ou  $\omega$ 6) et les oméga 3 (n-3 ou  $\omega$ 3). Il s'agit de l'acide linoléique (LA, chaîne carbonée de 18 pourvue de 2 insaturations, C18:2) et de l'acide alpha-linolénique (ALA, chaîne carbonée de 18 pourvue de 3 insaturations, C18:3) [1]. Ces acides gras conduisent, par une voie spécifique de désaturations-élongations successives, à la biosynthèse d'autres acides gras polyinsaturés (AGPI) dits à longues chaînes (LC, chaîne carbonée supérieure ou égale à 20) tels que les acides docosahexaénoïque (DHA, chaîne carbonée de 22 pourvue de 6 insaturations, C22:6 n-3) et arachidonique (ARA, chaîne carbonée de 20 pourvue de 4 insaturations, C20:4 n-6) qui sont indispensables pour le développement cognitif et l'acuité visuelle de l'enfant (Fig. 1). Une déficience en ces acides gras peut avoir d'importantes conséquences à long terme avec une augmentation des risques de développer

certaines maladies mentales et/ou métaboliques à l'âge adulte. Il est donc reconnu qu'il existe une importance à la fois quantitative et qualitative de l'apport lipidique durant la période de l'enfance. Bien que le lait maternel reste la référence en termes de nutrition du nourrisson puis du jeune enfant, des formules infantiles sont développées en tant que substituts en cas d'échec ou d'impossibilité de l'allaitement. La composition de ces formules est encadrée par une réglementation européenne précise qui a évolué en février 2020 en rendant obligatoire la supplémentation en DHA des formules de premier âge (destinées aux nourrissons de 0 à 6 mois) et des préparations de suite (destinées aux enfants de 6 à 12 mois). L'ajout d'ARA n'est, quant à lui, pas obligatoire mais fortement recommandé par la communauté scientifique. Du fait de leurs multiples insaturations, les AGPI-LC sont particulièrement sensibles au phénomène d'oxydation lipidique, principal phénomène à l'origine de l'altération des produits alimentaires et qui engendre une diminution de leur qualité nutritionnelle. De plus, les procédés de fabrication puis de stockage des formules sous formes de poudres peuvent être des paramètres propices à la dégradation des lipides. Par conséquent, une bonne résistance à l'oxydation des formules infantiles enrichies en AGPI-LC est un enjeu majeur. Ainsi, cette revue a pour objectifs de mettre en évidence l'importance des lipides pour le développement de l'enfant, de comparer la fraction lipidique du lait maternel mature et des préparations de suite en prenant en compte l'évolution de la réglementation européenne de 2020, et, enfin, d'identifier les points de vigilance pour la supplémentation des formules enrichies en DHA et ARA.



**Figure 1.** Voies biochimiques d'élargissement des acides linoléique (LA) et α-linolénique (ALA) en acides gras polyinsaturés à longue chaîne (AGPI-LC) [2,3].

## Besoins nutritionnels de l'enfant

### Besoins nutritionnels spécifiques en lipides

Les besoins en lipides alimentaires du nourrisson puis du jeune enfant sont très élevés au cours de la période de développement postnatal. Pour le nourrisson, ils sont plus de quatre fois supérieurs à ceux d'un homme adulte rapportés au poids corporel comme le montre la Fig. 2.

Pour couvrir ces besoins spécifiques, le lait maternel humain apporte en moyenne autour de 35 g de lipides totaux par litre. Ces derniers sont majoritairement sous la forme de triesters d'acides gras (98 % du poids total), les triacylglycérols (TAG) [4,5]. Ainsi, un nourrisson né à terme et allaité au sein pendant six mois ingérera une quantité de lipides équivalente à celle de son gain de poids soit 5,5 kg en moyenne. Qu'ils soient indispensables comme les AGPI ou non, tous les acides gras apportés par les lipides du lait maternel sont vitaux pour permettre la croissance rapide au cours de la première année de vie [2,6]. En outre, une part importante de l'acétyl-CoA produit suite à la dégradation β-oxydative des lipides est recyclée activement dans la synthèse *de novo* des lipides (acides gras, cholestérol, ...) mais également dans la voie des corps cétoniques, ces derniers étant les principaux substrats énergétiques du cerveau à cette période de la vie [7]. Ces voies de recyclage et de cétogenèse impliquent aussi bien les acides saturés et mono-insaturés que les AGPI indispensables. C'est le cas de l'ALA qui serait 200 fois plus activement recyclé que métabolisé en AGPI n-3 à longue chaîne comme le DHA. Les acides gras des lipides du lait maternel sont également impliqués dans le développement des tissus (lipides cellulaires), de l'intestin et l'acquisition du système immunitaire [8–10] et, lorsqu'ils sont indispensables, dans le développement des fonctions visuelles et cognitives du nourrisson et du jeune enfant [11].

Des données cliniques montrent qu'ils peuvent aussi réguler l'incidence de pathologies inflammatoires de l'enfant à l'âge de 12 mois [12]. En dehors de l'importance des acides gras estérifiés sur les TAG, de plus en plus de données expérimentales et cliniques soulignent également l'importance nutritionnelle des lipides mineurs qui rentrent dans la constitution des membranes des globules gras du lait maternel que sont les phospholipides, les sphingolipides, les gangliosides et le cholestérol [13,14]. Enfin, il est bien établi que les 1000 premiers jours de développement (*i.e.* depuis la conception jusqu'à l'âge de 2 ans) constituent une période de vulnérabilité vis-à-vis de l'alimentation, qui, lorsqu'elle est carencée ou déséquilibrée en certains nutriments, pourrait perturber les processus de programmation métabolique et par contre-coup, induire des altérations à long terme sur la santé de l'enfant et de l'adulte. On considère ainsi que l'alimentation lipidique serait à l'origine de déviations métaboliques chez le jeune enfant qui se maintiendraient jusqu'à l'âge adulte, favorisant alors l'émergence de pathologies comme l'obésité et ses complications métaboliques [15].

### Rôle et importance des AGPI

L'alimentation lipidique du nourrisson et du jeune enfant apporte donc une grande diversité d'acides gras dont les AGPI des deux séries n-6 et n-3. Chez l'Homme, les AGPI exercent de nombreuses fonctions vitales en tant que composants fondamentaux et régulateurs de l'architecture et de la fonction des membranes cellulaires, précurseurs de régulateurs endogènes de la signalisation cellulaire et de l'expression des gènes, précurseurs de multiples voies enzymatiques de synthèse de médiateurs lipidiques et de métabolites formés par auto-oxydation [16].

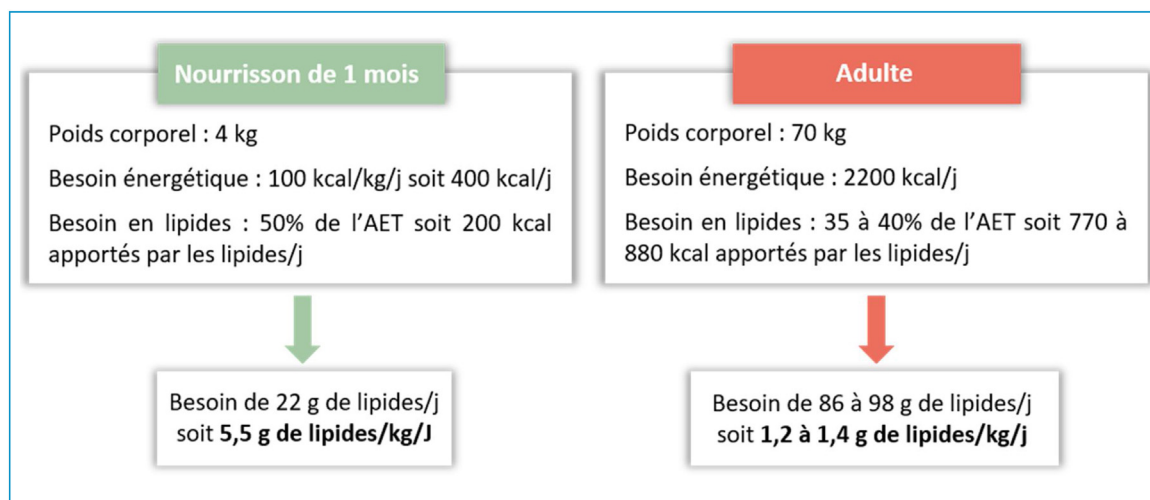


Figure 2. Besoins en lipides du nourrisson et de l'adulte [2,3].

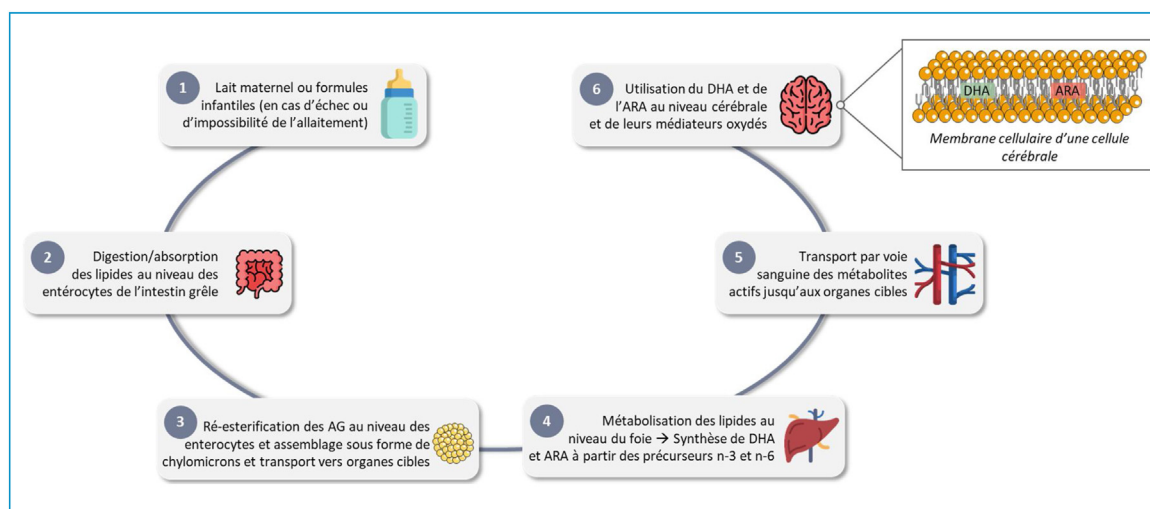


Figure 3. Métabolisation des acides gras polyinsaturés à longue chaîne (AGPI-LC) (ARA : acide arachidonique ; DHA : acide docosahexaénoïque).

Le caractère indispensable de LA a été démontré pour l'Homme dans les années 1960, précisément chez le nourrisson qui présentait un dessèchement et un épaississement de la peau et une desquamation cutanée après plusieurs mois d'allaitement avec des laits infantiles dépourvus de toute matière grasse alimentaire. L'ajout de LA à hauteur de 1 % de l'énergie faisait alors régresser rapidement puis disparaître ces symptômes cutanés [17]. Pour sa part, l'ARA est un acide gras ubiquitaire des membranes cellulaires impliqué dans l'architecture structurale, la signalisation cellulaire et la régulation génique ; il est également le précurseur de multiples médiateurs cellulaires (e.g. les prostanoïdes, endocannabinoïdes, epoxydes, isoprostanes, résolvines...) après métabolisation par plusieurs voies d'oxydation enzymatiques et non-enzymatiques. Certains de ces médiateurs comme la prostacycline I2 par exemple, activent le développement du tissu adipeux blanc [18]. L'ARA, obtenu à partir du LA (Fig. 1), est donc un acide gras très important métaboliquement pour l'homme et crucial pour la croissance du nourrisson et le développement de son cerveau.

Ainsi expérimentalement, des souris déficientes en ARA par invalidation de la voie de biosynthèse des AGPI à longue chaîne (KO pour l'étape de  $\Delta 6$  désaturation) développaient des altérations dans leur neurodéveloppement moteur que seul l'ARA alimentaire corrigeait [19]. Au contraire des AGPI n-6, le précurseur des AGPI n-3, l'ALA, n'exerce pas de rôle physiologique si ce n'est d'être métabolisé en dérivés supérieurs à longue chaîne soit en DHA qui, comme l'ARA, est un acide gras particulièrement abondant dans les phospholipides du système nerveux (cerveau, rétine) de tous les mammifères, y compris l'Homme (Fig. 3) [20]. Pour le cerveau, la période d'accumulation du DHA s'établit pendant sa période de croissance rapide, de synaptogenèse et de myélinisation, i.e. chez l'Homme du dernier trimestre de grossesse jusqu'à l'âge de 2 ans. Le caractère indispensable des AGPI n-3 a été principalement établi pour le nourrisson avec des modèles précliniques. Ainsi, il a été largement démontré que la diminution marquée de la teneur membranaire cérébrale en DHA chez le rongeur et le singe au cours de la période de développement périnatal



(gestation-lactation), généralement induite nutritionnellement par un régime maternel dépourvu d'AGPI n-3 et/ou déséquilibré dans la balance n-6/n-3, s'accompagnait d'une réduction des capacités d'apprentissage et de discrimination visuelle (acuité visuelle, amplitude de l'onde b de l'électrorétinogramme) [20]. Par ailleurs, la période d'accumulation du DHA constitue une fenêtre de grande sensibilité à l'alimentation lipidique, car il apparaît difficile de corriger entièrement chez l'adulte le déficit membranaire et les troubles fonctionnels décrits précédemment, en dépit de la réintroduction précoce des AGPI n-3 dans l'alimentation [21]. À partir de ces données pré-cliniques, le caractère indispensable des AGPI n-3, et plus particulièrement celui de l'ALA, a été admis pour le nourrisson en fixant un apport lacté compris entre 0,45 et 0,6 % de l'énergie [22]. Plus tardivement, il a été mis en évidence chez le nouveau-né humain (prématuré et né à terme) que la consommation de laits infantiles pauvres en AGPI n-3 et excessif en AGPI n-6 comparativement à des enfants allaités au sein, provoquait une diminution de la concentration en DHA dans les membranes érythrocytaires et cérébrales, et un retard de maturation des fonctions visuelles [11].

### Besoins physiologiques en AGPI du nourrisson et du jeune enfant et cadre réglementaire

Les besoins en AGPI du nourrisson et du jeune enfant, et donc les quantités fixées pour la formulation des laits infantiles de premier et deuxième âge, ont été établis pour les deux AGPI précurseurs mais également pour leurs principaux dérivés à longue chaîne [17]. Les besoins en LA et ALA ont été fixés respectivement à 2,7 et 0,45 % de l'énergie alimentaire totale, le ratio LA/ALA devant être équilibré et proche de 5. Une valeur maximale à ne pas dépasser dans les laits infantiles a été également proposée par l'agence européenne EFSA ( $5 < \text{LA/ALA} < 15$ ) [3], en s'inspirant des teneurs maximales retrouvées dans le lait maternel humain. En dépit d'un apport convenable en AGPI précurseurs, un grand nombre d'études cliniques menées à partir des années 80 ont systématiquement rapporté que les teneurs circulantes en ARA et surtout en DHA au cours de la première année de la vie restent systématiquement inférieures chez les enfants nourris avec des formules infantiles, en comparaison d'enfants recevant le lait maternel (enfants prématurés de faible poids et nés à terme) [11]. Les données obtenues chez les enfants victimes du syndrome de mort subite et ayant ingéré ce type de lait infantile ont confirmé ces observations, tout au moins pour le DHA. Le bilan global corporel en DHA est alors négatif chez ces enfants nés à terme au cours des six premiers mois de vie (- 0,9g), témoignant d'une mobilisation élevée des stocks corporels présents à la naissance afin de privilégier l'accumulation de DHA au niveau cérébral ; cette accumulation reste toutefois insuffisante (0,4g vs 1g chez les enfants allaités au sein) [23]. Ces différences de statut corporel trouvent leur explication dans la composition du lait maternel humain qui, au contraire de la majorité des laits infantiles commercialisés jusqu'aux années 2010, renfermait des quantités appréciables de dérivés à longue chaîne, et notamment du DHA préformé [18]. Ainsi, l'enfant allaité artificiellement couvre exclusivement ses besoins en dérivés à longue chaîne

par biosynthèse endogène et mobilisation de ses réserves corporelles, biosynthèse dont la capacité serait finalement insuffisante au regard des besoins physiologiques. Chez l'enfant prématuré de petit poids qui possède un système immature de bioconversion et pas de stock corporel en AGPI (précurseurs et longue chaîne), il apparaît de façon plus convaincante que la supplémentation de l'alimentation lactée avec du DHA permet de corriger le moindre niveau d'acuité visuelle [17]. Pour l'enfant né à terme, les données cliniques fonctionnelles sont controversées, liées notamment à une grande hétérogénéité des populations étudiées (variabilité des niveaux de désaturation et de réserve corporelle en AGPI à la naissance). Ces résultats ont amené les différentes agences gouvernementales à recommander l'ajout de DHA mais également d'ARA dans les préparations lactées pour les nourrissons, en s'inspirant des teneurs retrouvées dans le lait maternel ; à hauteur de 0,3 % des acides gras totaux pour le DHA, en équilibrant cet apport par la co-supplémentation avec de l'ARA (0,5 % des acides gras totaux) [17]. Depuis 2020, l'Union européenne impose également l'introduction de DHA dans les laits infantiles à un niveau plus élevé (0,5-1 %) [3], mais pas celle d'ARA et sans avancer d'argumentation scientifique précise [24]. L'intérêt de l'ajout d'ARA fait l'objet de débats bien détaillés dans la revue récente de Tounian et al. [25]. Tout d'abord, les formules infantiles ont pour objectif d'être biomimétiques du lait maternel, or ce dernier contient à la fois du DHA et de l'ARA à un ratio proche de 1:2. De plus, le taux de bioconversion du LA en ARA n'est pas suffisamment important pour permettre une couverture totale des besoins nutritionnels des enfants de cette tranche d'âge. L'ARA joue un rôle crucial, au même titre que le DHA, dans le développement cognitif et immunitaire et est en outre impliqué dans un large panel de fonctions biologiques dont certaines restent à identifier [25]. De nombreuses études ont donc démontré de manière concluante que l'ajout simultané d'ARA et de DHA dans les formules infantiles réduisait les occurrences d'inflammation et d'allergies dans l'enfance. La supplémentation des formules en ARA est ainsi fortement recommandée par la communauté scientifique. Rappelons que des apports nutritionnels recommandés sont également proposés pour la femme enceinte et allaitante [17]. Les enquêtes alimentaires montrent cependant que les niveaux de consommation en AGPI n-3 (ALA, DHA) sont très insuffisants dans ce groupe de femmes en France, comme dans la majorité des pays industrialisés, et restent très similaires à celui de la population générale [26].

### Produit commerciaux vs lait maternel : quelles différences ?

#### Types de produits commerciaux et réglementation liée à leur composition

Un apport nutritionnel adapté aux besoins évolutifs de l'enfant est donc fondamental pour garantir à la fois une croissance et un développement en bonne santé. Durant les six premiers mois, un allaitement exclusif est fortement recommandé, suivi jusqu'au deux ans de l'enfant par un allaitement de complément [27]. En effet, le lait maternel



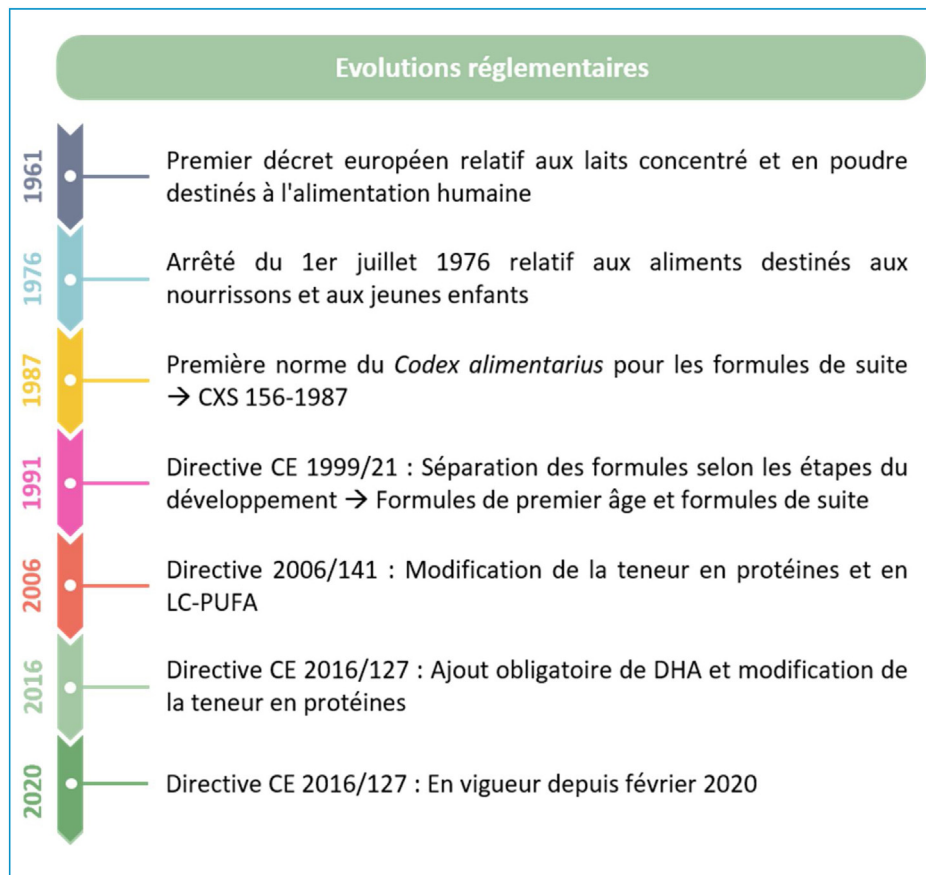


Figure 4. Évolutions réglementaires concernant les formules infantiles.

d'une femme ayant un régime alimentaire varié et équilibré est considéré comme « l'étalon d'or » en termes de nutrition néonatale. La composition du lait maternel est fortement influencée par le régime alimentaire de la mère. Cette influence est particulièrement importante sur les lipides (comparés à d'autres nutriments tels que les protéines ou les polysaccharides) et impacte ainsi la teneur en certains acides gras essentiels [4]. Néanmoins, le lait maternel reste la source de nutriments la plus adaptée aux besoins de l'enfant. En cas d'impossibilité et/ou d'échec de l'allaitement pour des raisons diverses (physiologiques, contraintes socioprofessionnelles, choix personnel...), des formules infantiles dites de premiers âges sont développées spécifiquement pour répondre aux besoins des nourrissons de 0 à 6 mois. Dans le cas de nourrissons prématurés, des formules enrichies en énergie, macro et micronutriments par rapport aux formules infantiles classiques sont proposées bien qu'une fois encore, la fortification du lait maternel ou de lait de donneuses soit recommandée. La deuxième étape du développement, de 6 à 12 mois, est caractérisée par un besoin énergétique plus important. Le lait maternel et les préparations de suite, spécifiques pour cette tranche d'âge, ne permettent plus de satisfaire entièrement les besoins nutritionnels de l'enfant. De plus, à ce stade une certaine maturité digestive est acquise [28] ce qui permet l'introduction progressive d'aliments dits de compléments. La phase de diversification alimentaire débute donc lors de cette période. Il s'agit d'une transition d'un

régime alimentaire exclusivement lacté à une alimentation diversifiée. Enfin, lors de la troisième étape du développement, de l'âge de 12 mois à 3 ans, la diversification alimentaire se poursuit en complément des formules infantiles de croissance jusqu'à devenir exclusive. Bien que de récentes modifications de ces stades aient été suggérées par des groupes de recherche [29] pour être davantage en accord avec la physiologie du nourrisson en développement, cette segmentation des préparations infantiles reste la référence qui correspond à la réglementation européenne.

