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APPLICATION TO PERMIT THE OPTIONAL USE OF MILKFAT GLOBULE MEMBRANE ENRICHED WHEY PROTEIN CONCENTRATE IN FORMULATED SUPPLEMENTARY FOODS FOR YOUNG CHILDREN

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Abbreviations

Abbreviation	Definition
ADPH	Adipophilin
AE	Adverse events
AFI	Arla Foods Ingredients P/S
Alk-SMase	Alkaline SMase
ANZ	Australia and New Zealand
ARA	Arachidonic acid
ASEBA	Achenbach System of Empirically Based Assessment
AUD	Australian dollars
BSSL	Bile salt-stimulated lipase
CAS	Chemical Abstracts Service
CCI	Confidential commercial information
CCP	Critical control point
Cer	Ceramide
CIP	Clean-in-place
cGMP	Current Good Manufacturing Practices
CGMP	Casein glycomacropeptide
ChP	Choline phosphate
GD3	Disialoganglioside
GPC	Glycerophosphocholine
GPE	Glycerophosphoethanolamine
DVFA	Danish Veterinary and Food Administration
ECCB	Exclusive capturable commercial benefit
EU	European Union
EV	Extracellular vesicles
FABP	Fatty acid binding protein
FDA	Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
FSFYC	Formulated supplementary foods for young children
FSC	Food standards code
GG	Gangliosides
HACCP	Hazard Analysis Critical Control Point
IAP	Intestinal alkaline phosphatase
IgG	Immunoglobulin G
MF	Microfiltration
MFG	Milk fat globules
MFGM	Milkfat globule membrane
MFGM-WPC	Milkfat globule membrane enriched whey protein concentrate
MPLs	Milk polar lipids
N-CDase	Neutral ceramidase

Abbreviation	Definition
Neu5Ac	N-acetylneuraminic acid
N-FA	N-fatty acyl
NIP	Nutrition Information Panel
PAGE	Polyacrylamide gel electrophoresis
PAS	Periodic acid/Schiff
PC	Phosphatidyl choline
PE	Phosphatidyl ethanolamine
PEAL	Plain English Allergen Labelling
PGD	Prostaglandin
PI	Phosphatidyl inositol
PL	Phospholipids
PLB	Phospholipase B
PS	Phosphatidyl serine
PUFA	Polyunsaturated fatty acid
RDA	Recommended dietary allowance
RID	Radial immunodiffusion
RO	Reverse osmosis
SA	Sialic acid
SAE	Serious adverse event
SM	Sphingomyelin
SMPR	Standard Method Performance Requirements
SMase	Sphingomyelinase
Sph	Sphingosine
SPL	Sphingosine lyase
SPLs	Soy polar lipids
sPLA2 IB	Secretory pancreatic phospholipase A2 IB
SPK	Sphingosine kinase
TAG	Triacylglycerol
UF	Ultrafiltration
UK	United Kingdom
UV	Ultra-violet
WPC	Whey protein concentrate
XDH/XO	Xanthine oxidase
α-LA	Alpha-lactalbumin
β-LG	Beta-lactoglobulin

Executive Summary

Arla Foods Ingredients P/S (AFI) is a Danish food ingredient manufacturer supplying dairy-based ingredients, using standard whey processing techniques, for a wide variety of food applications and medical nutrition formulations for all ages. Arla Foods Ingredients P/S has developed a dairy-derived milkfat globule membrane enriched whey protein concentrate (MFGM-WPC) known under the tradename Lacprodan® MFGM-10. Compared to typical WPC's, MFGM-WPC is enriched with phospholipids, glycolipids, membrane proteins, and sphingolipids. These stem from the three-layer milk fat globule membrane (MFGM) and single layer membrane of extracellular vesicles (EV) enriched in this product. MFGM is a component of all mammalian milks and is the primary delivery mechanism of fats in mammalian milk. For simplicity the MFGM and EV membrane materials are collectively referred to as MFGM as per the body of existing literature. All young children consuming any mammalian milk products, therefore consume MFGM and its lipid and protein components, albeit at different levels.

Arla Foods Ingredients P/S is requesting amendment to the Code to permit the addition of MFGM-WPC to formulated supplementary foods for young children (FSFYC) in Australia and New Zealand at maximum addition rate of 2.3 g per serve, and not more than 40 mg of sphingomyelin per serve.

The body of evidence presented demonstrates that MFGM-containing foods support immune-relevant function in young children, with studies in and contiguous to the 1–3-year age range showing reductions in the prevalence and severity of infection-related incidences, and mechanistic data providing biological plausibility for immune modulation during this critical window. Research across the lifespan further reinforces the immune-related benefits of MFGM, and its long history of safe use and tolerability in vulnerable populations—including infants and the elderly—supports the reasonable conclusion that MFGM will be safe and well tolerated in young children. Taken together, these findings provide a strong rationale for the inclusion of MFGM-WPC in FSFYC to deliver additional immune-related benefits in the 1-3 year old age group.