Afin de garantir que la composition des préparations infantiles de suite soit adaptée aux besoins nutritionnels de l'enfant, les réglementations internationales et européennes imposent des teneurs seuils en macro- et micronutriments. Ces différentes réglementations [30–32] se basent sur les recommandations établies selon la composition du lait maternel et des connaissances scientifiques. D'importantes évolutions réglementaires ont récemment été validées avec l'entrée en vigueur de la nouvelle réglementation européenne en février 2020 qui a mis à jour les critères de composition des formules infantiles de premier et deuxième âge (Fig. 4). Parmi les modifications majeures, la teneur en protéines a été diminuée afin de limiter le risque de développement d'un surpoids à l'âge adulte [33] et les valeurs seuils pour certaines vitamines et minéraux ont été modifiées. Enfin, les teneurs limites en certains acides gras indispensables ont été revues avec une augmentation de

**Tableau 1** Comparaison des valeurs nutritionnelles moyennes des préparations infantiles de suite avec les valeurs seuils imposées par les réglementations européenne et internationale et la composition typique du lait maternel mature [37].

| (/ 100 mL)      | Préparations infantiles de suite <sup>a</sup> | Réglementation UE<br>CE 2016/127 |       | Codex Alimentarius<br>CX 156/1987 |       | Lait maternel |
|-----------------|---|----------------------------------|-------|-----------------------------------|-------|---------------|
|                 |   | Min                              | Max   | Min                               | Max   |               |
| Energie (kcal)  | 67,2 ± 1,3                                    | 60                               | 70    | 60                                | 85    | 67            |
| Lipides (g)     | 3,3 ± 0,2                                     | 2,64                             | 4,2   | 1,8                               | 5,1   | 3,8           |
| AGS (g)         | 1,1 ± 0,4                                     | —                                | —     | —                                 | —     | 1,83          |
| AGMI (g)        | 1,5 ± 0,4                                     | —                                | —     | —                                 | —     | 1,51          |
| AGPI (g)        | 0,6 ± 0,1                                     | —                                | —     | —                                 | —     | 0,41          |
| LA (mg)         | 502,2 ± 0,1                                   | 300                              | 840   | 180                               | —     | 338,2         |
| ALA (mg)        | 53,7 ± 12,6                                   | 30                               | 70    | —                                 | —     | 45,6          |
| ARA (mg)        | 9,4 ± 5,6                                     | —                                | 42    | —                                 | —     | 27,4          |
| DHA (mg)        | 14,8 ± 4,0                                    | 12                               | 35    | —                                 | —     | 13,8          |
| Glucides (g)    | 7,8 ± 0,4                                     | 5,4                              | 9,8   | —                                 | —     | 7             |
| Protéines (g)   | 1,4 ± 0,2                                     | 1,08                             | 1,75  | 1,8                               | 4,7   | 1             |
| Caséines (g)    | 0,8 ± 0,3                                     | —                                | —     | —                                 | —     | 0,3           |
| Vitamine A (µg) | 61,5 ± 6,4                                    | 42                               | 79,8  | 45                                | 191,3 | 53            |
| Vitamine D (µg) | 1,4 ± 0,3                                     | 1,2                              | 2,1   | 0,6                               | 2,55  | 0,1           |
| Vitamine E (mg) | 1,2 ± 0,4                                     | 0,36                             | 3,5   | 0,28                              | —     | 0,54          |
| Vitamine C (mg) | 9,8 ± 2,3                                     | 2,4                              | 21    | 4,8                               | —     | 4,7           |
| Fer (mg)        | 0,976 ± 0,155                                 | 0,36                             | 1,4   | 0,6                               | 1,7   | 0,04–0,076    |
| Cuivre (mg)     | 0,052 ± 0,006                                 | 0,036                            | 0,070 | —                                 | —     | 0,02–0,04     |

<sup>a</sup> Valeurs nutritionnelles moyennes obtenues à partir de l'analyse statistique de la composition de 91 préparations infantiles de suite présentes sur le marché mondial.

la limite inférieure en LA, de la limite supérieure en ALA et une obligation de supplémenter les formules en DHA à hauteur de 20 à 50 mg pour 100 kcal (soit 0,5 à 1 % des acides gras totaux) ce qui était, jusqu'à présent, facultatif. Cependant, un vide réglementaire persiste concernant l'ajout d'ARA qui n'est toujours pas obligatoire malgré son importance avérée pour le développement de l'enfant [24].

La comparaison des valeurs nutritionnelles moyennes du lait maternel mature et celles de 91 préparations infantiles de suite présentes sur le marché a mis en évidence une adéquation au niveau des teneurs en macronutriments (Tableau 1), avec par exemple des teneurs en lipides respectivement de 3,8 et 3,3 g/100 mL [34]. Malgré ces similarités de teneurs et compositions globales, des différences persistent notamment au niveau de la composition en posés mineurs, du profil en acides gras et de leur répartition sur le squelette triglycéridique. Ces différences de composition et de structure entre les préparations infantiles de suite et lait maternel, ont été analysées en détail dans de précédentes revues [35,36].

### Différences en termes de structure et composition lipidique

D'un point de vue structural, comme le montre la Fig. 5, la fraction lipidique du lait maternel est sous forme de globule gras d'environ 4 µm de diamètre composé d'un cœur de TAG entouré d'une tricouche phospholipidique avec une importante diversité de composés à l'interface. Dans les formules infantiles, elle se présente sous forme de gouttelettes lipidiques d'environ 0,4 µm de diamètre avec un cœur

de TAG entouré d'une monocouche stabilisée par des protéines. Ces différences structurales ont une forte influence sur la digestibilité de leurs lipides du fait de la modification des cinétiques de protéolyse et lipolyse [36,38]. Le profil en acides gras ainsi que leur répartition sur les TAG ont également un impact sur l'absorption lipidique. Dans les formules infantiles commerciales de suite, la phase grasse résulte d'un mélange d'huiles majoritairement d'origine végétale. Selon la source d'huile végétale utilisée, le profil en acides gras varie fortement notamment au niveau des teneurs en saturés et de l'abondance en chaînes courtes (C4-C8) et moyennes (C9-C12). Ainsi, les huiles de palme, coco, tournesol et colza sont couramment utilisées. Les mélanges formulés à partir de ces huiles visent à mimer le taux de saturation du lait maternel (47 %), néanmoins une analyse statistique de la composition nutritionnelle moyenne de 91 formules infantiles de suite présentes sur le marché [1] réalisée par notre groupe a démontré que ce taux était plus bas (33 % environ) dans les produits commerciaux. En ce qui concerne les chaînes courtes et moyennes, à l'exception de l'huile de coco, dont l'acide gras majoritaire est l'acide laurique (C12:0), les huiles végétales n'en contiennent pas ou peu. Par conséquent, cette catégorie de lipides ayant l'intérêt de pouvoir être métabolisé très rapidement par la voie de la veine porte est peu représentée dans les formules infantiles à base d'huiles végétales comparé au lait maternel [39]. Outre le profil en acides gras des TAG, une différence notable entre préparations infantiles de suite et lait maternel est observée au niveau de la répartition des acides gras sur les TAG. Elle résulte d'une différence originelle entre lipides d'origine animale (saturés en

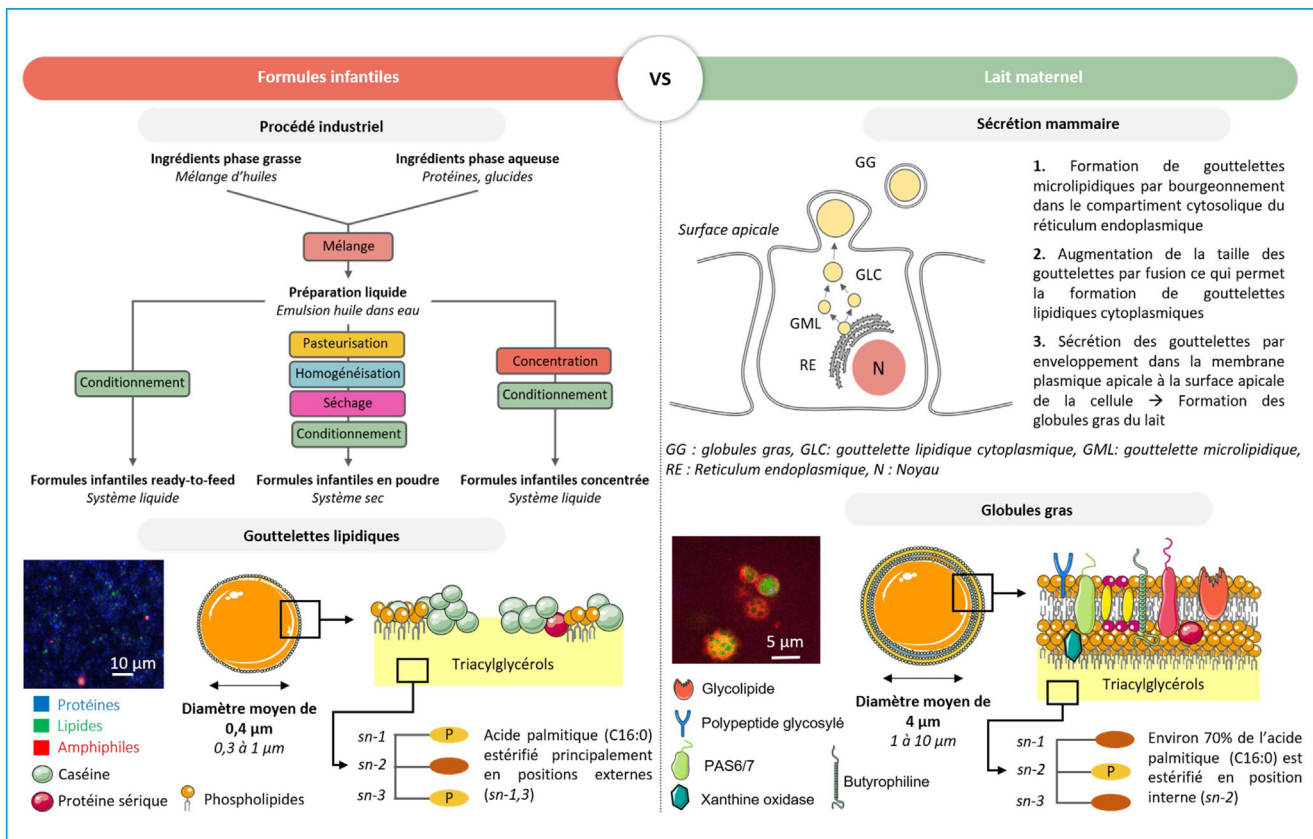


Figure 5. Comparaison de la structure lipidique des formules infantiles et du lait maternel. Adapté de [1].

position interne majoritaire) et végétale (saturés en position externe majoritaire). En effet, dans le lait maternel, environ 70 % de l'acide palmitique (C16:0), acide gras saturé majoritaire, est estérifié en position interne (*sn*-2) du TAG [40]. En revanche, dans les huiles végétales qui constituent la phase grasse des formules infantiles de suite, cet acide gras est principalement localisé en positions externes (*sn*-1 et 3) ce qui modifie les cinétiques de digestion et les taux d'absorption des lipides mais aussi de certains minéraux tels que le calcium ou le magnésium [41]. Il est admis que l'organisation des TAG du lait maternel, avec l'acide palmitique estérifié préférentiellement en position interne (*sn*-2), permet une meilleure absorption lipidique en évitant la formation de savons insolubles (palmitate de calcium) qui sont directement excrétés [40,42]. Ainsi, le coefficient d'absorption lipidique dans le lait maternel est globalement plus élevé que dans les formules infantiles (74 à 91 % pour le lait maternel vs 69 à 85 % pour les formules infantiles chez les enfants prématurés) [43]. De plus, l'étude de la distribution des positions des acides gras dans le lait maternel a montré que la majorité des AGPI sont estérifiés en positions externes (*sn*-1,3) alors que les AGPI-LC, tels que le DHA et l'ARA, sont quant à eux principalement localisés en position interne (*sn*-2) [44] ce qui permet d'améliorer leur biodisponibilité [45,46].

Afin de formuler des analogues de lipides du lait maternel d'un point de vue structural, différentes méthodes sont utilisées telles que l'interestérification qui permet

d'obtenir des lipides structurés avec des concentrations élevées d'acide palmitique en position *sn*-2 ou une estérification préférentielle du DHA et d'ARA en cette même position. Mais ces stratégies restent trop coûteuses et peu appliquées. À l'inverse, la recommandation de réintroduction de matière grasse laitière bovine à hauteur de 50 % des lipides totaux permet d'avoir une structure des lipides plus proches de celle d'un lait maternel. Cette approche permet en effet au-delà des TAG de se rapprocher de la complexité chimique globale du lait maternel en ajoutant des composés lipidiques mineurs tels que le cholestérol et d'autres lipides complexes comme les glycérophospholipides ou encore les sphingolipides. Ces composés mineurs ne sont pas présents dans la composition des lait infantiles commerciaux qui ne comprennent pas d'extraits de lipides polaires laitiers bovins [6].

## Difficultés liées à la supplémentation des formules en acide docosahexaénoïque

### Supplémentation en AGPI : les différentes sources

La réglementation européenne impose maintenant la supplémentation des préparations infantiles de suite en DHA qui doit être ajouté entre 20 et 50 mg pour 100 kcal. La supplémentation en ARA n'est pas obligatoire ; en revanche une

limite maximale est fixée pour ce dernier à hauteur de 1 % de la teneur totale en matières grasses (soit un maximum de 60 mg pour 100 kcal ou 42 mg pour 100 mL de formule) (Tableau 1). Afin d'enrichir les formules infantiles de suite en ces AGPI-LC et ainsi atteindre les valeurs réglementaires ou celles établies par les recommandations de la communauté scientifique, des huiles de poissons sont usuellement ajoutées aux formulations. Des huiles d'origine algale telles que les huiles issues de *Schizochytrium sp.* ou *Crythecodinium cohnii* ou fongique comme l'huile de *Mortierella Alpina* sont également de bonnes sources de DHA et ARA et peuvent être également utilisées.

## Sensibilité à l'oxydation et devenir des produits d'oxydation

Cependant, leur emploi peut s'avérer complexe du fait de leur fort degré d'insaturation qui les rend particulièrement sensibles à l'oxydation. Le phénomène d'oxydation lipidique est l'une des principales causes de dégradation des produits alimentaires et plus particulièrement des produits riches en AGPI tels que les formules infantiles. D'un point de vue chimique, il peut se produire selon trois voies : l'auto-oxydation, l'oxydation enzymatique et la photo-oxydation. De nombreux facteurs peuvent influencer sur les mécanismes d'initiation et de propagation, et ainsi accélérer l'oxydation lipidique : c'est par exemple le cas de la température, la concentration en oxygène, la présence d'insaturations sur la chaîne carbonée ou encore la présence d'espèces pro-oxydantes tels les métaux de transition. L'auto-oxydation, induite par l'oxygène triplet, est la voie d'oxydation majoritaire dans les produits transformés. Les traitements thermiques d'inactivation enzymatique ainsi que des emballages barrière à l'oxygène appliqués aux formules infantiles font que l'auto-oxydation est prédominante. Les produits résultants de cette auto-oxydation sont, dans un premier temps, des hydroperoxydes (composés primaires d'oxydation) qui réagissent ensuite pour générer entre autres des substances volatiles (composés secondaires d'oxydation), lesquels sont responsables de la perception d'un défaut organoleptique du produit concerné. Ces composés secondaires d'oxydation appartiennent pour la plupart à la famille des aldéhydes ou des cétones, mais le type de produits formés est tributaire de la nature des AGPI constituant le produit concerné (hexanal, (Z)-3-hexenal, (Z)-2-nonanal, 2-heptanone et 2-nonaone couramment détectés dans les formules infantiles). Ainsi, l'oxydation lipidique est non seulement à l'origine de la formation de saveurs et d'arômes indésirables mais également conduit à une perte de la valeur nutritionnelle du produit en raison de la dégradation en premier lieu des vitamines liposolubles (vitamine A plus sensible que vitamine E) puis des acides gras insaturés. L'oxydation touche aussi des antioxydants non lipidiques comme les protéines, des acides aminés, la vitamine C ou encore le cholestérol (production par auto-oxydation d'un oxystérol, le 7-kétocholestérol). De plus, cette dégradation chimique conduit également à l'apparition d'une multitude de produits néo-formés de structures chimiques diverses et dont certains sont avérés toxiques pour la santé humaine (e.g., 7-kétocholestérol,

[47]). Le lait maternel n'échappe pas à cette oxydation dès qu'il est stocké post-sécrétion [48].

## Les étapes clés : le procédé de fabrication et la conservation

De nombreux travaux de recherche s'intéressent au phénomène d'oxydation lipidique et aux moyens qui peuvent être mis en place afin de le limiter. L'une des stratégies la plus couramment étudiée est l'ajout d'antioxydants ou de mélange d'antioxydants dans le produit concerné. Cette stratégie est illustrée dans le Tableau 2. Cependant, la réglementation concernant leur utilisation dans les formules infantiles de suite est stricte, et définit explicitement les quantités pouvant être ajoutées et ce, suivant la nature de l'antioxydant mis en œuvre. Par exemple, des teneurs maximales respectivement de 5 et 30 mg pour 100 kcal d' $\alpha$ -tocophérol et de vitamine C, deux constituants développant une synergie, sont autorisées. La seconde stratégie toute aussi importante est la maîtrise des mécanismes de l'oxydation lipidique au sein de la matrice alimentaire concernée afin de pouvoir limiter ce phénomène de dégradation chimique. L'objectif est donc de garantir une résistance à l'oxydation des systèmes tout au long de leur date de durabilité minimale (DMM) tout en garantissant un profil nutritionnel adapté aux besoins de l'enfant.

Outre l'ajout d'antioxydants, il est possible d'intervenir sur la composition en acide gras et la structure des lipides (acides gras, triacylglycérols, glycérophospholipides). Bien évidemment, estérifier sur les TAG des groupements acyls saturés ou monoinsaturés pas ou peu sensibles à l'oxydation est un facteur stabilisant primordial. La forme moléculaire des lipides influence également leur sensibilité à l'oxydation. Ainsi, certains auteurs rapportent une oxydation plus rapide du DHA sous forme de TAG que sous forme d'esters éthyliques ou de phospholipides [55]. Les phospholipides sont néanmoins très souvent plus rapidement oxydés que les TAG du fait de leur plus grande concentration en AGPI. Les réactions d'hydrolyse générant des acides gras libres favorisent leur oxydation, via des phénomènes d'interactions avec des ions métalliques pro-oxydants [56]. Au sein des TAG, la sensibilité à l'oxydation des acides gras dépend de leur position (position centrale *sn*-2 mieux protégée) et des acides gras environnants. La conformation des TAG avec de l'acide palmitique majoritairement en position interne permet d'améliorer la métabolisation des lipides mais rend les mélanges d'huiles plus sensibles à l'oxydation du fait d'une plus large proportion des acides gras insaturés en position externe [57]. L'état physique (solide/liquide) des TAG et glycérophospholipides (liquide condensé, liquide expansé ou gel) qui détermine les partages de phases et concentrations locales en substrats, agents pro- ou antioxydants influence également les cinétiques d'oxydation. Une restructuration d'une partie des mélanges d'huiles utilisés pour les formules infantiles de suite permettrait d'augmenter les teneurs en acide palmitique en position interne tout en conservant la localisation AGPI-LC des huiles végétales (estérification en *sn*-2). En outre, l'état de la matière grasse libre (en surface du grain) ou emprisonné dans le grain de poudre modifie fortement sa susceptibilité à l'oxydation (plus forte si état libre) [51]. Néanmoins,



**Tableau 2** Résumé d'une sélection d'études emblématiques des paramètres de formulation, de structure ou environnementaux (température, espace de tête...) influençant les cinétiques d'oxydation des formules infantiles.

| Objectifs  | Principaux résultats   | Réf./année |
|--|--|------------|
| Déterminer les cinétiques d'oxydation à l'air de formules infantiles stabilisées par différentes sources protéines : laitières (L), de soja (S) ou hydrolysées (H) (stockage soit à 32 °C, soit à 55 °C) sur 12 mois   | Pertes moyennes en tocophérols(ppm/mois) de : -1,54 L, -1,94 H, -2,47 S (32 °C) et majoritairement plus élevées à 55 °C. Concentration en 7-kétocholestérol corrélée aux pertes en tocophérol. TBARS augmentant de 1,5 (T0, L et H) à 6–7 (12 mois) (mmol/100 g) vs 2.8 (T0, S) à ~17 (12 mois) à 32 °C. Perte de 3 % en moyenne des AG essentiels sur 12 mois | [49]/1998  |
| Déterminer l'effet de la lactoferrine sur la stabilité oxydative de deux formules infantiles (WF : formule à base de protéines sériques ; CF : formule à base de caséines) enrichies ou non en fer (0 ; 88 ; 172 ou 220 µM) et stockées à 50 °C pendant 20 jours                       | Oxydation plus rapide dans les échantillons CF. Activité antioxydante de la lactoferrine quelle que soit sa concentration. Plus forte capacité antioxydante pour un ratio molaire lactoferrine/fer de 2:1 qui permet une inhibition de plus de 75 % de la formation d'hydropéroxydes et d'hexanal dans l'ensemble des échantillons                             | [50]/2000  |
| Déterminer la stabilité à l'oxydation de formules infantiles à 25, 30 et 37 °C pendant 3 mois. Comparer l'état d'oxydation de la fraction libre de la matière grasse par rapport à l'ensemble de la matière grasse   | Pas d'évolution significative de la teneur en AGPI, de l'état d'oxydation ni des pertes en tocophérols pour la matière grasse totale sur 3 mois à 37 °C. En revanche, oxydation de la fraction matière grasse libre (7,5 % du total) à 3 mois et pertes en tocophérols variant entre -40 et -50 % (entre 25–37 °C)   | [51]/2010  |
| Etudier l'effet d'antioxydants autorisés à 50 ppm ( $\alpha$ -tocophérol, $\beta$ -carotène, palmitate d'ascorbyle, acide ascorbique, acide citrique, et leurs combinaisons) sur la stabilité à l'oxydation d'une formule contenant des TAG structurés (avec DHA) sur 28 jours à 37 °C | Effet des antioxydants variables suivant leur mécanisme d'action et polarité ; effet plus efficace du palmitate d'ascorbyle à 50 ppm mais moins efficace qu'une atmosphère inertée par N <sub>2</sub> ; synergie la plus forte observée entre $\alpha$ -tocophérol et $\beta$ -carotène  | [52]/2015  |
| Evaluer la stabilité à l'oxydation de 2 formules infantiles (FI1 : premier âge, FI2 : lait de suite) à base de protéines laitières sur 90 jours (42 ou 50 °C)  | Stabilité des AGPI (C18:1 n-9, C18:2 n-6, C18 :3 n-3, C20:4 n-6, C20:5 n-3 et DHA C22:6 n-3) de 0 à 90 jours excepté une perte en DHA à 90 jours dans FI1 (de -4 % à 42 °C et -7 % à 50 °C). Effet de la température sur les composés primaires et secondaires d'oxydation et la consommation d'O <sub>2</sub> à 90 jours                                      | [53]/2019  |
| Comparer la stabilité à l'oxydation de formules contenant ou non du DHA (0,5 g/kg), de l'ARA (1,25 g/kg), les deux, les deux en présence de métaux (Zn, Fe, Cu, Mn) et les deux en présence de vitamines C et E (2,5 et 0,125 g/kg)  | Beaucoup de variabilité sur les résultats mais l'addition de DHA et ARA augmente les TBARS à 3–6 et 12 mois, l'addition de métaux ou vitamines n'affecte pas ces TBARS. La teneur en matière grasse libre n'est pas impactée significativement par les changements de formulation  | [54]/2020  |

l'analyse de cette matière grasse libre dans un jeu restreint de formules infantiles sur 30 jours après l'ouverture de l'emballage a indiqué sa bonne stabilité sur ce temps correspondant aux recommandations de conservation post-ouverture des formules infantiles en poudre [58].

Dans ce contexte, la réintroduction de matière grasse laitière bovine, qui a été progressivement remplacée par des huiles végétales à partir des années 70 notamment pour des raisons économiques dans la formulation des mélanges

d'huiles des formules infantiles apparaît comme une stratégie très intéressante [59]. En effet, la matière grasse laitière présente des similarités avec les lipides du lait maternel à la fois au niveau de sa composition en acides gras et de leur localisation sur les TAG. Plusieurs études [36,60] ont souligné l'intérêt des matières grasses laitières bovines comme source d'acides gras saturés (y compris à chaînes courtes et moyennes) et d'acides gras saturés à très longues chaînes à la fois sous forme de TAG et de lipides polaires qui stabilisent

contre le processus d'oxydation. Ainsi, l'utilisation à la fois d'huiles végétales (pour leur teneur en acides gras insaturés), et d'huiles marine, algale ou fongique (pour leur teneur en AGPI-LC) et de matière grasse laitière (pour sa structure et sa composition, notamment en acides gras saturés), permettrait d'atteindre un certain équilibre entre la stabilité vis-à-vis de l'oxydation lipidique et l'amélioration du profil nutritionnel de la phase grasse des formules infantiles de suite.

Les formules infantiles de suite correspondent à des systèmes émulsionnés liquides ou séchés par pulvérisation. Afin de les stabiliser d'un point de vue physique, la réglementation autorise l'ajout aux formulations de certains agents émulsifiants tels que les lécithines, les mono- et diacylglycérols et les glycérophospholipides. Certaines protéines comme les protéines sériques ou les caséines ont également des propriétés émulsifiantes et peuvent être utilisées. Le type et la concentration d'émulsifiant employé exercent une influence notable sur la stabilité des préparations de suite. L'étude de Drapala et al. [61] a montré que 1 % w/w de lécithine de soja dans une émulsion modèle représentative des formules infantiles permettait de ralentir significativement l'oxydation. En revanche, au-delà de cette teneur, aucun effet positif n'était observé par ces mêmes auteurs. De manière générale, les émulsifiants à base de protéines et notamment les protéines sériques semblent montrer une meilleure efficacité que les lécithines pour limiter l'oxydation. Parmi les protéines sériques, la lactoferrine capable de piéger et de transporter le fer peut aussi être utilisée à certaines concentrations pour ses effets antioxydant et antimicrobien [50]. Ces résultats sur les effets antioxydants des émulsifiants au sein des formules sont cependant à considérer avec précautions en raison de la multi-factorialité de l'oxydation lipidique, laquelle implique de nombreux phénomènes de réactivités chimiques entre les espèces moléculaires présentes dans la formule ainsi que des phénomènes physico-chimiques liés à la nature hétérogène de ces mêmes formules [49,62].