Moreover, the optional addition of MFGM-WPC to FSFYC will increase the range of beneficial ingredients that can be used in these products. The addition of MFGM-WPC will provide additional nutritional benefits supporting normal development and immune function in young children. The addition of MFGM-WPC to FSFYC will increase consumer choice of products for young children that has seen limited innovation in Australia and New Zealand over a number of years.

1 General Requirements (3.1.1)

1.1 Purpose of the application

This application seeks permission under the Australia New Zealand Food Standards Code (FSC) for the optional addition of bovine milk derived MFGM-WPC as a nutritive substance, to foods regulated within the FSC Part 2.9 Special purpose foods, specifically Standard 2.9.3 Formulated meal replacements and formulated supplementary foods Division 4 Formulated supplementary foods for young children (FSFYC). The age of young children to which this regulation is applicable is 1 to 3 years, as defined in Standard 1.1.2-3 Definitions-particular foods. The nutritional purpose for the addition of MFGM-WPC replaces and supplements MFGM levels normally found in milk, but which are typically removed during milk processing, and contributes to the support of immune function in young children.

MFGM-WPC is a whey protein concentrate sourced from bovine milk containing approximately two to four-fold higher enrichment of MFGM lipid and protein components compared to a standard whey protein concentrate. It is widely used in formulas for young children throughout the world. AFI manufactures the ingredient under various trade names around the world, including Lacprodan® MFGM-10 which is available in Australia and New Zealand.

Permission to add nutritive substances to foods is regulated by Part 2.9 Special purpose foods, this application seeks to vary Standard 2.9.3 Division 4 Formulated supplementary foods for young children.

MFGM-WPC is a complex ingredient with a number of components that are common to other dairy and non-dairy ingredients added to FSFYC. Sphingomyelin is suitable as an analytical marker of MFGM-WPC addition. As there are no analytical methods to quantify the addition of MFGM-WPC, this can only be determined at formulation and manufacturing, therefore proposed limits are controlled through the quantification of sphingomyelin (SM). Proposed changes to the Code are laid out, as follows:

2.9.3—7 Compositional requirements for formulated supplementary foods for young children

2.9.3-7 (5) MFGM enriched whey protein concentrate (MFGM-WPC) may be used as a nutritive substance in a formulated supplementary food for young children at a maximum rate of 2.3 g/serving.

2.9.3-7 (6) When MFGM enriched whey protein concentrate (MFGM-WPC) is used as a nutritive substance in a formulated supplementary food for young children the total amount of sphingomyelin, both from added MFGM-WPC, and naturally occurring, must not be more than 40 mg/serving.

2.9.3-8 Labelling of formulated supplementary foods for young children

2.9.3-8 (7) If MFGM-WPC is added, the label on a package of formulated supplementary food for young children may include words indicating that the product contains MFGM enriched whey protein concentrate (MFGM-WPC) and /or total SM.

The purpose of this application is consistent with policy guidelines as set out by the Food Ministers' Meeting (previously the Australia and New Zealand Food Ministerial Forum on Food Regulation).

The relevant policy guideline for this application is the 'Recommended Policy Guideline on the intent of Part 2.9 – Special Purpose Foods', noting the 'Policy Guideline for the Addition to Food of Substances other than Vitamins and Minerals' does not apply to special purpose regulated under Part 2.9 of the Code.

The addition of MFGM-WPC is aligned with the 'High Order' Policy Principles:

(1) the protection of public health and safety, informed consumer choice and the prevention of misleading or deceptive conduct, and
(2) is based on risk analysis using sound scientific evidence available, will promote consistency between domestic and international food standards, will help promote an efficient and internationally competitive food industry and fair trading in food.

The application is further aligned with the 'Specific Policy Principles' in that the target population is clearly defined (young children aged 1 to 3 years) as is the composition, within Standard 2.9.3 Division 4 FSFYC. These principles also call for the provision of adequate information, including through labelling and advertising of special purpose foods, to:

- assist consumer understanding of the specific nature of the food, the intended population group and intended special purpose of the food; and
- provide for safe use by the intended population and to help prevent inappropriate use by those for whom the special purpose food is not intended.

FSFYC sold as "toddler milk" or "formula for young children" or potentially other formulated foods for young children, addresses these requirements in that the products are necessarily compliant with the FSC and clearly identified for the intended target age group (1 – 3 years) through labelling and product proposition. Manufactured and labelled according to the FSC they are safe for use by the intended population.

1.2 Justification for the application

The components of MFGM are naturally occurring in mammalian milks, including human milk and are associated with health benefits and supported by evidence supporting a key role in early life development. Milkfat globule membrane has been shown to support immune function in young children.

Milk-based FSFYC have lower levels of MFGM components than whole milk, as they are typically formulated using skim milk and / whey protein and are vegetable oil filled. The addition of MFGM-WPC to FSFYC can help deliver the benefits detailed in Section 2.5 in young children. The use of MFGM-WPC in aligned product categories in international markets is well established.