Un autre paramètre primordial pour assurer la stabilité des préparations de suite supplémenté en DHA est de choisir la qualité des matières premières avec un contrôle strict à réception de leur niveau d'oxydation et l'établissement de cahier des charges avec les différents fournisseurs. En effet, le niveau d'oxydation initial des produits manufacturés conditionne, en partie, leur stabilité durant le stockage. En effet, une fois l'étape d'initiation de l'oxydation achevée, le phénomène se propage rapidement à l'ensemble du produit. L'objectif est donc de retarder au maximum cette phase de latence.

Pour limiter l'occurrence du phénomène d'oxydation, il est également possible d'agir sur leur procédé de fabrication. Ce dernier implique une succession d'opérations unitaires dont une étape de traitement thermique [63] qui a pour objectif de garantir la salubrité des produits (Fig. 5). Suite à la pasteurisation des produits, une étape d'homogénéisation, le plus souvent réalisée à l'aide de hautes pressions, permet d'émulsionner le système. Enfin, l'étape de séchage, principalement réalisée par atomisation, permet de déshydrater le produit et d'obtenir ainsi une formule infantile sous forme de poudre. Ces différents traitements engendrent des augmentations de température parfois accompagnés de cisaillements/création d'interface

air/liquide pouvant favoriser l'oxydation des lipides et conduire à une perte significative de certaines vitamines et antioxydants, comme la vitamine A, la vitamine C et l' $\alpha$ -tocophérol bien que cette dernière soit supposée assez thermorésistante.

Par ailleurs, les conditions de stockage des formules infantiles de suite ont également un impact important sur la stabilité des produits et doivent donc être ajustées afin de retarder au maximum l'initiation de l'oxydation ou ralentir sa propagation. Il est ainsi possible d'agir par exemple sur la température de conservation, le taux d'oxygène dans l'espace de tête du produit ou encore le type d'emballage. L'atmosphère modifiée est largement utilisée pour le conditionnement des préparations infantiles en poudre et son efficacité a été démontrée dans plusieurs études [64,65]. Cependant, cette modification doit induire l'exclusion quasi-total de l'oxygène, car on considère que la présence d'oxygène résiduel (environ 2 %) peut tout de même initier les mécanismes d'oxydation lipidique [66]. Afin de limiter davantage l'occurrence du phénomène d'oxydation durant le stockage, de nouveaux emballages sont conçus avec par exemple l'inclusion d'antioxydants comme l'acide ascorbique ou l' $\alpha$ -tocophérol dans les matériaux. A titre d'exemple, les travaux de Jo et al. [67] ont montré que l'utilisation combinée d'atmosphère modifiée et d'emballages antioxydants sur lesquels ont été greffés de l'acide ascorbique et des tocophérols permettait d'améliorer significativement la stabilité oxydative d'une formule infantile en poudre.

## Conclusion

Un des défis majeurs pour la nutrition du nourrisson puis du jeune enfant est de concevoir des formules infantiles de suite stables vis-à-vis de l'oxydation lipidique et biomimétiques du lait maternel aussi bien en termes de composition que de structure. Dans ce contexte, la réglementation européenne a évolué pour encourager l'optimisation des formules de suite avec une attention particulière pour la composition en lipides du fait de leur rôle primordial durant la période de l'enfance. Ainsi, l'ajout désormais obligatoire de DHA et conseillé d'ARA rend les formules infantiles de suite plus sensibles au phénomène de dégradation qu'est l'oxydation. Cette revue a souligné l'importance des AGPI-LC pour le développement de l'enfant et les difficultés associées à leur introduction dans les produits de l'alimentation infantile. De nombreuses études se concentrent alors sur l'identification de paramètres permettant de maîtriser les mécanismes de l'oxydation lipidique. Il apparaît que la composition et la structure des lipides, la qualité des matières premières, le type d'émulsifiants utilisés et les procédés de fabrication puis de stockage des formules ont un impact important sur leur stabilité. L'oxydation lipidique est donc multifactorielle, cependant, du fait de la complexité des mécanismes, la majorité des études réalisées se concentrent sur l'optimisation d'un nombre restreint de paramètres en formulation ou génie des procédés. Des études complémentaires sont donc nécessaires afin de mesurer l'effet combinés de plusieurs paramètres et ainsi atteindre un équilibre entre profil



nutritionnel adapté aux besoins de l'enfant et stabilité vis-à-vis de l'oxydation lipidique.

## Déclaration de liens d'intérêts

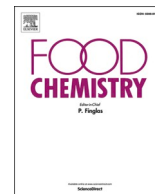
Les auteurs déclarent ne pas avoir de liens d'intérêts.

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# Comparison of the effect of various sources of saturated fatty acids on infant follow-on formulas oxidative stability and nutritional profile

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## ABSTRACT

Fortification of infant follow-on formulas (IFF) with docosahexaenoic acid (DHA), which is prone to lipid oxidation, is required by European regulation. This study aimed to identify lipid formulation parameters that improve the nutritional profile and oxidative stability of IFF. Model IFF were formulated using different lipid and emulsifier sources, including refined (POM) or unrefined red palm oil (RPOM), coconut oil (COM), dairy fat (DFOM), soy lecithin, and dairy phospholipids (DPL). After an accelerated storage, RPOM and DFOM with DPL had improved oxidative stability compared to other IFF. Specifically, they had a peroxide value twice lower than POM and 20% less loss of tocopherols for DFOM-DPL. This higher stability was mainly explained by the presence of compounds such as carotenoids in RPOM and sphingomyelin in DFOM-DPL very likely acting synergistically with tocopherols. Incorporation of dairy lipids and carotenoids into DHA-enriched IFF compositions seems promising to enhance their stability and nutritional quality.

## 1. Introduction

The infancy period and especially the first two years of life, which are included in the 1000-days concept, is a crucial period of human development (Martorell, 2017). At this stage of life, nutrition is essential to support the very rapid growth and development and the infant diet is highly involved in the programming effects (Koletzko et al., 2017). Among the nutrients, lipids are the dominant energy provider during infancy, with a supply of 45 to 55% of the calories via their  $\beta$ -oxidation. They are also structural membrane components and precursors of several cell mediators involved in the cerebral, visual, intestinal and immune development of infants (Delplanque et al., 2015).

To determine the optimal lipid supply for neonatal nutrition, breast milk is often considered as the “gold standard”. Although its composition is affected by many factors (one of the most influential being the mother’s diet), it remains the most appropriate source of nutrients for

infant’s needs (Jensen, 1999). In breast milk, lipids are found as the very specific form of milk fat globules, i.e. a trilayered membrane of polar lipids and proteins that stabilizes a core of complex triacylglycerols (TAG). These latter contain a significant amount of saturated fatty acids (SFA), but also essential fatty acids (FA) – linoleic acid (LA, C18:2n-6) and alpha-linolenic acid (ALA, C18:3n-3) – precursors of long-chain polyunsaturated fatty acids (LC-PUFA) such as arachidonic acid (ARA, C20:5n-6) and docosahexaenoic acid (DHA, C22:6n-3). When breast-feeding is impossible or fails for various reasons, infant formulas can be administrated and are developed specifically to meet the needs of infants at a given age. During the second stage of development, which extends from 6 to 12 months, the infant’s diet is mainly composed of a dairy fraction via breast milk and/or infant follow-on formulas (IFF). The IFF composition is governed by strict regulations and evolves to be in line with the recommendations, especially concerning the content of LC-PUFA. Since 2020, the addition of DHA to IFF has been mandatory in

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European countries (EU regulation CE 2016/127). The addition of ARA is not required yet, but it is strongly recommended by the scientific community (Tounian et al., 2021). It is therefore usually added in the composition of IFF.

Because of their multiple unsaturated double bonds, LC-PUFA are very prone to lipid oxidation and this degradation leads to the appearance of a multitude of neoformed products, some of which are detrimental to human health (Schaich, 2020). Because of this susceptibility to oxidation, a higher inclusion level of LC-PUFA in IFF is challenging. Oxidation also affects antioxidants such as fat-soluble vitamins (vitamins A and E) that are first degraded. This oxidation can be limited by several strategies of which the most common is the addition of antioxidants (Cancelon et al., 2022). Selecting the protein sources is also possible to limit oxidation using, for example, among whey proteins lactoferrin which is able to chelate metal ions and thus to block their pro-oxidant effect (Elias et al., 2008, Satué-Gracia et al., 2000). Recent regulatory evolutions have reinforced the need to identify efficient stabilization levers, including those related to the formulation and particularly to lipid formulation.

In commercial IFF, the oil phase is a mixture of oils mainly of vegetable origin in which palm oil is the most frequent source of SFA. Moreover, IFF are generally enriched in vitamins (especially vitamins A and E) and minerals (Delplanque et al., 2018). Depending on the source of oil used, the FA profile varies greatly, especially in terms of the amount of short (C4-C8) and medium (C10-C12) chains. Numerous studies have shown that these FA play an important role during infancy period, particularly because they are more easily lipolyzed and metabolized by the portal vein pathway than long-chain FA (Yuan et al., 2022). In this regard, dairy fats, whose most common commercial source worldwide is bovine source, are the most biomimetic of breast-milk lipids but they need to be supplemented in PUFA for their use in IFF. TAG fraction found in dairy fat can be also supplemented by dairy polar lipids, a concentrated source of sphingomyelin (SM) and LC-PUFA glycerophospholipids that have beneficial nutritional effects and may improve the IFF stability (Bourlieu et al., 2018).

The impact of several parameters (addition of antioxidants or proteins sources) on the resulting oxidative stability of IFF is largely documented. However, to the best of our knowledge, the comparison of various saturated fats sources on IFF oxidative stability and nutritional profile has never been undertaken. The presence and type of SFA lowers the melting point of oil mixtures possibly leading to partial crystallization, which in an emulsified system leads to the formation of a physical barrier that could then limit oxidation phenomena.

The present study aims at comparing different lipid sources of SFA in model IFF representative of marketed products (palm, red palm and coconut oils and dairy fat). In all these sources the SFA content account for more than 45% of their fatty acid profiles (supplementary table 1). The set hypothesis was therefore that variation in these lipid sources would modulate their oxidative stability.

Model IFF were thus formulated with equivalent chemical composition and structure, i.e. with a normalization of vitamins E and A, SFA, monounsaturated fatty acid (MUFA), PUFA, DHA and ARA contents and similar droplet size.

## 2. Materials and methods

### 2.1. Materials

Sunflower (*Helianthus annuus*) and rapeseed (*Brassica napus*) oils, from the Casino brand (Saint-Etienne, France), were purchased in local supermarkets. Coconut oil from BIO PLANÈTE - Huilerie Moog SAS (Bram, France) and red palm oil (*Elaeis Guineensis*) from La Pangée (Montpellier, France) were purchased in specialised grocery shops. High oleic sunflower oil was purchased from Cuisinor (Saint-Médard de Guzières, France). Fish oils (ARASCO® and DHASCO®) were generous gifts from DSM Nutritional products France (La Garenne Colombes,

France). The dairy fats and refined palm oil were also gifts from Corman (Limburg, Belgium) and Cargill (Paris, France). The fatty acid profiles of all these oils are presented in supplementary table 1. Soy lecithins and dairy phospholipids (DPL) were kindly provided by Novastell (Etrepagny, France) and Corman (Limburg, Belgium), respectively. The skim milk powder and serum protein isolate were generous gifts from Ingredia (Arras, France). A vitamin and mineral complex (VMC), made of a mixture of vitamin A as retinyl acetate (RA) (3593 µg RA/g), vitamin C as sodium ascorbate (81 µg/g), vitamin D as cholecalciferol (588 mg/g) and iron as dried iron sulfate (49 mg/g), was kindly supplied by DSM Nutritional Products South Africa (Isando, South Africa).

All reagents, analytical standards and solvents were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France).

### 2.2. Model system formulation

The model IFF composition was based on that of the marketed products. For this purpose, the labelled nutritional values of 91 IFF from the global market were collected and statistically analyzed (supplementary Fig. 1) (Cancelon et al., 2023). This analysis showed a low variability (low standard deviation) in macro- and micronutrient contents from one product to another. The target nutritional values of model IFF were therefore set to the average values obtained in the survey. The oil phase composition of the reference model IFF was determined by linear programming to reach the target nutritional values. The oil phase of this IFF consists of a mixture of refined palm oil, sunflower oil, high oleic sunflower oil, rapeseed oil and oils rich in DHA and ARA (Table 1, hereafter called DHA and ARA oils). This oil mixture was declined by linear programming, using Microsoft Excel Solver (Excel 2016, <https://www.microsoft.com/fr-fr/microsoft-365/excel>), into three other oils mixtures by varying the main source of SFA. Thus, four oil mixtures were formulated i.e. based on refined palm oil (POM), red palm oil (RPOM), coconut oil (COM) and dairy fat (DFOM). All the oil mixtures had equivalent contents of SFA, MUFA and PUFA but also in LA, ALA, DHA and ARA (Table 1).

### 2.3. Model IFF preparation

The aqueous and oil phases were prepared within 24 h before model IFF preparation according to Bourlieu-Lacanal et al. (2015) and Drapala et al. (2016) with slight modifications. The aqueous phase was made of a mixture of carbohydrates (20.5 and 39.0 g/L of lactose and maltodextrin, respectively) and proteins (32.9 and 3.5 g/L of skimmed milk powder and whey protein, respectively) in proportions to reach the targeted nutritional values (supplementary Fig. 1). Lactose was solubilized in ultrapure water at 50 °C and then all other components (i.e., maltodextrin, skimmed milk powder and whey proteins) were added and mixed at room temperature. The aqueous phase was then stored at 4 °C in a closed bottle. The oil phases consisted of a mixture of vegetable and/or animal fats as shown in Table 1. The oils with negative melting points (ARA, DHA, rapeseed, sunflower and oleic sunflower oils) were tempered at room temperature for 2 h. Those with positive melting points were melted for 10 min at a temperature equal to their melting temperature plus 10 °C and then blended with other components. Soy lecithin was added to the oil mixtures at 2 g/L. The DPL were also added to the oil mixtures at 8 g/L in order to have an equivalent emulsifier content (DPL powder purity of 25%). The oil blends were flushed under nitrogen in brown tubes closed and stored at 4 °C. Before model IFF preparation, VMC was added to the oil phase at 20 mg/100 mL. The contents of each mineral and vitamin were set according to European regulations and the average contents of products on the world market. Vitamins A and E contents were normalized in all model IFF (except in POM) by adding retinol and  $\alpha$ -tocopherol to the oil phase (Table 2). The aqueous phase was heated at 70 °C. Both phases were mixed and pre-emulsified twice for 5 min at 5000 rpm with a L5M Silverson (Silverson, Longmeadow, USA). Model IFF were then homogenized through

**Table 1**

Oil phase composition, fatty acids profile and initial peroxide value of model infant follow-on formulas. With POM: Palm oil mixture; VMC: Vitamin and mineral complement; A: Normalization of vitamin A content with retinyl acetate; COM: Coconut oil mixture; RPOM: Red palm oil mixture; DFOM: Dairy fat oil mixture; DPL: Dairy phospholipid; SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; LA: Linoleic acid; ALA:  $\alpha$ -linolenic acid; ARA: Arachidonic acid; DHA: Docosahexaenoic acid; PV: peroxide value.

|                                      | Target value | POM              | POM-VMC          | POM-A-VMC        | COM-VMC          | RPOM-VMC         | DFOM-VMC         | DFOM-DPL-VMC     |
|--------------------------------------|--------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| <b>Oil phase composition (% w/w)</b> |              |                  |                  |                  |                  |                  |                  |                  |
| Palm oil                             | –            | 67.81            | 67.81            | 67.81            | –                | –                | –                | –                |
| Rapeseed oil                         | –            | 18.41            | 18.41            | 18.41            | 18.56            | 15.42            | 12.63            | 12.63            |
| Sunflower oil                        | –            | 9.11             | 9.11             | 9.11             | 14.36            | 11.98            | 19.39            | 19.39            |
| High oleic sunflower oil             | –            | 3.09             | 3.09             | 3.09             | 34.91            | –                | 11.14            | 11.14            |
| Coconut oil                          | –            | –                | –                | –                | 30.59            | –                | –                | –                |
| Dairy fat                            | –            | –                | –                | –                | –                | –                | 55.26            | 55.26            |
| Red palm oil                         | –            | –                | –                | –                | –                | 71.02            | –                | –                |
| ARA oil                              | –            | 0.61             | 0.61             | 0.61             | 0.61             | 0.61             | 0.61             | 0.61             |
| DHA oil                              | –            | 0.97             | 0.97             | 0.97             | 0.97             | 0.97             | 0.97             | 0.97             |
| Soy lecithin                         | –            | 0.2              | 0.2              | 0.2              | 0.2              | 0.2              | 0.2              | –                |
| Dairy phospholipid                   | –            | –                | –                | –                | –                | –                | –                | 0.8              |
| <b>Fatty acid profile (% w/w)</b>    |              |                  |                  |                  |                  |                  |                  |                  |
| SFA                                  | 33.3         | 36.52 $\pm$ 0.55 | 35.92 $\pm$ 0.85 | 34.72 $\pm$ 0.11 | 37.60 $\pm$ 3.40 | 35.27 $\pm$ 0.13 | 34.98 $\pm$ 1.28 | 32.93 $\pm$ 0.48 |
| C12:0                                | –            | 0.18 $\pm$ 0.01  | 0.19 $\pm$ 0.01  | 0.12 $\pm$ 0.00  | 18.42 $\pm$ 2.65 | 0.12 $\pm$ 0.01  | 2.80 $\pm$ 0.16  | 1.90 $\pm$ 0.06  |
| C16:0                                | –            | 30.85 $\pm$ 0.54 | 30.31 $\pm$ 0.87 | 28.78 $\pm$ 0.10 | 6.74 $\pm$ 0.14  | 28.86 $\pm$ 0.11 | 16.55 $\pm$ 0.65 | 16.44 $\pm$ 0.38 |
| MUFA                                 | 45.5         | 44.59 $\pm$ 0.37 | 45.17 $\pm$ 0.51 | 46.02 $\pm$ 0.14 | 42.91 $\pm$ 2.99 | 45.46 $\pm$ 0.17 | 42.89 $\pm$ 1.13 | 46.81 $\pm$ 0.48 |
| PUFA                                 | 18.2         | 18.37 $\pm$ 0.19 | 18.37 $\pm$ 0.31 | 19.26 $\pm$ 0.20 | 19.40 $\pm$ 0.41 | 19.26 $\pm$ 0.10 | 21.44 $\pm$ 0.08 | 20.26 $\pm$ 0.16 |
| LA C18:2n-6                          | 15.63        | 16.27 $\pm$ 0.16 | 16.25 $\pm$ 0.24 | 16.70 $\pm$ 0.12 | 17.26 $\pm$ 0.37 | 17.06 $\pm$ 0.19 | 17.93 $\pm$ 0.18 | 17.66 $\pm$ 0.23 |
| ALA C18:3n-3                         | 1.61         | 1.48 $\pm$ 0.02  | 1.48 $\pm$ 0.03  | 1.57 $\pm$ 0.03  | 1.53 $\pm$ 0.03  | 1.60 $\pm$ 0.17  | 1.66 $\pm$ 0.18  | 1.54 $\pm$ 0.03  |
| ARA C20:4n-6                         | 0.29         | 0.27 $\pm$ 0.01  | 0.28 $\pm$ 0.02  | 0.44 $\pm$ 0.02  | 0.25 $\pm$ 0.02  | 0.25 $\pm$ 0.01  | 0.34 $\pm$ 0.01  | 0.61 $\pm$ 0.02  |
| DHA C22:6n-3                         | 0.45         | 0.31 $\pm$ 0.01  | 0.31 $\pm$ 0.02  | 0.27 $\pm$ 0.03  | 0.36 $\pm$ 0.03  | 0.35 $\pm$ 0.01  | 0.38 $\pm$ 0.01  | 0.30 $\pm$ 0.03  |
| LA/ALA                               | 9.71         | 10.99            | 10.97            | 10.63            | 11.28            | 10.66            | 10.8             | 11.47            |
| <b>PV(meqO<sub>2</sub>/kg)</b>       |              |                  |                  |                  |                  |                  |                  |                  |
| Initial PV                           | –            | 3.17 $\pm$ 0.22  | 2.47 $\pm$ 0.42  | 2.87 $\pm$ 0.35  | 2.96 $\pm$ 0.37  | 5.63 $\pm$ 0.37  | 3.17 $\pm$ 0.27  | 3.40 $\pm$ 0.25  |

**Table 2**

Levels of model infant follow-on formulas fortification with VMC and levels of normalization of tocopherols and vitamin A contents. With POM: Palm oil mixture; VMC: Vitamin and mineral complement; A: Normalization of vitamin A content with retinyl acetate; COM: Coconut oil mixture; RPOM: Red palm oil mixture; DFOM: Dairy fat oil mixture; DPL: Dairy phospholipid; RE: Retinol equivalent; RA: Retinyl acetate; RP: Retinyl palmitate.

|                                   | Target value | POM              | POM-VMC            | POM-A-VMC          | COM-VMC            | RPOM-VMC            | DFOM-VMC           | DFOM-DPL-VMC       |
|-----------------------------------|--------------|------------------|--------------------|--------------------|--------------------|---------------------|--------------------|--------------------|
| <b>Supplementation (mg/L)</b>     |              |                  |                    |                    |                    |                     |                    |                    |
| Retinol                           | –            | –                | 0.11               | –                  | 0.11               | 0.11                | –                  | –                  |
| Retinyl acetate                   | –            | –                | –                  | 0.14               | –                  | –                   | –                  | –                  |
| Tocopherol                        | –            | –                | 9.12               | 9.12               | –                  | 9.1                 | 6.06               | 6.06               |
| VMC                               | –            | –                | 200                | 200                | 200                | 200                 | 200                | 200                |
| <b>Tocopherol contents (mg/L)</b> |              |                  |                    |                    |                    |                     |                    |                    |
| Total                             | 22.99        | 11.97 $\pm$ 0.52 | 23.60 $\pm$ 1.23   | 26.34 $\pm$ 1.42   | 23.27 $\pm$ 2.13   | 18.92 $\pm$ 1.84    | 24.28 $\pm$ 2.53   | 23.74 $\pm$ 1.13   |
| $\alpha$ -tocopherol              | –            | 7.57 $\pm$ 0.43  | 10.02 $\pm$ 0.53   | 23.36 $\pm$ 2.86   | 17.65 $\pm$ 1.60   | 3.20 $\pm$ 2.07     | 11.11 $\pm$ 1.88   | 21.38 $\pm$ 1.01   |
| <b>Vitamin A content</b>          |              |                  |                    |                    |                    |                     |                    |                    |
| Total ( $\mu$ g RE/L)             | –            | –                | 493.44 $\pm$ 13.80 | 462.87 $\pm$ 29.63 | 298.81 $\pm$ 13.57 | 2029.86 $\pm$ 79.93 | 658.03 $\pm$ 46.43 | 586.72 $\pm$ 62.42 |
| Retinol ( $\mu$ g RE/L)           | –            | –                | –                  | –                  | –                  | –                   | –                  | –                  |
| Retinyl acetate ( $\mu$ g RA/L)   | 849.13       | –                | 565.97 $\pm$ 15.83 | 530.91 $\pm$ 33.99 | 342.73 $\pm$ 15.57 | 467.40 $\pm$ 18.99  | 708.10 $\pm$ 49.04 | 599.87 $\pm$ 70.22 |
| Retinyl palmitate ( $\mu$ g RP/L) | –            | –                | –                  | –                  | –                  | –                   | 74.58 $\pm$ 6.74   | 116.82 $\pm$ 2.21  |
| Carotenoid (mg/L)                 | –            | –                | –                  | –                  | –                  | 13.29 $\pm$ 0.55    | –                  | –                  |
| $\alpha$ -carotene (mg/L)         | –            | –                | –                  | –                  | –                  | 7.10 $\pm$ 0.32     | –                  | –                  |
| $\beta$ -carotene (mg/L)          | –            | –                | –                  | –                  | –                  | 5.12 $\pm$ 0.16     | –                  | –                  |
| 9-cis- $\beta$ -carotene (mg/L)   | –            | –                | –                  | –                  | –                  | 1.06 $\pm$ 0.06     | –                  | –                  |

eight cycles under high pressure using HHP SPX (SPXflow, Charlotte, North Carolina, USA) with a pressures couples of 350/40 bar. Five model IFF were obtained: POM-VMC and POM corresponds to the reference model IFF formulated from the oil mixture based on refined palm oil, enriched or not in VMC. COM-VMC, RPOM-VMC and DFOM-VMC corresponds to model IFF formulated from the oil mixture based on coconut oil, red palm oil or dairy fat, respectively and enriched in VMC.

Additional model IFF were studied to further investigate the effect of the vitamin A form used for normalization (retinol or retinyl acetate) on oxidative stability. Another series of samples with the reference (POM-VMC) have been prepared. POM-A in which the normalization of

vitamin A content was performed by adding retinyl acetate (unlike other model IFF in which normalization was performed by adding retinol) was formulated (Table 2). To further investigate the effect related to the combined addition of dairy fat and phospholipid, especially for its SM content, DFOM-DPL-VMC was formulated. It corresponds to the model IFF formulated from the oil mixture based on olein of dairy fat, enriched with VMC in which a DPL has been used as emulsifier instead of soy lecithin.

To avoid microbial proliferation 0.02% of azide was added to all model IFF. Model IFF were aliquoted in quadruplicate into 40 mL hermetically closed brown tubes with headspace of 3.82 mL containing O<sub>2</sub> at atmospheric pressure. Model IFF were stored for 20 days at either



40 °C with 110 rpm orbital stirring with a IKA KS 4000 i control incubator (IKA, Staufen, Germany) or at 25 °C without stirring. A sampling was carried out at 0, 1, 3, 6, 9, 15 and 20 days. Once collected, model IFF were kept under nitrogen at −20 °C until further analysis.

## 2.4. Structural characterization of model IFF

### 2.4.1. Droplet size distribution

The particle size distribution was determined using a Mastersizer 2000 (Malvern Instruments, Worcestershire, UK). Measurements were performed at refractive indices of 1.458 (milk fat; [Bourlieu et al., 2012](#)) and 1.33 (water) with agitation of 1500 rpm. About 500 µL of model IFF were diluted in 100 mL of distilled water in a measurement cell to reach an obscuration rate between 5 and 10%. The surface-weighted mean diameter ( $D[3,2]$ ) and the distribution mode were measured. Analysis was performed with three repeated measurements.

### 2.4.2. Confocal laser scanning microscopy (CLSM)

The model IFF microstructure was observed on inverted microscope using a CLSM system (Leica SP8, Heidelberg, Germany) as described by [Kergomard et al. \(2021\)](#) using a 40 × water-immersion objective. Three fluorescent dyes were used to localize proteins, apolar or polar lipids and were added in 200 µL model IFF at least 10 min before observation. 10 µL of model IFF was then deposited on a glass slide. The Leica LAS X software (Wetzlar, Germany) was used for images collection and analysis.

### 2.4.3. Differential scanning calorimetry

The crystallization behavior of oil phases was characterized using a differential scanning calorimeter (DSC Q200, TA Instruments, New Castle, USA). Cooling was carried out with a refrigerated cooling system (TA Instruments, New Castle, USA) with Nitrogen as purge gas. The TA Instruments Software was used to record and analyze the thermograms. 10 mg of each oil mixtures were loaded into aluminum DSC pans with inverted lids (TA Instruments, New Castle, USA) and sealed. An empty, hermetically sealed, aluminum pan was used as reference. The temperature range was adapted to each oil mixture as follow: isothermal during 10 min at 60 °C, 40 or 30 °C for POM, COM and RPOM or DFOM, respectively followed by cooling and isothermal at −40 °C for 5 min. The scan rate was 5 °C.min<sup>−1</sup>.

## 2.5. Chemical analysis of model IFF

### 2.5.1. Peroxide value

Primary oxidation compounds were quantified by measuring the hydroperoxide concentration named hereafter peroxide values (PV) according to [Shantha and Decker \(1994\)](#) adapted to microplate dimensions ([Ferreira da Silveira et al., 2021](#)). An extraction using a mixture of isooctane/isopropanol (3:1 v/v) was performed on 350 µL of model IFF. A dilution with methanol/butanol (3:7 v/v) was performed to give a final volume of 260 µL in the microplate. Then, 2.5 µL of aqueous ammonium thiocyanate (300 mg/mL) and 2.5 µL of ferrous solution (0.144 mol/L) were added. The mixture was incubated at 25 °C for 10 min. Absorbance was measured at 25 °C at 500 nm using an Infinite M1000 microplate reader (Tecan, Gröedig, Austria) equipped with the Magellan software. A standard calibration curve was prepared with cumene hydroperoxide. Results were expressed as meqO<sub>2</sub>/kg oil.

### 2.5.2. Thiobarbituric acid reactive substances (TBARS)

Secondary oxidation compounds were quantified by measuring the thiobarbituric acid reactive substances (TBARS) according to [Yi et al. \(2019\)](#), with slight modifications. 50 µL of model IFF were mixed with 200 µL of the reagent solution (150 mg/mL of trichloroacetic acid, 3.75 mg/mL of thiobarbituric acid and 0.25 mol/L of HCL) and heated at 95 °C for 15 min. Samples were then cooled in an ice bath for 5 min and centrifuged for 10 min at 5 000 rpm using a Pico 21 centrifuge

(ThermoFisher Scientific, USA). The supernatant absorbance was read with a microplate reader (Infinite M1000 microplate, Tecan, Gröedig, Austria) at 532 nm with the Magellan software. The calibration curves were set with 1,1,3,3-tetramethoxypropane. Results were expressed as mg MDA/kg oil.

### 2.5.3. Tocopherols and tocotrienols content

Four distinct tocopherols ( $\alpha$ ,  $\beta$ ,  $\delta$  and  $\gamma$ ) and  $\gamma$ -tocotrienol were quantified according to the ISO FDIS 9936 standard. Tocopherols were extracted with a Folch extraction method using a mixture of chloroform/methanol (2:1 v/v). High performance liquid chromatography (HPLC) analysis was performed with an Ultimate 3000 (Waltham, Massachusetts, USA) equipped with a silica column (250 mm × 4.6 mm i.d., 5 µm, Delaware USA) and a fluorescence detector with an HPLC Ultimate 3000 (Dionex, Sunnyvale, USA). The mobile phase consisted of hexane/dioxane (97:3 v/v) in isocratic conditions. The column temperature was maintained at 25 °C and flow rate was 1.3 mL.min<sup>−1</sup>. Fluorescence detection was set at 296 and 330 nm for excitation and emission, respectively. The injection volume was 100 µL and the calibration curves were realized with standard solutions of each tocopherols isomers.

### 2.5.4. Vitamin A content

Retinyl esters were quantified by HPLC measurement. The extraction was adapted from the method described by [Montúfar et al. \(2010\)](#). 1 mL of model IFF was mixed with 2 mL of hexane/isopropanol (3:2 v/v) extraction solvent. The samples were then centrifuged for 5 min at 1500 rpm with a CR412 centrifuge (Jouan Thermo Electron, Waltham, USA). The upper phase was collected and evaporated under nitrogen. The extracts were mixed with 1 mL of acetone and left for 3 h at −20 °C. After centrifugation at 900 g (Eppendorf 5810R, Hamburg, Germany) at −9 °C for 20 min, the upper phase was collected and filtered through a 0.2 µm Minisart SRP4 PTFE filter (Sartorius, Germany). 50 µL of extract was injected into a Thermo Scientific Ultimate 3000 HPLC system equipped with a YMC-30 column (250 × 4.6 mm, YMC) and a photodiode array detector (Vanquish PDA, Thermo Scientific) with the injection method described by [Moustiés et al. \(2019\)](#).

### 2.5.5. FA composition

The FA profiles were determined by gas chromatography (GC). A methylation of the FA was carried out according to the NF T30-233 standard with slight modifications. 500 µL sodium methylate solution with phenolphthalein were added to 200 µL of Folch extracts previously evaporated under nitrogen. Reaction medium was heated at 65 °C for 10 min. 500 µL chlorhydric methanol were added to phenolphthalein discoloration and the mixture was again heated at 65 °C for 10 min. 2 mL of hexane and water were added. After 5 min of centrifugation at 1500 rpm with a CR412 centrifuge (Jouan Thermo Electron, Waltham, USA), the organic phase was collected and analyzed by GC. A Focus GC (Thermo Electron Corporation, Massachusetts, USA) was used and equipped with a split injector (ratio of 1/20), a CP-Cil 88 Varian capillary column (50 m × 0.25 mm with 0.2 µm film thickness; Chrompack, Mid-delburg, Netherlands), and helium (1 mL.min<sup>−1</sup>) was used as carrier gas. Fatty acid methyl esters (FAME) were analyzed by flame ionization detector and ChromCard software data system (version 2005, Thermo FisherScientific, Massachusetts, USA). The column temperature started from 150 °C, reached 225 °C with a rise of 5 °C.min<sup>−1</sup> and was maintained for 10 min. The injector and detector temperatures were 250 and 270 °C, respectively. FAME were identified using an external standards of methyl esters mixture.

### 2.5.6. Carbonyl content

Oxidation reactions are also known to affect dairy proteins ([Hellwig, 2019](#)), therefore the protein-bound carbonyl content was analyzed according to the protocol adapted from Sante-Lhoutellier et al., 2007 ([supplementary materials](#) and methods). Briefly, after hydrazone derivatization with DNPH, samples were incubated with 6 M guanidine

overnight at 40 °C. After centrifugation, the absorbance of the supernatant was measured at 700 and 300 nm.

## 2.6. Statistical analysis

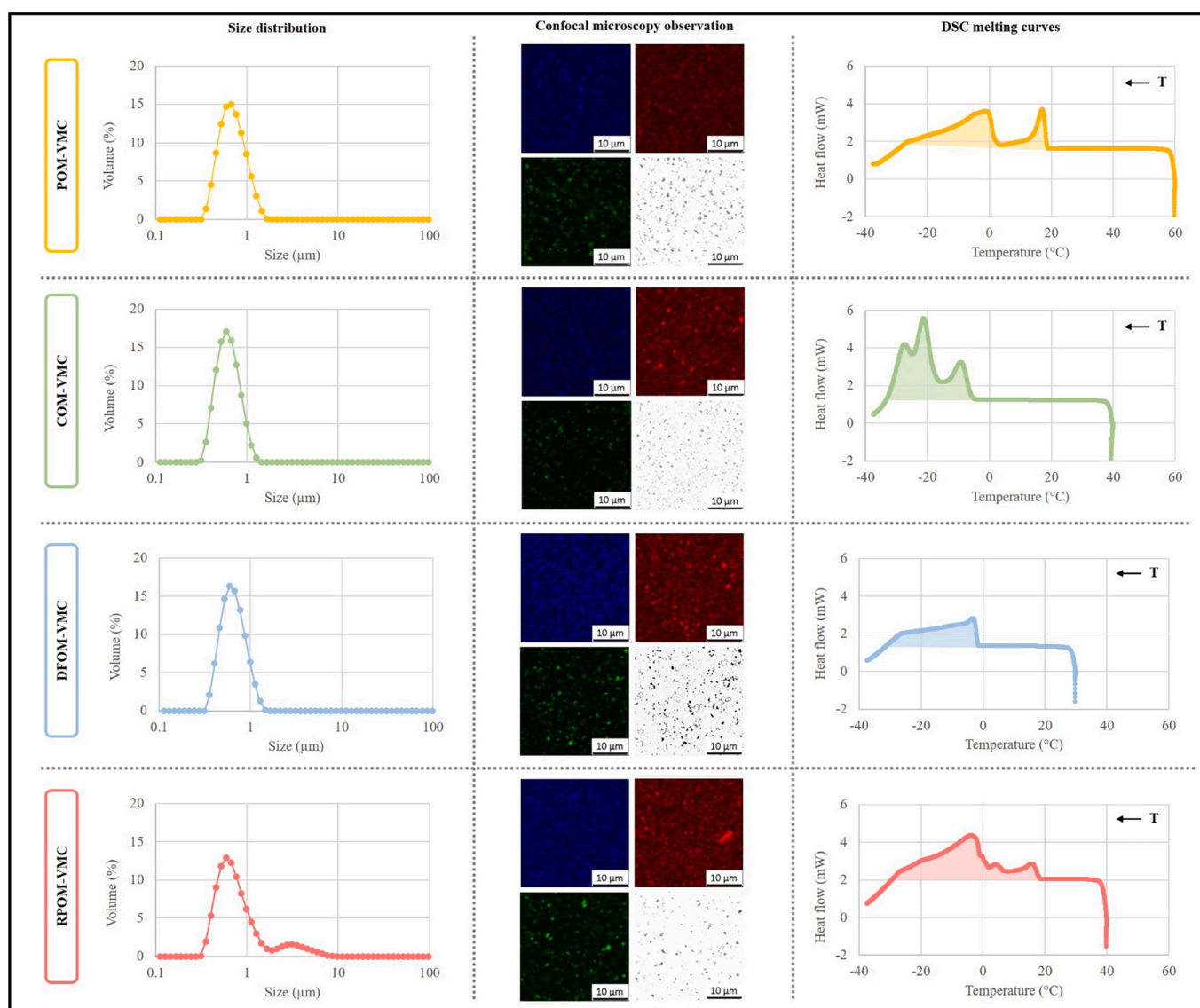
Model IFF were stored in quadruplicates and two repetitions of processing were done on reference model IFF (based on POM). Analyses were performed on each aliquot *i.e.* in quadruplicates and with possibly two repetitions of measurement. The results are presented as mean  $\pm$  SD. Statistical significance was determined by one-way ANOVA using R software (R.2.13.0, <https://cran.r-project.org>). Differences were considered statistically significant at  $P < 0.05$ .

## 3. Results

### 3.1. Model IFF development and standardization validation in term of chemical composition and structure

To develop model IFF abiding by the regulation and representative of marketed IFF, a survey of the nutritional values of 91 IFF was undertaken (supplementary Fig. 1) and used to establish target values. The oil phase compositions were then fixed by linear programming with constraints applied on the FA profile to standardize the contents of SFA, MUFA and PUFA (total and LA, ALA, ARA and DHA contents) and fit with the target. Thus, as shown in Table 1, four oil mixtures were formulated.

In order to evaluate the fit between the formulated model IFF by linear programming and the target values, the percentage of deviation was calculated. It represents the difference between the FA profiles measured and those targeted for the main classes (*i.e.* SFA, MUFA and PUFA contents) and was calculated for each class as follows (eq 1):



**Fig. 1.** Characterization of the structure and physical state of model infant follow-on formulas. Particles size distribution was assessed by laser light scattering (left panel); confocal laser scanning micrograph were collected using a 40  $\times$  water-immersion objective (middle panel) with each time four micrographs: top left blue colored micrograph: proteins; top right red colored: amphiphiles compounds; bottom left green colored: lipids; bottom right: transmission light micrograph; Melting points were assessed by differential scanning calorimetry (DSC) (left panel). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$$\text{Percentage of deviation} = \frac{|(\text{Target value} - \text{Measured value})| \times 100}{\text{Target value}} \quad (1)$$

The percentage of deviation was on average of 5.2%. Thus, model IFF could be validated and considered as representative of the marketed IFF embraced in the survey (supplementary Fig. 1).

The measurement of the initial FA profiles showed that the linear programming resulted in an equivalent SFA content ranging from 32.93% to 37.60% of the FA in all model IFF. The main SFA was palmitic acid for the palm oil-based reference model IFF (POM), the olein dairy fat-based model IFF (DFOM) and the red palm oil-based model IFF (RPOM), in which it represented 30.58%, 16.55% and 28.86% of FA, respectively. In the coconut oil-based model IFF (COM) the main SFA was lauric acid at a level of 18.42%. The contents of MUFA, PUFA, LA, ALA, ARA and DHA were also measured at the initial point and were equivalent in all model IFF. The LA/ALA ratio was on average of 10.97  $\pm$  0.31.

In addition, a structure standardization was also carried and targeted a droplet size distribution centred on 0.7  $\mu\text{m}$  in mode. All model IFF distribution and structures were assessed both by granulometry and CLSM (Fig. 1). The results showed a similar monomodal droplet size distribution centered on 0.7  $\mu\text{m}$  in all model IFF. Moreover, CLSM confirmed a good structure homogeneity with core of apolar lipids stabilized by proteins and amphiphile compounds. The distribution size was also followed at days 9 and 20. Creaming occurred especially at 40 °C but did not lead to coalescence and was reversible. Thus, before sampling, a redispersion was performed by repeated inversion of tubes and was effective. Creaming was similar in all model IFF and can be classically described following Stokes law.

The crystallization behaviour of the four oil mixtures was assessed by DSC (Fig. 1). The results showed that depending the SFA source the thermogram profiles varied strongly. The thermograms of POM showed an initiation of crystallization from 19.2 °C and two major crystallization events with extremes at 17.1 and −1.4 °C. COM had a crystallization that started from −4.5 °C and was characterized by three major

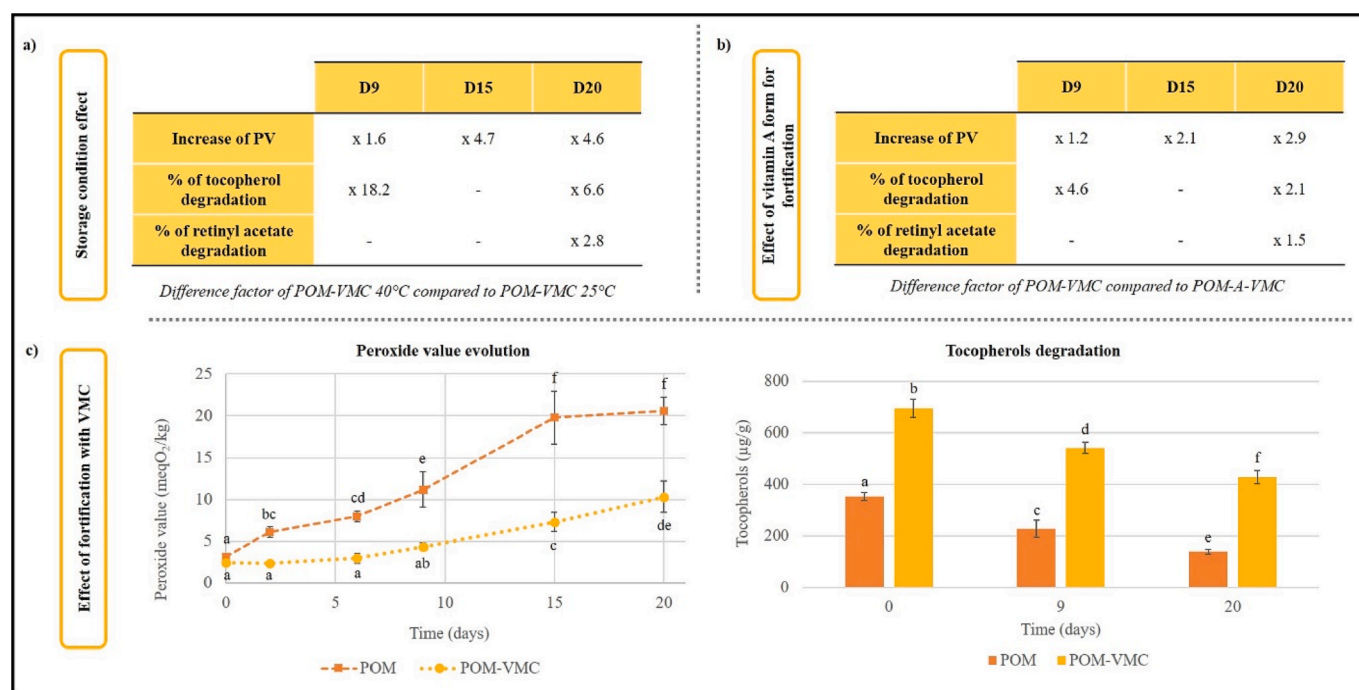
crystallization events with extremes respectively at −9.3, −21.2 and −27.4 °C. DFOM was characterized by a homogeneous and continuous crystallization range starting from −1.5 °C with an extremum at −3.2 °C. Finally, the crystallization initiation of RPOM occurred from 18.5 °C and was characterized by two major events at 15.6 and −4.0 °C.

In order to determine the storage conditions a preliminary test was carried out. POM model IFF were stored 20 days either at 40 °C under a constant stirring of 110 rpm or at 25 °C without stirring (Fig. 2.a). The results showed that under the least pro-oxidant conditions (25 °C without stirring) model IFF remained stable throughout the incubation period. In order to initiate oxidation for the purpose of the study, the temperature was set at 40 °C with a constant stirring.

All model IFF, except the negative control (POM), were enriched with a VMC. The enrichment in VMC aims at contributing to the nutritional intake of vitamins and minerals necessary for the development of infants. Moreover, addition of VMC is expected to alter the lipid oxidation kinetics. Indeed, VMC are made not only of antioxidant compounds (vitamins E and C) but also of iron sulfate that could have a potential pro-oxidant effect. In order to quantify the antioxidant capacity of VMC and evaluate its protective effect, the reference model IFF were either fortified (POM-VMC) or not fortified (POM). Fig. 2.b showed that the enrichment of model IFF delays oxidation and leads to a loss of tocopherols approximately 1.5 times less important (38.5% of loss in POM-VMC against 60.4% of loss in POM) and a PV twice lower at day 20. In the non-enriched model IFF, only the endogenous tocopherols of the raw materials acted as a barrier against oxidation which explains its faster degradation.

### 3.2. Selection of the most discriminating indicators of oxidation among the monitored parameters

To determine which indicators of oxidation level were the most informative (among: the evolution of PV, TBARS, FA profile and degradation of tocopherols and vitamin A), their statistical variations along the accelerated storage test and between model IFF were analysed. A correlation matrix was established (supplementary table 2). TBARS



**Fig. 2.** Effect of (a) storage condition (25 or 40 °C), (b) vitamin A form (retinol or retinyl acetate) used for fortification and (c) fortification in VMC on tocopherols loss and PV evolution in model infant follow-on formulas based on palm oil With POM: Palm oil mixture; VMC: Vitamin and mineral complement; A: Normalization of vitamin A content with retinyl acetate; D9: Sampling after 9 days of storage; D15: Sampling after 15 days of storage; D20: Sampling after 20 days of storage.



did not undergo any significant evolution during the kinetics (maximum value of 49.3 mg MDA/kg oil after 20 days of storage) although all model IFF contained 1.8% to 2.0% of FA of n-3 series (ALA and DHA). The oxidation level of the IFF model was not advanced enough, consequently, no significant evolution of the fatty acid profiles, especially the DHA content, was observed. At this stage, primary oxidation products are formed, and fat-soluble vitamins are degraded first, acting as sacrificial agents. The correlation matrix indicated that PV and tocopherols content underwent a significant evolution during storage and were strongly correlated throughout the storage.

### 3.3. Influence of vitamin A form

A preliminary characterization of the raw materials showed that they contained significant levels of vitamin E and various forms of vitamin A. In order to make all model IFF comparable,  $\alpha$ -tocopherol was added to normalize the vitamin E content in all model IFF. For vitamin A, the levels were normalized across all model IFF based on the content of DFOM-VMC, as this was the model IFF with the highest intrinsic vitamin A content (Table 2). Normalization was performed by adding retinol, the biologically active form of vitamin A, in POM-VMC, COM-VMC, DFOM-VMC and DFOM-DPL-VMC or with retinyl acetate, a retinol ester, in POM-A-VMC. The comparison of POM-VMC and POM-A-VMC showed that tocopherol losses and PV were significantly lower for model IFF in which normalization was performed with retinyl acetate (Fig. 2.c). In addition, a retinyl acetate degradation 1.5 times lower was observed in

model IFF normalized with retinyl acetate. POM-A-VMC would appear to be more resistant to oxidation than POM-VMC in which normalization had been performed by addition of retinol.

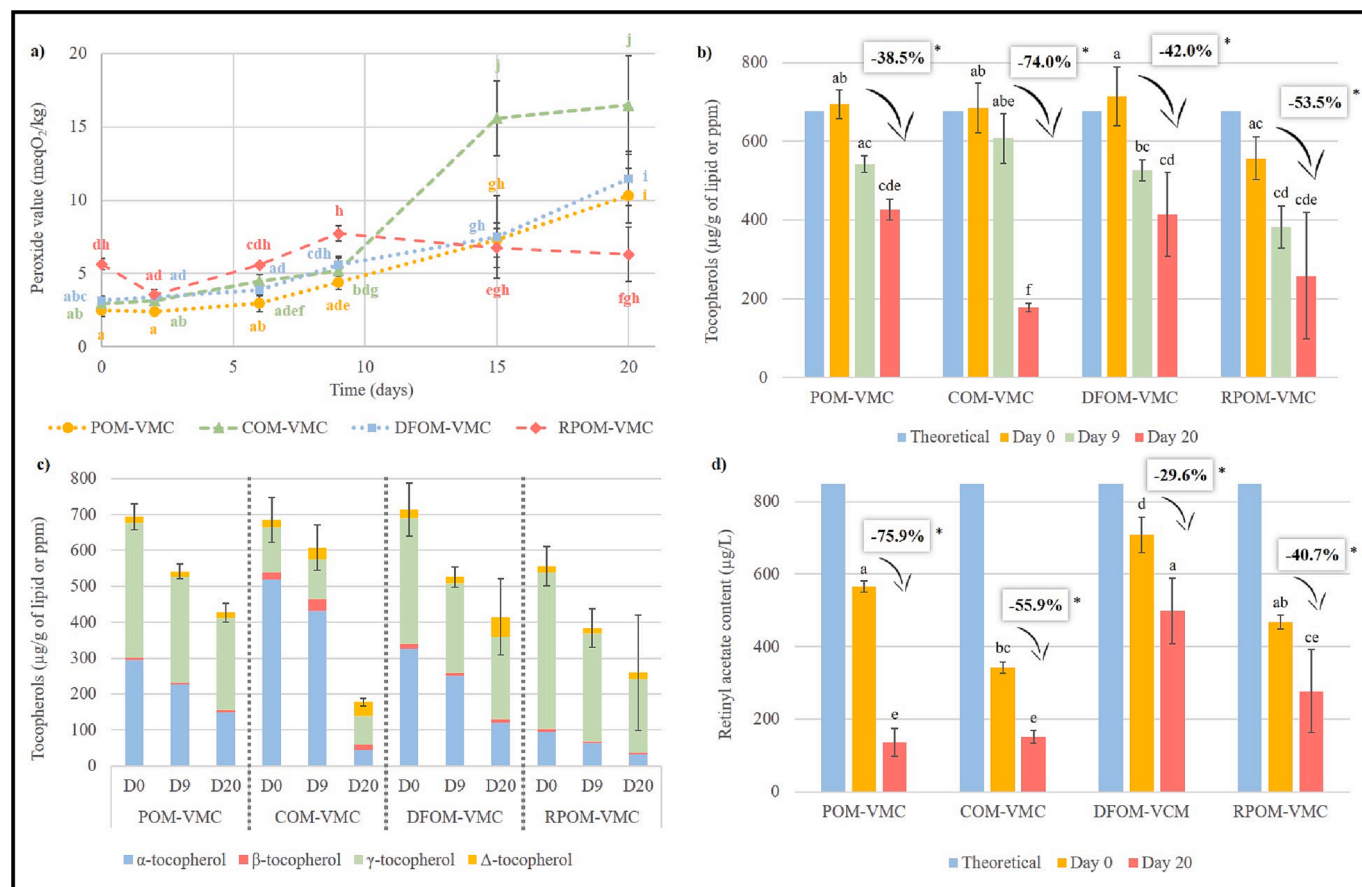
### 3.4. Comparison of palm oil with other SFA sources

#### 3.4.1. Comparison of palm oil with olein of dairy fat

POM-VMC was compared to a model IFF in which the main SFA source was dairy fat olein. As shown in Fig. 3.a, POM-VMC and DFOM-VMC have equivalent PV at day 20 of 10.31 and 11.48 meqO<sub>2</sub>/kg on average, respectively. In addition, tocopherol losses were 38.5% for POM-VMC versus 42.0% for DFOM-VMC (Fig. 3.b). The determination of tocopherol isomers (Fig. 3.c) showed that  $\gamma$ -tocopherol was the main isomer in POM-VMC and DFOM-VMC and represented 54.0 and 49.3% of the total tocopherol, respectively followed by  $\alpha$ -tocopherol (42.5 and 45.8% of total tocopherol, respectively). During the kinetic, the loss of tocopherol isomer followed the same behavior in each of these model IFF.  $\alpha$ -Tocopherol is mostly degraded and the fraction of  $\gamma$ -tocopherol thus increased at day 20. A higher loss of retinyl acetate was observed in POM-VMC compared to DFOM-VMC after 20 days of storage and was of 75.9 and 29.6%, respectively (Fig. 3.d).

#### 3.4.2. Comparison of palm oil with coconut oil

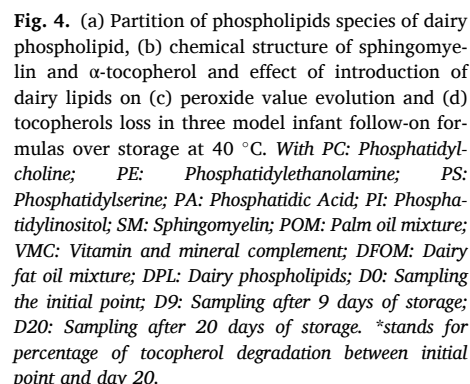
POM-VMC was compared to a model IFF in which SFA were mainly provided by coconut oil (COM-VMC) and their oxidative stability was evaluated. As shown in Fig. 3.a, at day 20 the PV of COM-VMC was 1.5



**Fig. 3.** Effect of refined palm oil substitution by other sources of saturated fatty acids (coconut oil, dairy fat or non-refined palm oil (red palm oil)) on (a) peroxide value evolution, (b) tocopherols loss, (c) tocopherols isomers partition and (d) retinyl acetate content in model infant follow-on formulas over storage at 40 °C. With POM: Palm oil mixture; VMC: Vitamin and mineral complement; A: Normalization of vitamin A content with retinyl acetate; COM: Coconut oil mixture; RPOM: Red palm oil mixture; DFOM: Dairy fat oil mixture; DPL: Dairy phospholipid; D0: Sampling the initial point; D9: Sampling after 9 days of storage; D20: Sampling after 20 days of storage. \*stands for percentage of tocopherol degradation between initial point and day 20. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

COM-VMC, this isomer then represented only 24.4% of total tocopherols. The important degradation of tocopherols in COM-VMC may relate to the loss of  $\alpha$ -isomer.

The impact of the refining process on oxidative stability and nutritional properties was evaluated by comparing POM-VMC with the red palm oil-based model IFF (RPOM-VMC). The measurement of PV is



based on a colorimetric method. The PV results were overestimated due to the coloration of red palm oil and therefore not representative. At the initial point the PV of RPOM-VMC, which amounted to 5.63 meqO<sub>2</sub>/kg (Table 1), was more than two times higher than the initial value of POM-VMC. Despite this overestimation, the PV of RPOM-VMC at day 20 was 1.6 times lower than that of POM-VMC (6.29 vs. 10.31 meqO<sub>2</sub>/kg, respectively).

Almost no PV evolution was observed during the storage in RPOM-VMC while this value more than quadrupled in POM-VMC. A degradation of 53.5% of total tocopherols was observed in RPOM-VMC versus 38.5% in POM-VMC (Fig. 3.b). At initial point,  $\gamma$ -tocopherol was the main isomer and thus represented 54.0 and 78.2% of total tocopherols in POM-VMC and RPOM-VMC, respectively (Fig. 3.c). The second most abundant isomer was  $\alpha$ -tocopherol in each model IFF and represented for 42.5 and 16.9% of the total tocopherols in POM-VMC and RPOM-VMC, respectively. A decrease in  $\alpha$ -tocopherol content was observed at day 20 in these two model IFF. The degradation of tocopherols may therefore be attributed to a loss in the  $\alpha$ -isomer. Red palm oil is one of the richest vegetable oils in tocotrienols. Therefore,  $\gamma$ -tocotrienol was also one of the major forms of vitamin E found in RPOM-VMC. At initial point an average content of 170.2 ppm of  $\gamma$ -tocotrienols was present in the RPOM-VMC. At day 20, 58.3% of  $\gamma$ -tocotrienols were degraded in these model IFF (average content of 71.0 ppm at day 20). A loss of 75.9% of retinyl acetate was observed in POM-VMC compared to 40.7% in RPOM-VMC, i.e. almost two times less important (Fig. 3.d).

### 3.5. Combined use of dairy fat and phospholipids to stabilize IFF

To further investigate the effect related to the introduction of dairy raw materials in model IFF composition, another challenge test was performed. The objective was to substitute soy lecithin by a DPL in DFOM-DPL-VMC. The use of DPL as an emulsifier leads to a higher content of some PL species like SM (Fig. 4.a) (Chai et al., 2022). This model IFF was then compared to both POM-VMC and DFOM-VMC which are stabilized with soy lecithin. The results first confirmed the ones obtained concerning the comparison of POM-VMC and DFOM-VMC (Fig. 3). Thus, despite faster oxidation kinetics than for the previous model IFF (resulting in higher PV and tocopherol degradation at day 20), these two model IFF seemed to have equivalent oxidative stabilities. DFOM-DPL-VMC had PV twice lower than POM-VMC (Fig. 4.c). Moreover, the degradation of tocopherols in DFOM-DPL-VMC was less important than in model IFF with soy lecithin with a loss of 67.5% of tocopherols at day 20 against about 89% for POM-VMC and DFOM-VMC.

It is expected that the composition of the oil droplets surface depends on the amount and type of PL used, which will directly alter the amount of dairy proteins adsorbed at this locus. In turn, protein localization in emulsions is known to be related to the propensity of proteins to oxidize (Berton et al., 2012), which is why protein-bound carbonyls were measured in selected model IFF. This analysis showed that the amount of carbonylated proteins was overall fairly low in all tested model IFF (supplementary Fig. 2), initially and throughout storage (<13  $\mu$ mol/g soluble protein). Yet, the carbonyl content was higher in the DPL-stabilized IFF model than in those stabilized with soy lecithin, even in freshly prepared IFF.

## 4. Discussion

An important result of our study is that in the presence of antioxidants (added under the form of VMC), model IFF showed an overall good stability despite the addition of LC-PUFA (DHA and ARA in particular) and an acceptable nutritional profile (n-6/n-3 ratio below 15). More specifically, our study indicates that the lipid sources can modulate the oxidative stability and the nutritional profile quality of IFF with an improvement with the introduction of dairy lipids (dairy fat olein and PL). The lipid sources also influence the chemical composition of fat-

soluble vitamins. Among them, vitamin A and tocopherol isomers were key factor influencing model IFF oxidation stability and some isomers and chemical forms were more stable than other (interest of retinyl acetate, carotenoids and  $\gamma$ -tocopherol). In addition, among the oxidation descriptive indicators studied, once again and in agreement with literature, the evolution of PV and the tocopherols degradation were reliable descriptors of oxidation kinetics.

### 4.1. Tocopherol content and isomers composition are key element of IFF stabilization

The indicators choice to evaluate the oxidation level of model IFF, i.e. the evolution of PV and tocopherol contents, and their strong negative correlation is in agreement with Jiang et al. (2021). These authors also demonstrate that the measurement of volatile compounds and the monitoring of vitamin C contents were reliable indicators for estimating the oxidation rate. In our study, we confirm that tocopherols act as sacrificing agents and are able to limit oxidation by several reaction pathways (Barouh et al., 2021). Tocopherols can react with the radicals formed during the oxidation process by donating their phenolic hydrogen. This ability is related to the low dissociation energy of the phenolic O—H bond. Tocopherols can also react with singlet oxygen to induce a return to the triplet oxygen ground state. In addition, tocopherols are able to develop synergic effects with several compounds (PL like SM, vitamin C or carotenoids for example) with different physico-chemical interactions resulting in tocopherol regeneration. Finally, they can chelate transition metals by forming a complex that will block the pro-oxidant activity of these compounds.

The results showed that the oxidation kinetics differed according to the tocopherols isomers composition, for instance illustrated on COM-VMC that has a lower resistance to oxidation than POM-VMC. These results are consistent with Bruni Let et al. (2005) who compared the ability of different antioxidants ( $\alpha$  and  $\gamma$ -tocopherols, ascorbyl palmitate and EDTA) to prevent oxidation in fish-oil-enriched milk emulsion. These authors showed that when added individually,  $\gamma$ -tocopherol inhibits oxidation more effectively than  $\alpha$ -tocopherol with a concentration dependent efficacy (maximum at 660 ppm). This study also demonstrated that, in these systems, ascorbyl palmitate was very effective in limiting oxidation.

As described by Barouh et al. (2021), in bulk system the regeneration capacity of tocopherols isomers is quite easily predictable on a given range of concentration (<500 ppm) and is mainly based on the dissociation energy of their phenolic O—H bond. The lower the binding energy, the higher the hydrogen donating power and follows the order:  $\alpha < \beta < \gamma < \delta$ . However, in more complex biphasic systems such as ours, the chemical environment influences the antioxidant efficiency of the different isomers. The results suggest that in our model IFF,  $\gamma$ -tocopherol would have a better regenerative capacity than  $\alpha$ -tocopherol.

### 4.2. Vitamin A form is a key parameter of formulation for the IFF stabilization

The results showed that the vitamin A form used to fortify model IFF has an impact on the oxidative stability. Model IFF normalized with retinyl acetate has a better oxidation resistance than those with retinol. This result agrees with the literature in which it is demonstrated that retinol esters are less prone to oxidation than unesterified retinol. Esterification increases the lipophilicity of the retinyl esters and thus modulates its location at the oil–water interface where lipid oxidation mainly occurs (Thorsteinn Loftsson, 2014).

Our results seem to indicate that RPOM-VMC has a better oxidative stability compared to POM-VMC. Refining processes have a strong impact on the chemical composition of palm oil and in particular on its composition in vitamins with antioxidant activities and nutritional benefits for infants. The study of Abdullah (2018) had shown that red palm oil had a highest antioxidant activity due to its higher carotenoids



and vitamin E (tocopherols and tocotrienols) contents compared to refined palm oil. These results indicated that refining processes lead to the loss of antioxidant compounds and are in line with our results.

Red palm oil contains high amount of provitamin A carotenoids, the most abundant of which are  $\alpha$ - and  $\beta$ -carotenoids (41.3 and 41% of total carotenoids, respectively (Loganathan et al., 2017)). In addition, an important part of the vitamin E in red palm oil is in the form of tocotrienols. It is well documented that these compounds have important antioxidant properties. Moreover, the presence of both carotenoids and vitamin E provides synergistic effects for protection against oxidation. This effect was demonstrated in the study of Schroeder et al. (2006) that aimed to compare the oxidative stability of liposome as a function of antioxidants ( $\alpha$  and  $\gamma$ -tocopherols and tocotrienols and  $\beta$ -carotene) added individually or in combination at three different concentrations (100, 500 or 1000 ppm). It showed that the induction period of oxidation was significantly increased for systems with combined addition of vitamin E (1000 ppm) and  $\beta$ -carotene (100 ppm). These results indicate that these compounds interact synergistically. To explain this observation, the authors suggest that tocopherols and tocotrienols would promote the regeneration of carotenoids.

Thus,  $\alpha$ -tocopherol acts as a sacrificial agent for carotene regeneration which could explain its faster degradation in our study. However, these results disagree with Yi et al. (2011) who compare the oxidative stability of fish oil mixed with refined or unrefined palm olein at concentrations of 0, 20 or 50%. These authors showed that red palm olein mixture was more susceptible to oxidation than refined palm olein. These unexpected results, which go against the hypothesis that the intrinsic antioxidant composition of red palm oil makes it less sensitive, could be explained by the study of Henry et al. (1998) who showed that above a certain concentration (>500 ppm) carotenoids could have a pro-oxidant effect. The study of Yi et al. (2011) also showed that in their systems  $\alpha$ -tocopherol and tocotrienol were depleted antioxidants, while  $\gamma$ -tocotrienol concentrations remained stable.

From a nutritional point of view, the inclusion of red palm oil in IFF conduct to a greater incorporation of provitamin A carotenoids which are more bioaccessible in such lipid environment and quite efficiently converted into the active form of vitamin A, i.e. retinol. Several studies have shown that supplementation of the infant and adults diet (especially pregnant and lactating women) with red palm oil leads to an increase in retinol and  $\beta$ -carotene levels in serum but has no effect on  $\alpha$ -carotene level (Dong et al., 2017). Red palm oil supplementation is therefore an effective strategy to fight against vitamin A deficiency disease. However, there are different forms of carotenoids that do not all have the same nutritional properties and antioxidant activity. In addition, the presence of tocotrienol has been reported to have health benefits. They may participate in the regulation of the cholesterol synthesis in the liver and could play an important role in the reduction of the progression of certain cancers (Aggarwal et al., 2019).

#### 4.3. SFA profile modulates the physical state and influences the oxidation kinetics

Despite standardized levels of total SFA in all model IFF, the type of SFA varies depending on the fat source used. Indeed, while in POM-VMC and RPOM-VMC the main SFA was palmitic acid and represented 30.31 and 28.86% of total FA, respectively. These same FA represented only 16.55% in DFOM-VMC (Table 1). DFOM-VMC also provides capric (C10:0), lauric (C12:0) and myristic (C14:0) acids with average contents of 1.97, 2.80 and 6.79% of the total FA, respectively. The olein dairy fat has the specificity to be rich in short and medium chains SFA. COM-VMC also provides a medium chain SFA due to its lauric acid content. These FA, which are found in minimal quantities or even absent in POM-VMC, are more easily digested and metabolized very quickly by the portal vein (Menard et al., 2014). The introduction of dairy fat and coconut oil in IFF lipid fraction have therefore a nutritional interest, especially in the case of infant.

The more important sensitivity to oxidation of COM-VMC compared to POM-VMC could also be related to the difference in the chain lengths of the most abundant SFA in each model IFF. The melting point of the oil mixture constituting COM-VMC is lower than that of POM-VMC which impact the physical state of lipid especially at 40 °C. Calligaris et al. (2006) reported in their study that lowering the melting point of an oil mixture makes it more susceptible to lipid oxidation phenomena. From a nutritional point of view, a lower melting point improves the lipid absorption rate.

Based on these results, COM-VMC is more adapted to the infant nutritional needs than POM-VMC due to the profile and the bio-accessibility of its FA, however this model IFF may be more sensitive to lipid oxidation.

#### 4.4. Interest of dairy raw materials to optimize IFF

The results appear to show that the substitution of palm oil by olein dairy fat did not impact the oxidative stability. It also showed that the combined substitution of palm oil and soy lecithin by dairy fat olein and DPL respectively, would lead to a higher oxidative resistance. The oil mixture based on olein dairy fat provides, for equivalent SFA contents, a more diversified FA profile especially of short and medium saturated chains that is more suitable for infant nutrition. Moreover, a notable difference is observed in the organization of FA on the TAG backbone between lipids of animal and vegetable origin (saturated preferentially in internal or external positions). Indeed, in dairy fat the *sn*-2 position is mostly occupied by long chain SFA, while short chain and unsaturated FA are mainly esterified in external positions (*sn*-1 and 3). The study of Jensen (2002) on the composition of bovine milk lipids, the most represented TAG in dairy fat was identified as 18:1–16:0–4:0. In vegetable oils that constitute the fat phase of POM-VMC, SFA are mainly located in the external positions (*sn*-1 and 3). The TAG organization, with palmitic acid preferentially esterified in the internal position (*sn*-2), as found in dairy fat, is similar to that in breast milk. Furthermore, it is admitted that this organization conduct to a better lipidic absorption by avoiding the formation of insoluble soaps (calcium palmitate) which are directly excreted (Dubois et al., 2008). The TAG structure could also have an influence on the sensitivity to oxidation with a protective effect of the PUFA in the central position because less accessible (Wijesundera et al., 2012).

The inclusion of DPL provides very long chains SFA that form rigid domains at the interface which is then more biomimetic of breast milk. It also provides different PL species like SM (Fig. 4.a) (Chai et al., 2022). It has been reported in the literature that these compounds can react with tocopherols and promote their regeneration. The study of Shimajiri et al. on the synergistic antioxidant activity of milk SM and  $\alpha$ -tocopherol on fish oil TAG showed that the higher the number of amino groups, the greater the synergy (Shimajiri et al., 2013). Thus, the synergy between tocopherols with SM enhanced their antioxidant activity. In this study, two interaction mechanisms were proposed. The first would involve a transfer of hydrogen or electrons between an amino group and the tocopherols, thus promoting their regeneration (Fig. 4.b). The second mechanism would involve an interaction between oxidized lipids and the amino group of polar lipids. This positive synergy could explain the better oxidative stability of DFOM-DPL-VMC than other model IFF.

#### 4.5. Optimization of IFF formulation

IFF aim to mimic the composition and structure of breast milk while ensuring the stability of nutrients throughout storage. In order to optimize the lipid fraction of IFF, the substitution of palm oil by other sources of SFA seems to be a viable strategy. The use of red palm oil conduct to an increase of the oxidation initiation phase due to its antioxidant composition while improving the nutritional status of provitamin A compounds. However, it did not increase the intake of short and medium SFA and minor lipids compounds. The inclusion of bovine dairy

fat and DPL provides a lipid structure and a global chemical complexity closer to that of breast milk, in particular thanks to its composition of minor lipids such as cholesterol and other complex lipids (glycerophospholipids or sphingolipids). These minor compounds are lacking in commercial IFF which do not include polar lipid extracts from bovine milk (Delplanque et al., 2015). In this context, the reintroduction of bovine dairy fat in the oil fractions of IFF, which was progressively replaced by vegetable oils from the 1970s onwards particularly for economic reasons - appears to be a viable strategy (Delplanque & Baudry, 2015; Viriato et al., 2022). Thus, the use of vegetable oils - for their unsaturated FA content -, marine, algal or fungal oils - for their LC-PUFA content - and dairy fat - for its structure and composition, especially in terms of SFA - would lead to reach a balance between lipid oxidation stability and improvement of the nutritional profile of IFF lipid fraction.

## 5. Conclusion

This study compared the oxidative stability of model IFF according to the source of saturated fat used. The importance of the balance between stability and nutritional properties to meet the infant nutritional needs was highlighted. The results showed that in all model IFF, the oxidation phenomena affected primarily the fat-soluble vitamins (A and E) which acted as sacrificial agents. It has also shown that the lipid formulation parameters were key levers to optimize IFF from a chemical and nutritional properties point of view. Based on the tocopherol degradation kinetics and PV evolution the use of coconut oil as the main SFA does not seem to give convincing results in terms of stability. The red palm oil would delay oxidation, and olein dairy fat would conduct to a similar stability to that obtained with palm oil. However, the combined use of dairy fat and DPL led to an improvement in both oxidative stability and nutritional profile by providing a wide variety of components playing a role in the proper development of infant.

## CRediT authorship contribution statement

**Mathilde Cancalon:** Methodology, Investigation, Formal analysis, Writing – review & editing. **Youna M. Hemery:** Methodology, Formal analysis, Validation, Writing – review & editing. **Nathalie Barouh:** Methodology, Validation, Writing – review & editing. **Bruno Baréa:** Resources. **Claire Berton-Carabin:** Investigation. **Lucie Birault:** Investigation. **Erwann Durand:** Writing – review & editing. **Pierre Villeneuve:** Supervision, Writing – review & editing. **Claire Bourlieu-Lacanal:** Supervision, Methodology, Validation, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Formulation was based on a survey on follow-on infant formula made by the author and made available as data at Recherche Data Gouv. <https://doi.org/doi:10.57745/CLER60>.

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## Appendix A. Supplementary data

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## RESEARCH ARTICLE

# Stabilization of infant formulas against lipid oxidation: What are the key structural levers?

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## Abstract

Infant follow-on formulas (IFF) mimic human milk. This study aimed to identify the key structural levers that influence oxidative stability of IFF. Representative model IFF of marketed products in term of lipid composition were formulated with varying droplet size, lipid droplet core composition, and interfacial composition using different emulsifiers (soy lecithin or dairy phospholipids [DPL]). The oxidative stability of model IFF was assessed in accelerated storage conditions. No significant stabilization effect based on the lipid droplet core composition was observed. However, the nature of the interface, influenced by the emulsifier type, had an impact. Model IFF with DPL showed no loss of tocopherols and peroxide value was up to twice lower than those with soy lecithin after 20 days. This effect was particularly pronounced for the 0.4 µm droplets. These results suggest that model IFF with DPL had a greater oxidative stability, likely due to the presence of sphingomyelin and the formation of a rigidified domain at the droplet surface. Model IFF with 0.4 µm droplets stabilized with soy lecithin, especially when added to the water phase, showed a tocopherols loss twice as high as that of IFF with DPL. These results indicate that oxidative stability of IFF is more influenced by the chemical environment rather than droplet size.

**Practical Application:** Infant follow-on formulas (IFF) aim to ensure an adequate nutritional intake to support the proper development and infant growth. Therefore, IFF must be stable against degradation phenomena such as lipid oxidation and have a composition and structure that are as biomimetic as possible to mature breast milk. This study provides key information for the development of IFF with a lipid composition and structure that are suitable for the infant nutritional needs and have an acceptable resistance to lipid oxidation. More generally, these results can be applied to all dispersed systems in the form of oil-in-water emulsions with a similar composition.

**Abbreviations:** ALA, α-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; DLDC, different lipid droplet composition; DPL, dairy phospholipid; FAME, fatty acids methyl esters; HM, human milk; HMP, high melting point; IFF, infant follow-on formulas; IMP, intermediate melting point; LA, linoleic acid; LC-PUFA, long chain polyunsaturated fatty acid; LWP, lecithin in water phase; MUFA, monounsaturated fatty acid; PL, phospholipid; PUFA, polyunsaturated fatty acid; PV, peroxide value; SFA, saturated fatty acid; TAG, triacylglycerol; Tbars, thiobarbituric acid reactive substances; VMC, vitamin and mineral complex; WHO, World Health Organization.

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## KEYWORDS

antioxidant, emulsifier, infant formula, lipid oxidation, lipid structure

## 1 | INTRODUCTION

Human milk (HM) is a complex and dynamic biofluid recognized as the reference for infant nutrition even if its composition is strongly impacted by many external factors, with one of the most influential being the mother's diet.<sup>[18,35,37]</sup> Its specific composition is adapted to the evolving nutritional needs of infants, particularly with regard to its lipid content.<sup>[17]</sup> HM provides a complex lipid profile with a majority of saturated fatty acids (SFAs) (mainly palmitic acid C16:0) that account for more than half of the total FAs. HM is also rich in polyunsaturated FAs (PUFA) including essential FAs, such as linoleic (LA, C18:2 *n*-6) and  $\alpha$ -linolenic (ALA, C18:3 *n*-3) acids, and also provides their longer chain derivatives, namely, arachidonic (ARA, C20:5 *n*-6) and docosahexaenoic (DHA, C22:6 *n*-3) acids, which are notably involved in the infant brain development. HM is also a source of various bioactive compounds that promote optimal development and provide long-term benefits, such as a reduced risk of developing type II diabetes or obesity in adulthood.<sup>[1]</sup> The World Health Organization (WHO) recommends exclusive breastfeeding for the first 6 months, followed by mixed breastfeeding until the age of 2 years. However, 48% of infants aged under 6 months are exclusively breastfed, and only 70% of them are still breastfed up to the age of 12 months.<sup>[42]</sup> Infant follow-on formulas (IFF), which are designed for the period ranging from 6 to 12 months, are therefore widely consumed throughout the world. At the industrial scale, the lipid composition of IFF is submitted to very specific regulations that are regularly updated to align with the nutritional recommendations. For instance, the addition of DHA to IFF is mandated by the EU regulation CE 2016/127 since 2020. These regulatory changes aim to improve the composition of IFF to meet infant nutritional needs.

However, differences in composition and structure between mature HM and IFF remain.<sup>[10]</sup> The lipids in HM are mainly found as triacylglycerols (TAG) (98%) and phospholipids (PL) (1%) and have a particular structural organization in the form of milk fat globules (MFG), ranging from 0.1 to 10  $\mu\text{m}$  droplet size (4  $\mu\text{m}$  on average).<sup>[6,22]</sup> MFGs are composed of a core of TAG stabilized by a trilayered biological membrane composed mainly of polar lipids and proteins. However, IFFs are composed of submicron droplets (0.3–0.8  $\mu\text{m}$ ) stabilized by milk proteins and vegetable emulsifiers (mainly lecithin).<sup>[11]</sup> The regiodistribution of FAs on the TAG backbone also differs widely due to the use of a mixture of fats almost exclusively of vegetable origin. It has been reported that these differences in structure and composition lead to differences in the efficiency of digestion and assimilation of nutrients, especially of lipids.<sup>[4]</sup> Some studies also showed that these differences could impact the oxidative stability, with a greater resistance of HM compared to IF.<sup>[29]</sup> Various studies have focused on structural parameters that modulate oxidative stability at different levels: first, at lipid droplet core level with the FA redistribution by formulat-

ing HM fat analogues.<sup>[44]</sup> But also depending of their esterification as PL or TAG with an increased stability attributed to PL due to the presence of the phosphate group and the polar head group.<sup>[27]</sup> In emulsions, it is now well known that the interface is the lipid oxidation initiation site. Therefore, many studies have focused on the interface properties such as the size and composition to modulate the oxidative stability.

However, to the best of our knowledge, no studies have yet proposed a multiparameter approach to screen the most efficient structural levers ranging from droplet size, core lipid composition, emulsifier source, and its mode of incorporation in model IFF having similar chemical composition.

Therefore, the present study aims at identifying structural parameters that influence oxidative stability of model IFF representative of marketed products. Additionally, particular attention is given to the biomimetic aspect related to these parameters, which may improve the nutritional profile. Model IFFs were formulated with equivalent chemical composition with a normalization of their lipid profile (similar contents in SFA, monounsaturated FA [MUFA], PUFA, DHA, ARA, vitamins E and A). The influence of the lipid structure was evaluated by adjusting the droplet size, the core lipid composition the emulsifier source, and its mode of incorporation.

## 2 | MATERIALS AND METHODS

### 2.1 | Materials

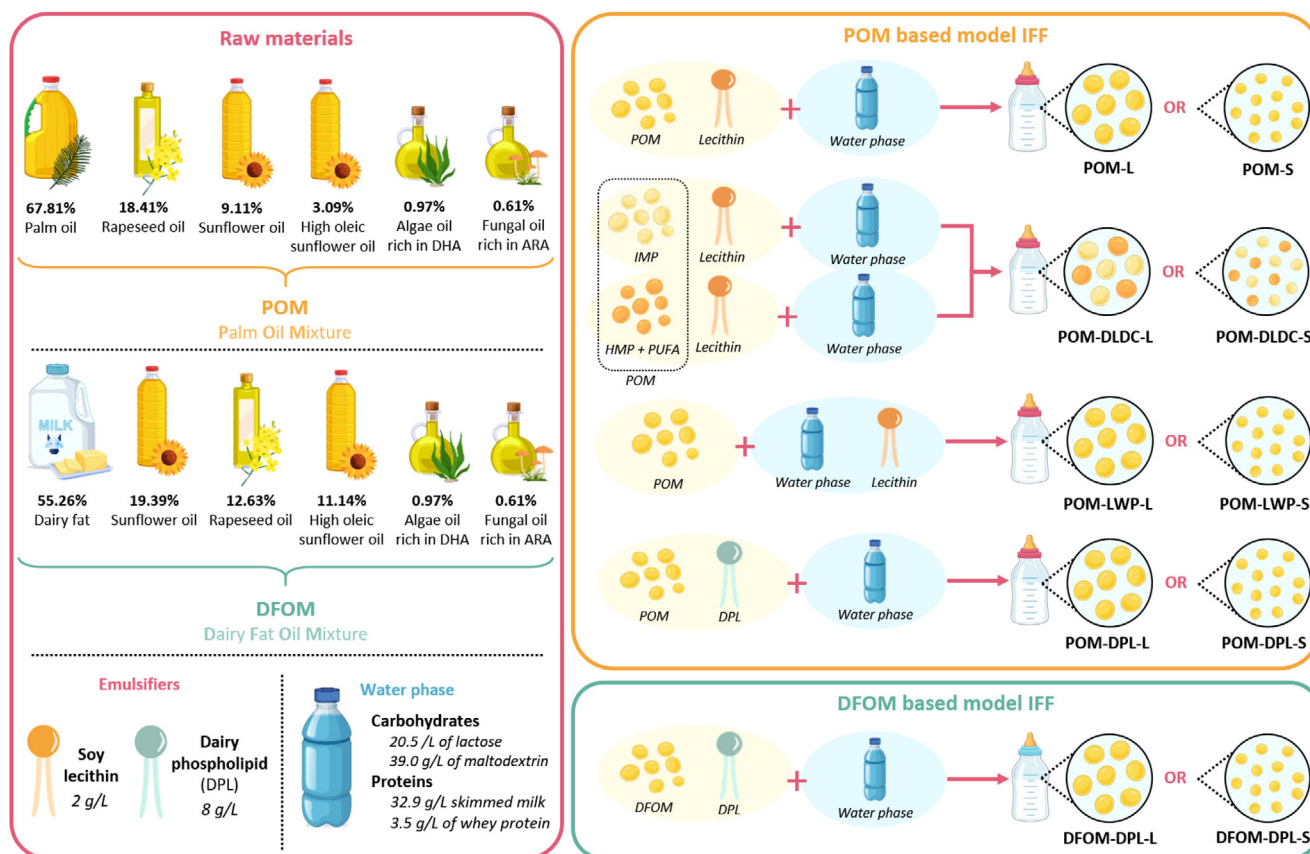
Sunflower (*Helianthus annuus*) and rapeseed (*Brassica napus*) oils were purchased in local supermarkets (Casino). High oleic sunflower oil was purchased from Cuisinor. Fungal and algae oils (ARASCO and DHASCO), dairy fat, and refined palm oil (POM) were generous gifts from DSM Nutritional products France, Corman, and Cargill, respectively. Soy lecithin and dairy PL (DPL) were kindly provided by Novastell and Corman, respectively. The skim milk powder and serum protein isolate were gifts from Ingredia. A vitamin and mineral complex (VMC), corresponds to a mixture of vitamin A (retinyl acetate, 3593  $\mu\text{g RA g}^{-1}$ ), vitamin C (sodium ascorbate, 81  $\mu\text{g g}^{-1}$ ), vitamin D (cholecalciferol, 588  $\text{mg g}^{-1}$ ), and iron (dried iron sulfate, 49  $\text{mg g}^{-1}$ ), which was kindly supplied by DSM Nutritional Products South Africa.

All analytical standards, reagents, and solvents were purchased from Sigma-Aldrich.

### 2.2 | Formulation and preparation of model IFF

As shown in Figure 1, model IFF based on two oil mixtures with standardized lipid profiles were formulated as described in a previous work





**FIGURE 1** Oil and water phases composition and preparation of model infant follow-on formulas (IFF). L is used for model IFF with droplet size of 0.7 µm and S for model IFF with a droplet size of 0.4 µm. POM-L/POM-S: POM-based IFF with a droplet size of 0.7 or 0.4 µm. POM-DLDC-L/POM-DLDC-S: POM-based IFF with heterogeneous lipid droplet core composition (high and low melting point oils droplets, that is, palm with arachidonic acid (ARA) and docosahexaenoic acid (DHA) oils or intermediate melting point oils droplets, that is, sunflower, oleic sunflower, and rapeseed oils) with a droplet size of 0.7 or 0.4 µm. POM-LWP-L/POM-LWP-S: POM-based IFF with soy lecithin previously homogenized with the aqueous phase with a droplet size of 0.7 or 0.4 µm. POM-DPL-L/POM-DPL-S: POM-based IFF with a DPL as emulsifier with a droplet size of 0.7 or 0.4 µm. DFOM-DPL-L/DFOM-DPL-S: DFOM-based IFF with a DPL as an emulsifier with a droplet size of 0.7 or 0.4 µm. DFOM, dairy fat oil mixture; DLDC, different lipid droplet composition; DPL, dairy phospholipids; HMP, high melting point oil; IMP, intermediate melting point oils (rapeseed, sunflower, and high oleic sunflower oils); LWP, lecithin in water phase; POM, palm oil mixture; PUFA, polyunsaturated fatty acids.

to be representative of commercial IFF.<sup>[8]</sup> The first oil mixture was based on POM and the second on dairy fat olein (DFOM). Both were prepared by mixing oils in proportions determined by linear programming using Microsoft Excel Solver. Ten model IFF were studied, including eight based on POM and two on DFOM:

- POM-L/POM-S: POM-based IFF with a droplet size of 0.7 or 0.4 µm, respectively (L stands for large and S for small)
- POM-DLDC-L/POM-DLDC-S: POM-based IFF with heterogeneous lipid droplet composition (blend of two pre-emulsions: one with high and low melting point oils, i.e., palm with ARA and DHA oils, the other with intermediate melting point [IMP] oils, i.e., sunflower, oleic sunflower, and rapeseed oils) with a droplet size of 0.7 or 0.4 µm.
- POM-LWP-L/POM-LWP-S: POM-based IFF with assembly of two pre-emulsions: one composed of the oil phase homogenized with the aqueous phase (stabilization with proteins) and the other of soy lecithin homogenized with the aqueous phase (i.e., lecithin previously

homogenized in water phase) with a droplet size of 0.7 or 0.4 µm.

- POM-DPL-L/POM-DPL-S: POM-based IFF with a DPL as emulsifier with a droplet size of 0.7 or 0.4 µm.
- DFOM-DPL-L/DFOM-DPL-S: DFOM-based IFF with a DPL as emulsifier with a droplet size of 0.7 or 0.4 µm.

Oil and water phases were prepared 24 h before model IFF formulation. The oil phases were obtained by mixing oils in proportions described in Figure 1. For POM-DLDC-L and POM-DLDC-S two pre-mixes of oils were initially prepared, one consisting of a mixture of oils with intermediate melting points (IMPs include rapeseed, sunflower, and high oleic sunflower oils) and the other consisting of a mixture of oils with low (ARA and DHA oils) and high (HMP includes POM) melting points.

Soy lecithin was added at 2 g L<sup>-1</sup> (40% PL, i.e., 0.8 g L<sup>-1</sup> of PL) in the oil phase except for POM-LWP-(S and L) in which soy lecithin was

added in the water phase. DPL was also added in the oil phase at  $8 \text{ g L}^{-1}$  (11% PL, i.e.,  $0.88 \text{ g L}^{-1}$  of PL) and concomitantly increases protein content ( $+0.4 \text{ g/100 mL}$ ). The oil phases were flushed under nitrogen and stored in hermitically sealed brown tubes at  $4^\circ\text{C}$ . All model IFF were prepared using the same water phase which corresponded to a mixture of carbohydrates ( $20.5 \text{ g L}^{-1}$  of lactose and  $39.0 \text{ g L}^{-1}$  of maltodextrin) and proteins ( $32.9 \text{ g L}^{-1}$  of skimmed milk powder and  $3.5 \text{ g L}^{-1}$  of whey proteins). This water phase was prepared as described in a previous work<sup>[7]</sup> and stored at  $4^\circ\text{C}$  in a closed bottle before use. Prior to model IFF preparations, VMC was added to the oil phases (to be representative of industrial practices) at  $20 \text{ mg/100 mL}$ . Vitamins A and E contents were then normalized by adding retinol (the active form of vitamin A) and  $\alpha$ -tocopherol to the oil phase as described in Table 1. Both phases were mixed and pre-emulsified twice for 5 min at 5000 rpm with an L5M Silverson (Silverson). Model IFF were then homogenized at two different pressures using an APV-1000 lab homogenizer (SPXFlow) to reach two droplet size. The first homogenization process corresponded to eight cycles at pressures of 100/30 bars and lead to a droplet size of  $0.7 \mu\text{m}$ , the second used eight cycles at pressures of 350/40 bars and lead to a droplet size of  $0.4 \mu\text{m}$ .

Sodium azide of 0.02% was added to all model IFF that were then aliquoted in quadruplicate into 40 mL brown tubes, with a headspace of 3.82 mL. The samples were then stored for 20 days at  $40^\circ\text{C}$  with 110 rpm orbital stirring using a IKA KS 4000 i-control incubator (IKA). After their sampling (0, 1, 3, 6, 9, 15, and 20 days), model IFF were flushed under nitrogen and stored at  $-20^\circ\text{C}$  until further analysis.

## 2.3 | Structural characterizations of model IFF

### 2.3.1 | Droplet size distribution

The particle size distribution of model IFF was assessed using a Mastersizer 2000 (Malvern Instruments). Model IFF of  $500 \mu\text{L}$  were placed in a measurement cell (obscuration rate between 5% and 10%). Refractive indices of 1.458 and 1.33 were used and 1500 rpm stirring was applied. The surface-weighted mean diameter ( $D[3,2]$ ) and the distribution mode were measured.

### 2.3.2 | Confocal laser scanning microscopy (CLSM)

Model IFF were observed on an inverted microscope using a confocal laser scanning microscopy (CLSM) system (Leica SP8). A  $40\times$  water-immersion objective was used and three fluorescent dyes were added to  $200 \mu\text{L}$  model IFF at least 10 min before observation in order to localize proteins (Fast green FCF, 6:100 v/v), nonpolar (Lipidtoxi, 0.2:100 v/v,  $\lambda_{\text{ex}}$  488 nm to  $\lambda_{\text{em}}$  590 nm), or polar lipids (Rd DOPE, 1:100 v/v,  $\lambda_{\text{ex}}$  543 nm to  $\lambda_{\text{em}}$  590 nm).<sup>[20]</sup> The images collection and analysis were performed using the Leica LAS X software.

## 2.4 | Chemical characterizations of model IFF

### 2.4.1 | Lipid oxidation monitoring by a measurement of peroxide value (PV) and thiobarbituric acid reactive substances (TBARS)

The amounts of primary oxidation compounds monitored by the measurement of the peroxide value (PV) were determined according to Ferreira da Silveira, Laguerre et al.<sup>[16]</sup> Briefly, after an extraction on  $350 \mu\text{L}$  of model IFF using  $750 \mu\text{L}$  of isooctane/isopropanol (3:1 v/v), a dilution of the supernatant with methanol/butanol (3:7 v/v) was performed. Then,  $2.5 \mu\text{L}$  of aqueous ammonium thiocyanate ( $300 \text{ mg mL}^{-1}$ ) and ferrous solution ( $0.144 \text{ mol L}^{-1}$ ) were added to give a final volume of  $265 \mu\text{L}$  in the microplate UV star 96 well COC F-bottom (Greiner Bio-One). Each microplate was first incubated at  $25^\circ\text{C}$  for 10 min with a stirring of 1000 rpm in PHMP-4 microplate thermoshaker a (Grant Instruments Ltd) and then placed in an Infinite M200 microplate reader (Tecan). Absorbances were measured at 500 nm. Data acquisition was made using Magellan software (Tecan). PVs were determined using a standard calibration curve of cumene hydroperoxide and were expressed as  $\text{meqO}_2 \text{ kg oil}^{-1}$ .

Secondary oxidation compounds were measured using the thiobarbituric acid reactive substances (TBARS) following the procedure as described in a previous work.<sup>[7]</sup> Briefly,  $50 \mu\text{L}$  of model IFF were mixed with  $200 \mu\text{L}$  of the reagent solution ( $150 \text{ mg mL}^{-1}$  of trichloroacetic acid,  $3.75 \text{ mg mL}^{-1}$  of thiobarbituric acid, and  $0.25 \text{ mol L}^{-1}$  of HCL) and heated at  $95^\circ\text{C}$  for 15 min. Model IFF were then cooled in an ice bath for 5 min and centrifuged for 10 min at 5000 rpm using a Pico 21 centrifuge (Thermo Fisher Scientific). The absorbance of the supernatant was read with an Infinite M200 microplate, Tecan) at 532 nm with the Magellan software. TBARS were determined using a standard calibration curve of 1,1,3,3-tetramethoxypropane and were expressed as  $\text{mg MDA (kg oil)}^{-1}$ .

### 2.4.2 | Retinyl esters and tocopherols contents

Retinyl esters contents were determined as described in a previous work<sup>[7]</sup> by high performance liquid chromatography (HPLC) using a Thermo Scientific Ultimate 3000 HPLC system equipped with a YMC-C30 column ( $250 \times 4.6 \text{ mm}^2$ , YMC) and a photodiode array detector (Vanquish PDA, Thermo Scientific) with the injection method described by Ref. [31].

Tocopherols isomers ( $\alpha$ ,  $\beta$ ,  $\delta$ , and  $\gamma$ ) were quantified according to the ISO-FDIS 9936 normalized procedure. They were extracted with a Folch mixture (chloroform/methanol 2:1 v/v) and analyzed by HPLC with an Ultimate 3000 equipped with a silica column ( $250 \text{ mm} \times 4.6 \text{ mm}$ , i.e.,  $5 \mu\text{m}$ ) and a fluorescence detector (Dionex). The mobile phase consisted of hexane/dioxane (97:3 v/v) in isocratic conditions with a flow rate of  $1.3 \text{ mL min}^{-1}$  and a column temperature of  $25^\circ\text{C}$ . Fluorescence detection was set at 296 for excitation

**TABLE 1** Model infant follow-on formulas fortification with VMC, normalization of tocopherols, and vitamin A contents and peroxides values.

| Supplementation (mg L <sup>-1</sup> )    | Target value | POM-L        | POM-S        | POM-DLDC-L   | POM-DLDC-S   | POM-LWP-L    | POM-LWP-S    | POM-DPL-L    | POM-DPL-S    |
|--|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Retinol                                  | -            | 0.11         | 0.11         | 0.11         | 0.11         | 0.11         | 0.11         | 0.11         | 0.11         |
| Retinyl acetate                          | -            | -            | -            | -            | -            | -            | -            | -            | -            |
| Tocopherol                               | -            | 9.12         | 9.12         | 9.12         | 9.12         | 9.12         | 9.12         | 9.12         | 9.12         |
| VMC                                      | -            | 200          | 200          | 200          | 200          | 200          | 200          | 200          | 200          |
| Tocopherols contents (ppm)               |              |              |              |              |              |              |              |              |              |
| Total                                    | 676.2        | 694.2 ± 36.1 | 688.9 ± 55.6 | 672.0 ± 3.6  | 615.5 ± 11.9 | 651.3 ± 80.4 | 556.1 ± 15.4 | 570.8 ± 79.9 | 535.1 ± 58.6 |
| α-Tocopherol                             | -            | 294.8 ± 15.5 | 293.4 ± 24.1 | 265.3 ± 2.0  | 224.1 ± 6.1  | 229.6 ± 36.6 | 140.8 ± 7.7  | 161.1 ± 35.2 | 122.0 ± 24.8 |
| Vitamin A content                        |              |              |              |              |              |              |              |              |              |
| Total (µg RE L <sup>-1</sup> )           | -            | 493.4 ± 13.8 | 126.9 ± 28.7 | 383.3 ± 27.1 | 179.3 ± 31.1 | 440.6 ± 68.4 | 189.1 ± 27.4 | 651.2 ± 66.1 | 351.8 ± 85.1 |
| Retinyl acetate (µg RAL <sup>-1</sup> )  | 849.1        | 566.0 ± 15.8 | 145.5 ± 33.0 | 439.6 ± 31.1 | 205.7 ± 35.6 | 505.4 ± 78.5 | 216.9 ± 31.4 | 746.9 ± 75.8 | 403.5 ± 97.6 |
| PV (meqO <sub>2</sub> kg <sup>-1</sup> ) |              |              |              |              |              |              |              |              |              |
| Initial PV (day 0)                       | -            | 2.5 ± 0.4    | 1.9 ± 0.4    | 2.5 ± 0.3    | 2.2 ± 0.3    | 1.3 ± 0.1    | 1.3 ± 0.2    | 1.1 ± 0.1    | 0.6 ± 0.1    |
| Final PV (day 20)                        | -            | 10.3 ± 1.8   | 9.4 ± 1.2    | 10.9 ± 1.9   | 11.5 ± 1.3   | 11.8 ± 0.4   | 16.4 ± 1.9   | 7.1 ± 0.5    | 4.4 ± 0.3    |

Note: L is used for model IFF with droplet size of 0.7 µm and S for model IFF with a droplet size of 0.4 µm. POM-L/POM-S: palm oil mixture-based IFF with a droplet size of 0.7 or 0.4 µm. POM-DLDC-L/POM-DLDC-S: POM-based IFF with heterogeneous lipid droplet core composition (high and low melting point oils droplets, that is, palm with ARA and DHA oils or intermediate melting point oils droplets, i.e., sunflower, oleic sunflower, and rapeseed oils) with a droplet size of 0.7 or 0.4 µm. POM-LWP-L/POM-LWP-S: POM-based IFF with soy lecithin previously homogenized with the aqueous phase with a droplet size of 0.7 or 0.4 µm. POM-DPL-L/POM-DPL-S: POM-based IFF with a DPL as emulsifier with a droplet size of 0.7 or 0.4 µm. DFOM-DPL-L/DFOM-DPL-S: dairy fat oil mixture based IFF with a DPL as an emulsifier with a droplet size of 0.7 or 0.4 µm.

Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; DLDC: different lipid droplet composition; DPL, dairy phospholipid; IFF, infant follow-on formulas; LWP: lecithin in water phase; PV, peroxide value; RE, retinol equivalent; RA: retinyl acetate; VMC: vitamin and mineral complement.

and 330 nm for emission. Tocopherols content was determined using standard calibration curves of each tocopherol isomers.

### 2.4.3 | Fatty acid composition

The FA compositions of model IFF were determined by gas chromatography (GC) after an FA methylation as described in our previous work.<sup>[7]</sup> Analyses were performed using a Focus GC (Thermo Electron Corporation) equipped with a split injector (ratio of 1/20), a CP-Cil 88 Varian capillary column (50 m × 0.25 mm with a 0.2-μm film thickness; Chrompack) with an initial temperature of 150°C to reach 225°C at 5°C min<sup>-1</sup>. Final temperature (225°C) was maintained for 10 min. The injector and detector temperatures were 250 and 270°C, respectively. Helium was used as carrier gas at a flow rate of 1 mL min<sup>-1</sup>. Flame ionization detector was used and FA methyl esters were identified using standard solution of methyl esters mixture. ChromCard software (version 2005, Thermo Fisher Scientific) was used to collect and analyze data.

### 2.5 | Statistical analysis

Results are presented as mean ± SD. Statistical analyses were conducted using R software (R.2.13.0, <http://cran.r-project.org>). Statistical differences were evaluated using a one-way ANOVA test and considered significant for  $p < 0.05$ .

## 3 | RESULTS

The aim of this study was to compare the impact of different chemical and physicochemical parameters on oxidative stability of model IFF. In that context, various model IFF were designed with similar FA composition and vitamin content but varying in their structure (droplet size, core lipid composition, emulsifier source, and its mode of incorporation). These model IFF were formulated in compliance with the regulations and were intended to be representative of the marketed IFF. The oxidation rates were monitored during an accelerated storage test of 20 days at 40°C through the evaluation of several indicators of lipid oxidation (PV, TBARS, FA profile evolution, and vitamin A and E contents).

### 3.1 | Standardization of model IFF and selection of descriptive indicators of oxidation

Model IFF size droplet distribution and structure were assessed both by granulometry and CLSM (Figure 2 and Figure S1). All model IFF show a similar monomodal droplet size distribution centered on 0.4 or 0.7 μm depending on the homogenization pressures applied. CLSM observations confirmed good structure homogeneity with a core of

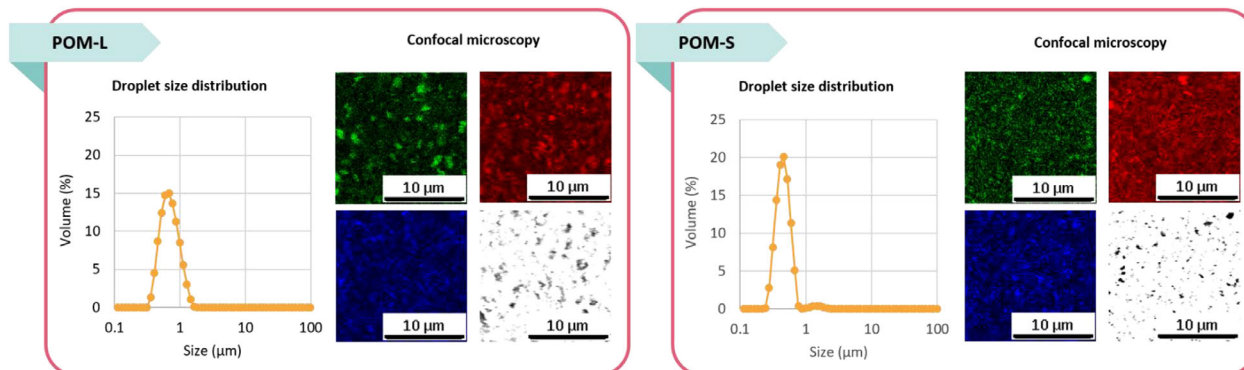
nonpolar lipids stabilized by proteins and amphiphilic compounds. The size droplet distribution and the CLSM observations showed that creaming occurred but did not lead to coalescence and was reversible. Therefore, before sampling an effective redispersion was achieved by repeated inversion of tubes. Creaming was similar in all model IFF and can be classically described following Stokes law.

The FA compositions of model IFF were designed to be representative of marketed products.<sup>[7]</sup> As shown in Figure 3, the linear programming used to design the various model IFF the resulted in similar initial FA profiles with equivalent SFA (ranging from 32.9% to 38.8% of total FA), MUFA (ranging from 43.3% to 46.9% of total FA), and PUFA (ranging from 17.0% to 20.3% of total FA) contents in all model IFF. The contents of LA, ALA, ARA, and DHA were also relatively equivalent and the LA/ALA ratio was  $10.7 \pm 0.6$  on average. Total DHA and ARA content in DFOM-DPL-(S and L) is slightly higher (+0.3%), making them potentially more sensitive to oxidation.

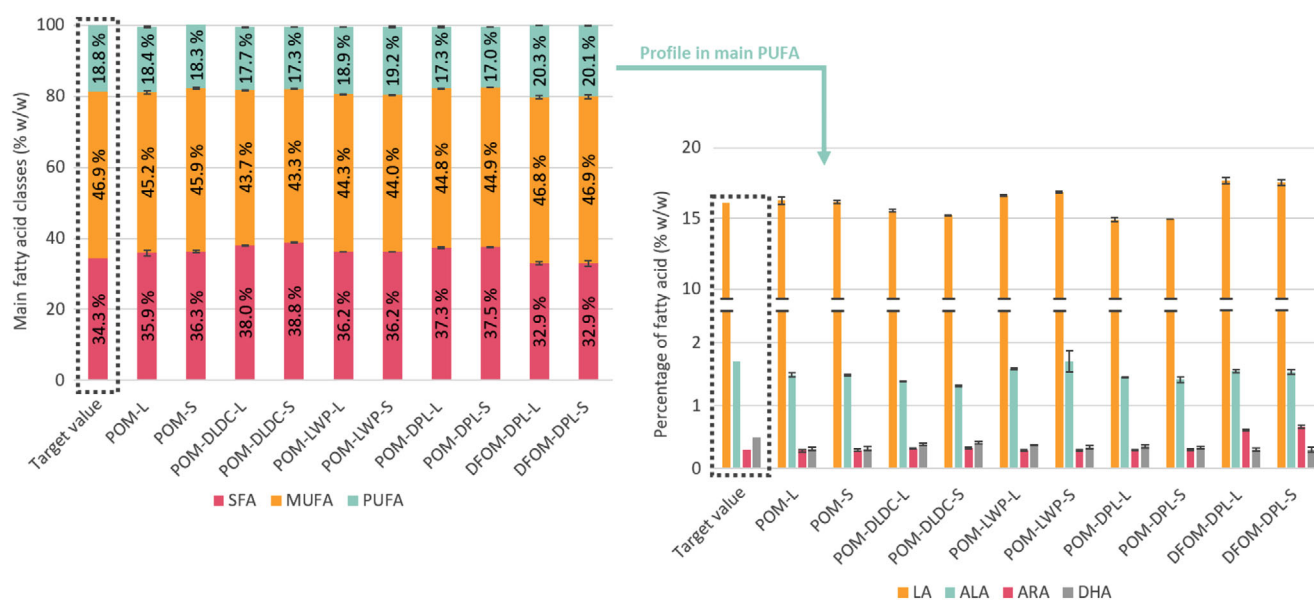
Normalization of initial vitamin A and E contents was performed by supplementation with VMC and the addition of α-tocopherol and retinol (Tables 1 and 2). The normalization in vitamin A (retinyl acetate with the VMC and retinol) was made prior to emulsification to be representative of industrial-scale practices. All model IFF had an equivalent initial total tocopherol content ( $653.0 \pm 67.6$  ppm) (Tables 1 and 2). However, depending on the emulsification process especially the pressure applied, significant variations of the initial vitamin A content were observed. Indeed, the more drastic homogenization process (to obtain small droplets), the more important loss in vitamin A contents during the process (Figures 4 and 5).

Concerning the evaluation of lipid oxidation rates in model IFF in accelerated storage conditions, five different measurements were performed: evolution of PV, TBARS, FA profile and degradation of tocopherols and vitamin A. The measurement of primary oxidation compounds (here, PV value) is generally considered to be relevant to evaluate lipid oxidation rates at early stage of oxidation, where the formation of secondary oxidation compounds is not yet important. In our case, lipid oxidation was not advanced enough for any model IFF studied in our chosen accelerated conditions. Therefore, TBARS which is a measurement of some secondary oxidation compounds<sup>[24]</sup> did not significantly increase after 20 days at 40°C. Moreover, TBARS assay is designed to measure malondialdehyde that preferentially forms from FA containing at least three double bonds.<sup>[9]</sup> In our case, the amount of such FA especially ALA and DHA ranged from 1.7% to 2.0% of the total FA content. This amount could be too low to guarantee a relevant TBARS measurement. Similarly, the FA profile did not show any significant evolution during storage in all model IFF. This can be attributed to the fact that advanced oxidation stages were not reached during our chosen storage conditions. Finally, a correlation matrix was established to determine the most relevant indicators of the oxidation rate in our model IFF. It is also worth noting that the tocopherols presence in all model IFF induced a lag phase (up to 9 days) and that peroxidation increased when tocopherols were partially or totally consumed.





**FIGURE 2** Typical droplet size distribution of model infant follow-on formulas and physical organization in palm oil (POM)-L and POM-S. Particles size distribution was assessed by laser light scattering. Confocal laser scanning micrographs were collected using a 40x water-immersion with each time four micrographs: blue colored: proteins; red colored: amphiphiles compounds; green colored: lipids; white colored: transmission light micrograph. L is used for model infant follow-on formulas (IFF) with a droplet size of 0.7  $\mu\text{m}$  and S for model IFF with a droplet size of 0.4  $\mu\text{m}$ . POM-L/POM-S: palm oil mixture-based IFF with a droplet size of 0.7 or 0.4  $\mu\text{m}$ , respectively.



**FIGURE 3** Fatty acid profiles of model infant follow-on formulas (IFF) (SFA, MUFA, PUFA, LA, ALA, ARA, and DHA) at the initial point (D0). Target values were set abiding by the regulation and to be representative of marketed infant IFF.<sup>[7]</sup> L is used for model IFF with droplet size of 0.7  $\mu\text{m}$  and S for model IFF with a droplet size of 0.4  $\mu\text{m}$ . POM-L/POM-S: palm oil mixture-based IFF with a droplet size of 0.7 or 0.4  $\mu\text{m}$ . POM-DLDC-L/POM-DLDC-S: POM-based IFF with heterogeneous lipid droplet core composition (high and low melting point oils droplets, i.e., palm with ARA and DHA oils or intermediate melting point oils droplets, i.e., sunflower, oleic sunflower, and rapeseed oils) with a droplet size of 0.7 or 0.4  $\mu\text{m}$ . POM-LWP-L/POM-LWP-S: POM-based IFF with soy lecithin previously homogenized with the aqueous phase with a droplet size of 0.7 or 0.4  $\mu\text{m}$ . POM-DPL-L/POM-DPL-S: POM-based IFF with a DPL as emulsifier with a droplet size of 0.7 or 0.4  $\mu\text{m}$ . DFOM-DPL-L/DFOM-DPL-S: dairy fat oil mixture-based IFF with a DPL as emulsifier with a droplet size of 0.7 or 0.4  $\mu\text{m}$ . ALA,  $\alpha$ -linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; DLDC, different lipid droplet composition; DPL, dairy phospholipid; LA, linoleic acid; LWP, lecithin in water phase; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; VMC: vitamin and mineral complement.

### 3.2 | Impact of model IFF droplets size on oxidation

In order to evaluate the impact of droplet size on model IFF oxidative stability, they were systematically homogenized at two pressures to obtain emulsions with either 0.4 or 0.7  $\mu\text{m}$  droplet sizes. As presented

previously, depending on the chosen emulsification process, significant variations of the initial retinyl acetate content were observed (Figures 4 and 5). In average the loss of retinyl acetate loss was twice as important for the more drastic homogenization process compared to the homogenization process that was applied to obtain larger droplets. Interestingly, this loss seems to be even more pronounced for model



**TABLE 2** Model infant follow-on formulas fortification with VMC (vitamin and mineral complement), normalization of tocopherols, and vitamin A contents and peroxides values for the additional storage test.

|  | Target value | POM-L        | POM-S        | DFOM-DPL-L   | DFOM-DPL-S   |
|--|--------------|--------------|--------------|--------------|--------------|
| Supplementation (mg L <sup>-1</sup> )      |              |              |              |              |              |
| Retinol                                    | –            | 0.11         | 0.11         | –            | –            |
| Retinyl acetate                            | –            | –            | –            | –            | –            |
| Tocopherol                                 | –            | 9.12         | 9.12         | 6.06         | 6.06         |
| VMC  | –            | 200          | 200          | 200          | 200          |
| Tocopherols contents (ppm)                 |              |              |              |              |              |
| Total                                      | 676.2        | 740.0 ± 18.6 | 710.5 ± 17.7 | 698.1 ± 33.1 | 703.1 ± 42.9 |
| α-Tocopherol                               | –            | 592.3 ± 15.2 | 567.3 ± 13.0 | 628.8 ± 29.6 | 634.2 ± 36.1 |
| Vitamin A content                          |              |              |              |              |              |
| Total (μg RE L <sup>-1</sup> )             | –            | 456.3 ± 31.8 | 422.3 ± 21.3 | 586.7 ± 62.4 | 491.5 ± 63.8 |
| Retinyl acetate (μg RA L <sup>-1</sup> )   | 849.1        | 450.1 ± 28.1 | 419.8 ± 21.2 | 599.9 ± 70.2 | 537.7 ± 69.0 |
| Retinyl palmitate (μg RP L <sup>-1</sup> ) | –            | –            | –            | 116.8 ± 2.2  | 41.7 ± 6.7   |
| PV (meqO <sub>2</sub> kg <sup>-1</sup> )   |              |              |              |              |              |
| Initial PV (day 0)                         | –            | 3.6 ± 0.5    | 2.8 ± 0.3    | 3.40 ± 0.3   | 3.11 ± 0.3   |
| Final PV (day 20)                          | –            | 33.4 ± 3.6   | 21.8 ± 2.3   | 16.6 ± 1.0   | 4.8 ± 0.6    |

Note: L is used for model IFF with droplet size of 0.7 μm and S for model IFF with a droplet size of 0.4 μm. POM-L/POM-S: Palm oil mixture based IFF with a droplet size of 0.7 or 0.4 μm. POM-DPL-L/POM-DPL-S: POM based IFF with a DPL as an emulsifier with a droplet size of 0.7 or 0.4 μm. Abbreviations: DPL, dairy phospholipid; PV, peroxide value; RA, retinyl acetate; RE, retinol equivalent.

IFF designed with soy lecithin as emulsifier, with for instance an initial retinyl acetate content up to four times lower for POM-S compared to POM-L versus twice lower for POM-DPL-S compared to POM-DPL-L (Table 1). Moreover, in the case of model IFF stabilized with soy lecithin, a higher loss of tocopherols was observed after 20 days of storage for the smaller droplet sizes with for instance 62.8% loss for POM-S against only 38.5% for POM-L. The substitution of soy lecithin with DPL in model IFF resulted in a very limited tocopherols loss after 20 days whatever the droplet size. These results suggest that the coverage of interfacial area by DPL is efficient, offering a good protection of tocopherols versus oxidation whatever the droplet size. On the contrary, with soy lecithin, the larger interfacial area obtained with smaller droplet may have been imperfectly covered by this emulsifier, resulting in a weaker protection of tocopherols. This result may also be related to the slightly higher protein content of model IFF with DPL. Concomitantly, no significant differences in the PV evolution were observed as a function of droplet size in the case of model IFF stabilized with soy lecithin (POM-(S and L) and POM-DLDC-(S and L)). However, for model IFF with a droplet size of 0.4 μm, the addition of soy lecithin to the water phase conducts to a 1.7-fold increase in PV. In the case of DPL-stabilized model IFF, the evolution of PV was significantly less important especially for the smaller droplets ( $4.4 \pm 0.3$  meqO<sub>2</sub> kg<sup>-1</sup> for POM-DPL-S) than for the larger ones ( $9.4 \pm 1.2$  meqO<sub>2</sub> kg<sup>-1</sup> for POM-S) after 20 days.

These results suggest that the droplet size cannot be considered individually to anticipate the resulting model IFF oxidative stability. Indeed, the chemical environment at the interface probably has a more significant influence than the droplet size alone on the oxidative stability.

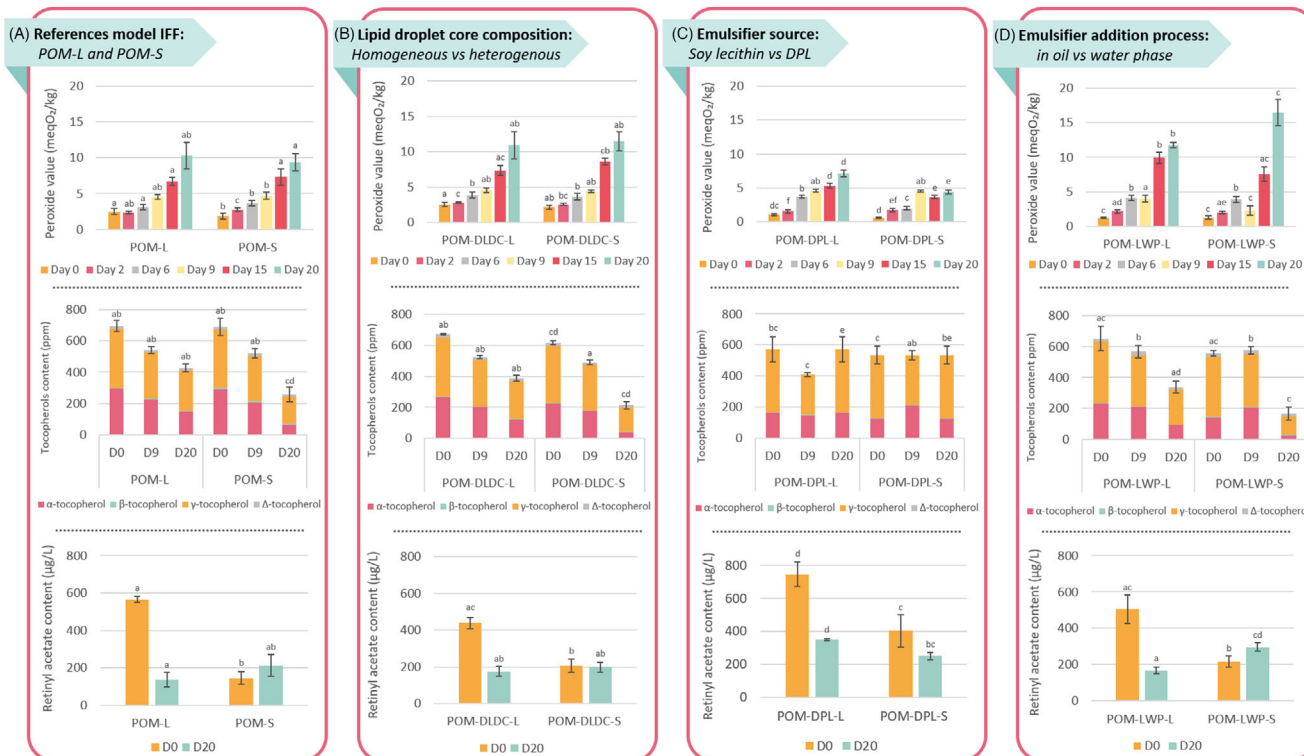
### 3.3 | Impact of homogenous or heterogeneous droplet core composition

The impact of the droplet core composition (homogeneous or heterogeneous) on the oxidative stability was evaluated by comparing reference model IFF, that is, POM-(S and L) with model IFF in which the individual core composition of the droplets was heterogeneous (PUFA and HMP oils droplets or IMP oils droplets), that is, POM-DLDC-(S and L) (comparison of Figure 4a,b). The results show no significant differences in the PV evolution after 20 days and reach on average  $10.5 \pm 0.9$  meqO<sub>2</sub> kg<sup>-1</sup>. The tocopherols loss was similar but higher for model IFF with a droplet size of 0.4 μm (62.8% and 65.3% of total tocopherol loss for POM-S and POM-DLDC-S, respectively) compared to the one with a size of 0.7 μm (38.5% and 42.6% of total tocopherol loss for POM-L and POM-DLDC-L, respectively). According to the tocopherols isomers composition, the tocopherols loss was mainly attributed to a loss of α-tocopherol that was the second most abundant isomer after the γ-tocopherol in these model IFF. Significant differences in retinyl acetate content were measured after processing, but levels after storage were equivalent.

### 3.4 | Impact of the interfacial composition

#### 3.4.1 | Impact of emulsifier type

Lipid oxidation is well known to occur at the lipid/water interface in emulsified system.<sup>[3]</sup> Therefore, the interface composition and

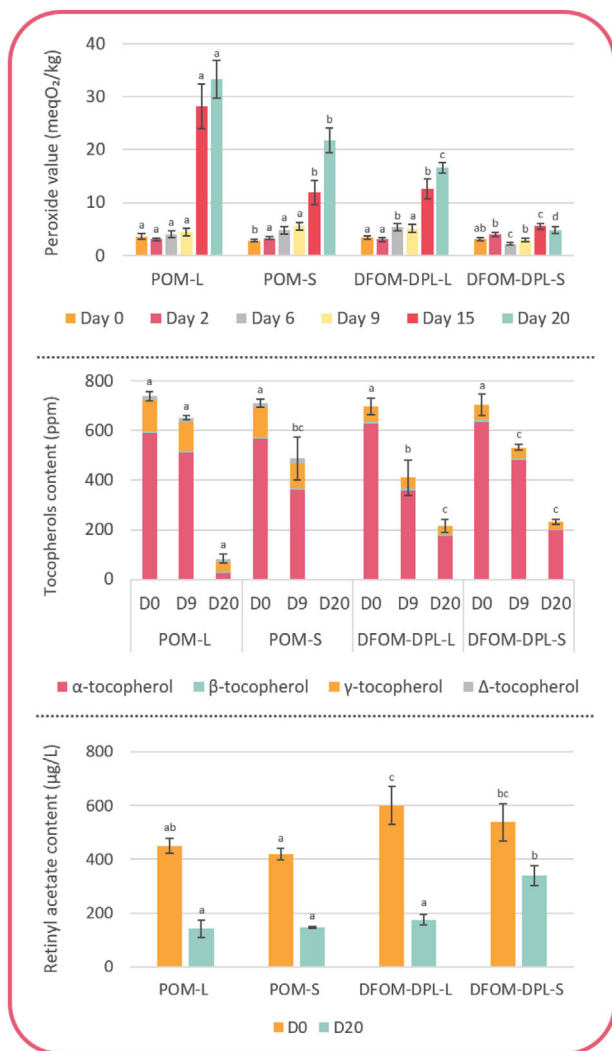


**FIGURE 4** Peroxide values, tocopherols, and retinyl acetate contents evolution (a) in the reference model infant follow-on formulas (IFF) (palm oil mixture [POM]-S and POM-L) and depending on (b) the lipid droplet core composition (homogeneous or heterogeneous), (c) the source of emulsifier (soy lecithin or dairy phospholipid), and (d) the emulsifier addition process (in the oil or water phase) during accelerated storage conditions. Oxidative stability is systematically compared with model IFF references: POM-S and POM-L. The different letters indicate a significant difference ( $p < 0.05$ ) between two model IFF at a given time. L is used for model IFF with droplet size of  $0.7 \mu\text{m}$  and S for model IFF with a droplet size of  $0.4 \mu\text{m}$ . POM-L/POM-S: POM-based IFF with a droplet size of  $0.7$  or  $0.4 \mu\text{m}$ . POM-DLDC-L/POM-DLDC-S: POM-based IFF with heterogeneous lipid droplet core composition (high and low melting point oils droplets, i.e., palm with arachidonic acid [ARA] and docosahexaenoic acid [DHA]) oils or intermediate melting point oils droplets (i.e., sunflower, oleic sunflower, and rapeseed oils) with a droplet size of  $0.7$  or  $0.4 \mu\text{m}$ . POM-LWP-L/POM-LWP-S: POM-based IFF with soy lecithin previously homogenized with the aqueous phase with a droplet size of  $0.7$  or  $0.4 \mu\text{m}$ . POM-DPL-L/POM-DPL-S: POM-based IFF with a DPL as emulsifier with a droplet size of  $0.7$  or  $0.4 \mu\text{m}$ . DPL, dairy phospholipid; DLDC, different lipid droplet composition; LWP: lecithin in water phase; VMC: vitamin and mineral complement.

physicochemical properties are crucial regarding oxidation rates and are directly correlated with the type of the emulsifier used. Therefore, model IFF that prepared either with soy lecithin, that is, POM-(S and L) or DPL, that is, POM-DPL-(S and L) were compared to evaluate the impact of the emulsifier source on the oxidative stability. According to PV evolution, model IFF stabilized with a DPL were significantly more stable than the ones stabilized with soy lecithin (comparison of Figure 4a,c). This tendency was even more pronounced for the small droplets size with PV of  $4.4 \pm 0.3 \text{ meqO}_2 \text{ kg}^{-1}$  for POM-DPL-S after 20 days versus  $9.4 \pm 1.2 \text{ meqO}_2 \text{ kg}^{-1}$  for POM-S. In parallel, the total tocopherols content remains constant after 20 days for model IFF with DPL as an emulsifier, whereas losses of 62.8% for POM-S and 38.5% for POM-L were observed. As expected, these results show that the type of emulsifier modulates the oxidative stability. In our case, DPL demonstrates superior protection against lipid oxidation compared to soy lecithin. Moreover, retinyl acetate losses were significantly lower when using DPL.

### 3.4.2 | Impact of the emulsifier partition between the lipid and the water phases

The oxidation rate in emulsions can be also related to the processing conditions.<sup>[28]</sup> Thus, the way the emulsifier is added during emulsification process plays an important role as it will determine the potential presence of emulsifier micellar or colloidal structures. These particular structures are known to play an important role on mass transport phenomena in lipid oxidation.<sup>[23,40]</sup> They could hypothetically be carriers of antioxidants, prooxidant, or oxidation compounds in emulsion. The oxidative stability of POM-(S and L) in which soy lecithin was added to the oil phase prior to emulsification processing was compared to POM-LWP-(S and L) in which the same emulsifier was added to the water phase also prior to emulsification processing. Depending on the addition process, it is expected that the emulsifiers are more prone to be localized at the interface or to form colloidal structures in the emulsions. The comparison of Figure 4a,d shows that the incorporation of soy lecithin in the water phase (POM-LWP-(S and



**FIGURE 5** Impact of introduction of dairy lipids (dairy fat olein and dairy phospholipids) in model infant follow-on formulas (IFF) composition on peroxide value, tocopherols, and retinyl acetate contents. Different letters indicate a significant difference ( $p < 0.05$ ) between two model IFF at a given time. L is used for model IFF with droplet size of 0.7  $\mu\text{m}$  and S for model IFF with a droplet size of 0.4  $\mu\text{m}$ . Palm oil mixture (POM)-L/POM-S: POM-based IFF with a droplet size of 0.7 or 0.4  $\mu\text{m}$ . POM-DPL-L/POM-DPL-S: POM-based IFF with a DPL as emulsifier with a droplet size of 0.7 or 0.4  $\mu\text{m}$ . DPL, dairy phospholipid; VMC, vitamin and mineral complement.

L)) leads to a higher PV evolution after 20 days whatever the droplet size. Similarly, the tocopherols loss was significantly higher when the emulsifier was added in the water phase. This loss was even more important for small droplets (70.2% loss for POM-LWP-S vs. 48.2% for POM-LWP-L).

### 3.5 | Effect of dairy lipids introduction in model IFF

An additional storage test was performed to further investigate the impact on oxidative stability of the substitution of POM by dairy

lipids (including DPL and DFOM) (Figure 5). Despite a faster oxidation kinetics than for the previous model IFF (resulting in higher PV and tocopherol degradation after 20 days of storage for POM-(S and L), model IFF based on dairy lipids were more stable than the ones with POM. PVs were indeed significantly lower, especially for the smaller droplets for which PV were 9.5 times lower in comparison with model IFF made from POM ( $4.8 \pm 0.6$  and  $21.8 \pm 2.3$  meqO<sub>2</sub> kg<sup>-1</sup> for DFOM-DPL-S and POM-S, respectively). Moreover, the tocopherols loss in DFOM-DPL-(S and L) was less important than POM-(S and L) with a loss up to 1.5 times lower. The difference in oxidative stabilities with the prior model IFF studied could be attributed on the fact that in these additional model IFF,  $\alpha$ -tocopherol was the most abundant tocopherol isomers (85% of the total tocopherols). However, in the prior model IFF, the most abundant isomer was the  $\gamma$ -tocopherol and represented at least 54% of the total tocopherols. The post-processing retinyl acetate content was significantly higher in DFOM-(S and L) and a lower loss after storage was measured in DFOM-DPL-S.

## 4 | DISCUSSION

Results presented in the current study showed that the studied model IFF have an overall good oxidative stability despite the addition of LC-PUFA (in particular DHA and ARA). Among the structural levers that we evaluated regarding their impact on oxidative stability, the most influential being the interface nature that can be modulated depending on the type of the used emulsifier (in our case soy lecithin vs. DPL). On the opposite, the droplet size and their core composition homogeneity have a limited impact which is mostly related to the chemical environment, especially to the presence of colloidal vesicles.

### 4.1 | Droplet size alone has no strong impact on oxidative stability over the range of model IFF sizes tested

Our results show that the droplets size (0.4 or 0.7  $\mu\text{m}$ ) impacts mainly the initial vitamin A content with a more important loss when model IFF were designed with smaller droplets. This observation is most likely explained by higher shearing forces that lead to higher local heating of vitamin A molecules favoring their oxidative degradation during process.

Over the droplet sizes range tested no strong effect on oxidative stability was observed considering this parameter alone illustrated for instance by POM-(S and L). The hypothesis most often encountered in literature is that smaller droplets oxidized faster due to their larger specific surface area in comparison with larger droplets. However, it is difficult to distinguish between the effect of droplet size and that of the chemical environment. In fact, homogenization conditions not only modulate droplet size but also have an impact on other crucial parameters involved in the oxidation reaction rates, such as the emulsifier partitioning with more or less adsorption at the interface.<sup>[39]</sup> The present study shows that the type of emulsifier and the chemical

environment determine stability rather than the droplet size. There are many contradictory results in the literature about the effect of droplet size. Some studies demonstrate that decreasing droplet sizes resulted in increased oxidation,<sup>[26]</sup> whereas others show that there was no effect of droplet size on lipid oxidation initiation or propagation,<sup>[14]</sup> or the opposite, an increased stability with a decreasing of the droplets size.<sup>[25]</sup> This dissensus supports our hypothesis that other factors, such as the interface composition, are more important than the total surface area itself.

From a nutritional point of view, droplet size has a very strong impact on the bioaccessibility of lipid compounds with accelerated lipolysis kinetics for smaller droplet sizes and a structure less close to that of HM.<sup>[4,5]</sup> Moreover, processing conditions to obtain smaller droplet may result in a loss of added vitamin A in model IFF has seen in the present study.

## 4.2 | Substitution of soy lecithin by DPL improves oxidative stability of model IFF

In emulsified systems, the interface area is known to be one of the main site of lipid oxidation phenomena.<sup>[3,40,41]</sup> Our study shows that, for an equivalent lipid composition, the substitution of soy lecithin by DPL in model IFF improves their oxidative stability (illustrated by the comparison of POM-DPL-(S and L) and POM-(S and L)). A first hypothesis to explain this result is that the thickness and density of the interface is more important for model IFF formulated with DPL. Indeed, DPL provides very long chains SFA (beyond C20:0) that create rigid domains at the interface which may form a steric protective barrier at the interface limiting the prooxidant effects of water-dissolved molecules such as metals. DPL also provides different PL species such as sphingomyelin which are known to have an antioxidant effect themselves or have synergistic effect with tocopherols.<sup>[38]</sup> The efficiency of the synergy could be related to the number of amino groups, which may regenerate tocopherols after a series of rearrangement reactions.<sup>[2]</sup> Another hypothesis that could explain these results is that the normalization of PL content has concomitantly slightly increased the protein content for model IFF with DPL compared to soy lecithin that can exhibit antioxidant properties.<sup>[32]</sup>

The results obtained in our study show that in presence of DPL the smaller the droplet size, the greater the stability. Moreover, the addition of DPL concomitantly increases protein content. Similar results were obtained in the study of<sup>[25]</sup> in which fish oil and/or rapeseed oil were homogenized with milk at different temperatures and pressures to obtain droplets from 0.5 to 1.36  $\mu\text{m}$  (D[3,2]). The authors show that oxidative stability was improved for smaller droplet sizes and hypothesized that the adsorption of proteins and their rearrangement at the interface was more important in the case of smaller droplets, enabling an interface more fully covered and facilitating the contact with amino groups.

The results showed that stability was even enhanced when DPL was used in combination with dairy fat. Dairy fat provides short and medium saturated chains and minor lipids such as cholesterol and other

complex lipids (glycerophospholipids or sphingolipids) that are suitable for infant nutrition. In addition, the organization of its FA on the TAG backbone, with long-chain SFA and LC-PUFA mainly esterified in *sn*-2 position, leads to better lipid absorption by avoiding the formation of insoluble soaps (calcium palmitate) which are directly excreted.<sup>[13]</sup> This organization is also suspected of influencing oxidative stability, with a protective effect for PUFA in the central position, as they are less accessible.<sup>[43]</sup> Complexifying the chemical composition by introducing dairy lipids is more relevant to mimic HM, and therefore more adapted to the needs of infants.<sup>[10]</sup> Moreover, despite a slightly lower oxidative stability than POM-DPL-S, droplets of 0.7  $\mu\text{m}$  would be closer to the average size of MFG and avoid overprocessing leading to significant losses of vitamin A in particular.

## 4.3 | The addition of lecithin to the water phase in model IFF may favor lipid oxidation

Our results show that the presence of colloidal structures in the water phase illustrated by POM-LWP-(S and L) was not inert regarding lipid oxidation and result in a decrease of the stability compared to POM-(S and L). Lipid oxidation depends not only on the amount of PUFA but also on the overall reactivity and interactions of the many molecules involved in lipid oxidation, and their location and mobility in the emulsion. More and more evidences show that colloidal structures such as the one studied in POM-LWP-(S and L) play a crucial role in mass transport phenomena in lipid oxidation pathway.<sup>[21,40]</sup> According to literature, micelles and colloidal structures may have two antagonistic effects regarding lipid oxidation in emulsified systems. They could act as antioxidant reservoirs, leading to a better diffusion of these molecules at the interface area and thus limiting oxidation. On the contrary, colloidal structure may serve as carriers of oxidation products, leading to their transfer from one droplet to another and promoting the oxidation. The results obtained in the present study seem to agree with the second pathway, as we observed that the additions of lecithin to the water phase diminish oxidative stability of model IFF. Moreover, this decrease in stability could also be attributed to the fact that the addition of lecithin in the water phase concomitantly affects the interfacial composition, by reducing its concentration at the oil–water interface. Consequently, interface coverage and the steric barrier would be lower. This would thus favor contacts between oxidizing compounds (transition metals or oxidation products such as hydroperoxides) and consumption of lipid-soluble antioxidants such as tocopherols leading to their fast degradation. Moreover, PL and tocopherols are known to have synergistic effect that favor the regeneration of tocopherols.<sup>[12,19,34]</sup> The lack of lecithin at the interface due to colloidal structure formation may also limit this synergy effect and could also explain the more important sensitivity to oxidation of POM-LWP-(S and L) compared to POM-(S and L). Finally, due to functional groups such as phosphates and amines, lecithin can also act as metal chelators that contribute to limit the prooxidant effects of iron or copper ions. The lower concentration of lecithin at the interface in POM-LWP-(S and



L) may also limit this effect at the oxidation initiation site, which could explain their higher susceptibility to oxidation. However, the transition metals in their free form are located in the water phase in direct contact with the colloidal structures, this hypothesis seems limited.

#### 4.4 | The individual core lipid droplets composition in a population is difficult to control and stabilize

MFG core composition of HM is heterogeneous, with the smallest globules containing a higher content of medium-chain SFA (C8–C12) and lower amount of long-chain SFA than the largest globules.<sup>[15]</sup> LC-PUFAs are therefore “encapsulated” in large globules surrounded by long-chain SFA, with high melting point leading to a partial surface crystallization.<sup>[30]</sup> Instinctively, these parameters could impact the oxidative stability due to the formation of a lipid shell protecting LC-PUFA (steric barrier). In our study, the individual composition of the lipid core droplet in POM-DLDC-(S and L) was expected to be heterogeneous to mimic the HM as closely as possible. Our results show no significant effect on oxidative stability. However, Okubanjo, Loveday et al.<sup>[33]</sup> studied the oxidative stability of oil-in-water emulsions by forming a lipid shell and demonstrated that structuring the interface with solid lipids improved oxidative stability. The authors suggest that this kind of system leads to limited contact between prooxidant and oxidizable compounds. Similar results were obtained by Salminen, Helgason et al.<sup>[36]</sup> who found that the thicker the crystallized shell, the better the oxidative stability. These findings were attributed to a reduced diffusion and mobility of prooxidants compounds and oxygen at the interface.

Contrary to the previous studies, our results show no significant effect of the lipid core heterogeneity. One hypothesis to explain this contradictory result would be that a homogenization of the composition of the lipid droplet cores occurred during storage. Physical instability, possibly due to the stirring and reduction in viscosity caused by storage at 40°C, could favor collisions between droplets and concomitantly the transfer of lipids between droplet cores. Further investigations will be necessary to determine whether the individual lipid core droplet can be controlled in a such complex food matrix and used to delay lipid oxidation.

## 5 | CONCLUSIONS

This study showed that the oxidative stability of model IFF depends on their chemical composition and can be modulated according to different structural levers. Based on the tocopherol degradation kinetics and PV evolution, the most representative indicators of oxidation in our systems, the results showed that in a such complex food matrix it is difficult to manage the individual lipid core droplet composition. The impact of droplet size on lipid oxidation in IFF is limited and highly dependent on other factors such as the chemical environment, and in particular the nature of the interface. The interface quality could be

modulated according to the source of emulsifier with a more important oxidative stability when soy lecithin is substituted by DPL. Moreover, the presence of colloidal structures in IFF has an impact on stability, probably by promoting the diffusion of oxidized compounds or limiting the synergy with tocopherols. Thus, considering both issues related to lipid oxidation and those related to nutrition, IFF enriched in LC-PUFA and incorporating dairy lipids may be a reliable strategy to guarantee oxidative stability and an adequate nutritional profile.

#### AUTHOR CONTRIBUTIONS

*Methodology; investigation; formal analysis; writing—review and editing:* Mathilde Cancalon. *Methodology; validation, writing—review and editing:* Nathalie Barouh and Youna Hemery. *Resources:* Bruno Baréa. *Writing—review and editing:* Erwann Durand. *Supervision; methodology; validation, writing—review and editing:* Claire Bourlieu-Lacanal. *Supervision; writing—review and editing:* Pierre Villeneuve.

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#### CONFLICT OF INTEREST STATEMENT

The authors have declared no conflicts of interest.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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## Data Article

# Dataset of the nutritional composition of follow-on infant formulas commercialized worldwide in 2021



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## ABSTRACT

The main objective of infant follow-on formulas, consumed from the age of 6 to 12 months, is to mimic the composition of breast milk in order to meet the nutritional needs of infant. In this context, their composition is governed in Europe by a strict regulation that has evolved in 2020 to force manufacturers to improve the nutritional profile of the formulas. The objective of this dataset was to collect the ingredient lists and nutritional values of infant follow-on formulas present on the world market with a focus on the lipid fraction. The data collection was carried out from December 2020 to April 2021 directly on the product packaging or on the websites of the different brands. Only “classic” infant follow-on formulas that are widely consumed were listed. Thus, the ingredient lists and nutritional values of 91 infant formulas were collected. The nutritional values are systematically presented for 100 g of powder, for 100 Kcal and for 100 mL of formula. The sources of fats, emulsifiers and vita-

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mins A and E were also extracted from the ingredient lists. This dataset can be used as a tool for the formulation of infant follow-on formulas or to situate the positioning of products in relation to the market.

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Specifications Table

|                          |   |
|--------------------------|---|
| Subject                  | Health and medical sciences: Nutrition  |
| Specific subject area    | Composition and nutritional values of infant follow-on formulas   |
| Data format              | Raw and, Filtered   |
| Type of data             | Table   |
| Data collection          | The data were collected from brand websites or directly from product labels according to three selection criteria: <ul style="list-style-type: none"><li>- The products had to be follow-on infant formulas, i.e. consumed between the ages of 6 and 12 months</li><li>- They had to be so-called "classic" formulas (not hypoallergenic, anti-reflux ...)</li><li>- The formulas had to be widely consumed to cover at least 60 % of the market share</li></ul> All data were anonymized via a coding of the products from IF1 to IF91 and a standardization of the ingredients lists (decreasing weight order not necessarily respected). |
| Data source location     | Global  |
| Data accessibility       | Repository name: Dataverse INRAE<br>Data identification number: <a href="https://doi.org/10.57745/CLER60">10.57745/CLER60</a><br>Direct URL to data: <a href="https://doi.org/10.57745/CLER60">https://doi.org/10.57745/CLER60</a>  |
| Related research article | M. Cancalon, Y. M. Hemery, N. Barouh, B. Baréa, C. Berton-Carabin, L. Birault, E. Durand, P. Villeneuve and C. Bourlieu- Lacanal, Comparison of the effect of various sources of saturated fatty acids on infant follow-on formulas oxidative stability and nutritional profile. Food Chem. <a href="#">[1]</a>   |

1. Value of the Data

- These data are useful to companies or researchers in the field of infant nutrition
- The dataset is a tool to help in the formulation of infant formulas for companies or representative model systems for laboratory research
- Companies or intervention programs aimed at improving infant formulas composition can use our data as a benchmark
- Our data are useful to companies to position their product in relation to the market and regulations
- The dataset can be used to identify the most used raw materials with a focus on the lipidic fraction (oils mixtures, sources of vitamins A and E fortification, type of emulsifiers)
- These data constitute a support for the estimation of the nutritional intake and the contribution of each ingredient. These data are complementary to the CIQUAL, USDA or FCEN databases

2. Data Description

This dataset was generated to provide an overview of the ingredient lists and nutritional values of 91 infant follow-on formulas covering more than 60 % of the market share by volume. The nutritional characteristics of infant follow-on formulas have been compared with European and international regulations. This overview was also intended to identify the sources of fat,



emulsifiers and fat-soluble vitamin fortification (A and E) most commonly used in this type of product (Table 1).

**Table 1**

Nutritional characteristics of infant follow-on formulas from the dataset and European and international regulations.

| (/100 mL)                            | Infant follow-on formulas<br>Mean $\pm$ SD | EU regulation<br>CE 2016/127 |       | Codex Alimentarius<br>CX 156/1987 |       |
|--------------------------------------|--|------------------------------|-------|-----------------------------------|-------|
|                                      |  | Min                          | Max   | Min                               | Max   |
| <b>Energy (kcal)</b>                 | 67,2 $\pm$ 1,3                             | 60                           | 70    | 60                                | 85    |
| <b>Lipids (g)</b>                    | 3,3 $\pm$ 0,2                              | 2,64                         | 4,2   | 1,8                               | 5,1   |
| SFA (g)                              | 1,1 $\pm$ 0,4                              | –                            | –     | –                                 | –     |
| MUFA (g)                             | 1,5 $\pm$ 0,4                              | –                            | –     | –                                 | –     |
| PUFA (g)                             | 0,6 $\pm$ 0,1                              | –                            | –     | –                                 | –     |
| LA (mg)                              | 502,2 $\pm$ 0,1                            | 300                          | 840   | 180                               | –     |
| ALA (mg)                             | 53,7 $\pm$ 12,6                            | 30                           | 70    | –                                 | –     |
| ARA (mg)                             | 9,4 $\pm$ 5,6                              | –                            | 42    | –                                 | –     |
| DHA (mg)                             | 14,8 $\pm$ 4,0                             | 12                           | 35    | –                                 | –     |
| <b>Carbohydrates (g)</b>             | 7,8 $\pm$ 0,4                              | 5,4                          | 9,8   | –                                 | –     |
| <b>Proteins (g)</b>                  | 1,4 $\pm$ 0,2                              | 1,08                         | 1,75  | 1,8                               | 4,7   |
| Casein (g)                           | 0,8 $\pm$ 0,3                              | –                            | –     | –                                 | –     |
| <b>Vitamin A (<math>\mu</math>g)</b> | 61,5 $\pm$ 6,4                             | 42                           | 79,8  | 45                                | 191,3 |
| <b>Vitamin D (<math>\mu</math>g)</b> | 1,4 $\pm$ 0,3                              | 1,2                          | 2,1   | 0,6                               | 2,55  |
| <b>Vitamin E (mg)</b>                | 1,2 $\pm$ 0,4                              | 0,36                         | 3,5   | 0,28                              | –     |
| <b>Vitamin C (mg)</b>                | 9,8 $\pm$ 2,3                              | 2,4                          | 21    | 4,8                               | –     |
| <b>Iron (mg)</b>                     | 0,98 $\pm$ 0,16                            | 0,36                         | 1,4   | 0,6                               | 1,7   |
| <b>Copper (mg)</b>                   | 0,052 $\pm$ 0,06                           | 0,036                        | 0,070 | –                                 | –     |

SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; LA: Linoleic acid; ALA:  $\alpha$ -linolenic acid; ARA: Arachidonic acid; DHA: Docosahexaenoic acid.

The dataset is provided as a text file (.txt) and three Excel Spreadsheet (.xlsx) files, each including one sheet:

**Summary.txt** provides information about the aim and the method used to generate this dataset in three points. The “aim of the dataset” section describes the objective, the “Selection criteria” section lists the criteria for data inclusion and the “Data collection method” section defines the data collection method used. This file also gives additional information such as the abbreviations list and the specificities on the list of ingredients (decreasing order of weight of ingredients not necessarily respected) and the sources of vitamins A and E (addition of an asterisk when the source is not specified).

**Nutritional values.xlsx** provides information about the age group in which the product is consumed, the list of ingredients, the composition of the oil mixture, the source of emulsifier and vitamin A and E fortification, the preparation conditions per 100 mL and the nutritional values per 100 g of powder, 100 mL of preparation or 100 kcal. Thus energy, fats content with details on the content of saturated fatty acids (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) and specific fatty acids (linoleic (LA),  $\alpha$ -linolenic (ALA), docosahexaenoic (DHA) and arachidonic acids (ARA)) are listed. The carbohydrates and proteins contents (with casein and serum protein contents) are also provided. Finally, the contents of vitamins A, E, C, D and iron and copper are presented. When the values were not available the boxes are left empty. This file is organized as described in Fig. 1.

**Overview.xlsx** provides information about the number of values available on the product labels or on the website, the average (with standard deviations), maximum and minimum nutritional values for 100 g of powder, 100 ml of preparation or 100 kcal.

**Examples of regulation.xlsx** provides information concerning the range of contents in certain macro and micronutrients governed by the international regulation (Codex Alimentarius STAN 156/1987) and the European regulation (UE regulation 2016/127).

| General information | Product ID   | Age                                  | Ingredients list   | Lipid fraction composition                                     | Emulsifiers  | Vitamin E   | Vitamin A   | Formulas preparation g/100mL                  |
|---------------------|--|--------------------------------------|--|--|--|---|---|---|
|                     | Products name<br>All formulas have been anonymized | Age at which the product is consumed | List of ingredients of the products<br>All ingredient lists have been standardized to have a single format | Fat composition<br>Data extracted from the list of ingredients | Emulsifier source<br>Data extracted from the list of ingredients | Vitamin E fortification source<br>Data extracted from the list of ingredients | Vitamin A fortification source<br>Data extracted from the list of ingredients | Amount of powder to prepare 100 mL of formula |

| Nutritional values | Nutritional values for 100 g of powder, 100 mL of preparation or 100 kcal                  |  |   |  |   |   |
|--------------------|--|--|---|--|---|---|
|                    | Energy (kcal)  | Fats (g)   | Carbohydrates (g)   | Proteins (g)   | Vitamins  | Minerals  |
|                    | Energy in kcal per 100 g of powder, 100 mL of preparation or values expressed per 100 kcal | Fats content in grams per 100 g of powder, 100 mL of preparation or 100 kcal   | Carbohydrates content in grams per 100 g of powder, 100 mL of preparation or 100 kcal | Proteins content in grams per 100 g of powder, 100 mL of preparation or 100 kcal | Vitamins content per 100 g of powder, 100 mL of preparation or 100 kcal | Minerals content per 100 g of powder, 100 mL of preparation or 100 kcal |
|                    |  | SFA (g)<br>MUFA (g)<br>PUFA (g)<br>LA (mg)<br>ALA (mg)<br>ARA (mg)<br>DHA (mg) |   | Caseins (g)<br>Serum proteins (g)  | A (µg)<br>D (µg)<br>E (mg)<br>C (mg)                                    | Iron (mg)<br>Copper (mg)  |

Fig. 1. Organization and information provided in the “Nutritional values.xlsx” file.

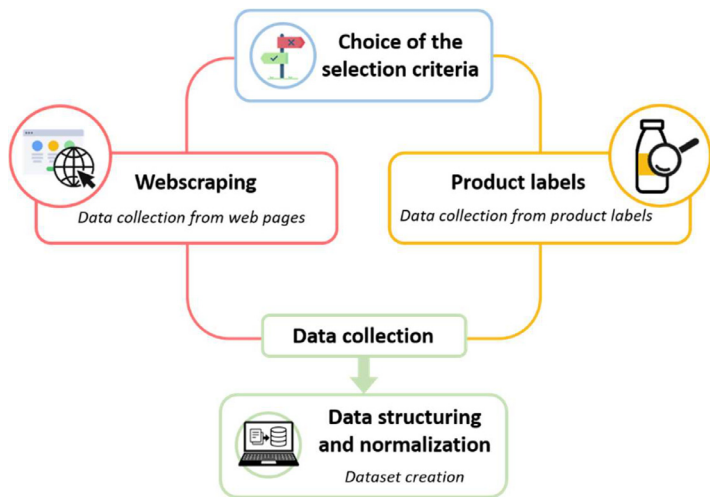


Fig. 2. Collection data method.

3. Experimental Design, Materials and Methods

The data were collected according to a three-phase method as shown in Fig. 2. In a first step, selection criteria were established. Thus, the products included in the dataset had to be infant formulas of second age also named follow-on formulas. They had to be classic formulas (no anti-reflux or hypoallergenic formulas...) and widely consumed. The data were then collected in two ways. Either via a webscraping method (information collected on websites either of brands or merchants trading products) or directly on the labels of the products. Once the data collected, it was anonymized by attaching a product code to each formulas and then structured to form the dataset. The lists of ingredients were standardized in order to preserve the anonymization and to present the data systematically in the same format. In each list of ingredients, data were extracted when they were indicated, namely the sources of fat, emulsifier and vitamin A and E used.

## Limitations

The data included in the dataset is subject to change, particularly in case of regulatory changes, and therefore needs to be regularly updated.

## Ethics Statement

The authors have read and follow the ethical requirements for publication in Data in Brief and confirming that the current work does not involve human subjects, animal experiments, or any data collected from social media platforms.

## Data Availability

[Panorama of the nutritional composition of follow-on infant formula commercialized worldwide in 2021 \(Reference data\)](#) (Dataverse)

## CRediT Author Statement

**Mathilde Cancalon:** Conceptualization, Methodology, Investigation, Writing – review & editing; **Youna M. Hemery:** Conceptualization, Methodology, Writing – review & editing, Validation; **Nathalie Barouh:** Conceptualization, Methodology, Writing – review & editing, Validation; **Rallou Thomopoulos:** Resources; **Bruno Baréa:** Resources; **Erwann Durand:** Writing – review & editing, Validation; **Pierre Villeneuve:** Supervision, Writing – review & editing, Validation; **Claire Bourlieu Lacanal:** Supervision, Conceptualization, Methodology, Writing – review & editing, Validation.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Reference

- [1] M. Cancalon, et al., [Comparison of the effect of various sources of saturated fatty acids on infant follow-on formulas oxidative stability and nutritional profile](#), Food Chem. 429 (2023) 136854.