Currently, the Code does not explicitly permit the addition of MFGM-WPC, as a nutritive substance, to FSFYC. Therefore, this application seeks to clarify the permission to use and seeks to amend Standard 2.9.3 of the Code to allow MFGM-WPC as a permitted nutritive substance in FSFYC.

The optional addition of MFGM-WPC to FSFYC will extend the options of beneficial ingredients that may be used in the manufacture of FSFYC and hence provide additional benefits and increase

consumer choice. Young Australian and New Zealand children will have the opportunity to consume FSFYC that deliver the benefits of MFGM, similar to young children around the world.

Arla Food Ingredients P/S has not identified any disadvantages to permitting the addition of MFGM-WPC to FSFYC. Permission in the Code will be for optional addition. MFGM-WPC is sourced solely from bovine milk and is therefore required to be listed on product labels in accordance with allergen labelling requirements in ANZ (Standard 1.2.3). Allergen labelling requirements apply to existing standard milk-based products manufactured as FSFYC.

1.2.1 Regulatory impact

This application will provide regulatory clarification in Australia and New Zealand (ANZ) with regards to the permitted use of MFGM-WPC in formulated supplementary foods for young children.

Outcomes of this application may require consideration if in the future a standard for Young child formula (as per P1066) is introduced.

As this application is for a Nutritive Substance, P1024 – revision of the Regulation of Nutritive Substances & Novel Foods (currently on hold pending Food Standards Australia New Zealand (FSANZ) Act Review) may hold some relevance if in the future it is progressed. Currently there is no impact of that review affecting this application.

1.2.1.1 Cost and benefits

Manufacturers, suppliers and importers will be able to market and import MFGM-WPC and MFGM-WPC fortified products for sale in the Australian and New Zealand markets.

The facilitation of export trade of FSFYC, and equivalent product categories in other international markets, manufactured by domestic manufacturers in Australia and New Zealand will be of direct benefit to them by increasing market opportunities and ease of access to those markets.

Consumers in Australia and New Zealand will benefit from the availability of FSFYC with MFGM and the benefits associated with its consumption.

The incremental cost of the addition of MFGM-WPC is normally passed on as a nominal price premium for products to which it is added. In ANZ the FSFYC has generally been slower to keep pace with innovations in optional ingredients which support the on-going development of young children in this category.

In the range of available FSFYC, historically the use of optional ingredients and associated price premiums has resulted in market segmentation into standard and premium product ranges, that have provided consumers with product and price range options.

The cost of addition of MFGM-WPC to FSFYC products is dependent on ingredient cost and addition rate. Cost expectations are that the addition of MFGM to FSFYC would add approximately 0.10 to 0.15 Australian dollars (AUD) per serve. Products containing MFGM-WPC are expected to be at the premium end of the market and attract a higher price differential.

Several commercial sources of MFGM-WPC are available, both domestically and internationally. Permission to use MFGM-WPC in FSFYC domestically will enable manufacturers the option to use and does not raise any issues related to fair trading at any point in the manufacture and sale of FSFYC containing MFGM-WPC.

1.2.1.2 Impact on International trade

The permitted addition of MFGM-WPC to formulated supplementary foods for young children (regulated under Standard 2.9.3 Division 4) will better align products made in Australia and New Zealand with existing standards in other countries, facilitating international trade among jurisdictions in which MFGM-WPC is already or is soon to be permitted.

Domestic manufacturers wishing to export MFGM-WPC fortified products to overseas markets will benefit from permission in the Code to add MFGM-WPC through alignment with international standards and no requirement to apply for exemptions under export regulations. There are significant market opportunity advantages with the ability to export locally compliant value-added products to various markets around the world, for example under Certificates of Free Trade.

1.3 Assessment procedure

Based on the criteria provided in the FSANZ "Application Handbook" (1 July 2024), AFI considers this application should be assessed by FSANZ under the general procedure. This application is for the variation of food regulatory measures required for the addition of a new nutritive substance to foods and the requirement for pre-market approval of the substance. Arla Foods Ingredients P/S notes that MFGM is a component of mammalian milks; and no genetic modification has taken place, which may reduce complexity of assessment.

1.4 Confidential commercial information

Information is submitted separately under the terms of Confidential Commercial Information (CCI).

1.5 Other confidential information

Information is submitted separately under the terms of Confidential Information (CI).

1.6 Exclusive capturable commercial benefit

This application is not subject to any request for an Exclusive capturable commercial benefit (ECCB).

1.7 International and other national standards

1.7.1 International standards

The addition of MFGM-WPC to FSFYC is consistent with the intent and recommendations of relevant internationally recognised codes of practice and guidelines, and in particular with those by the Codex Alimentarius Commission.

The Codex Alimentarius Commission (Codex)[1] provides a number of standards, codes of practice and guidelines relevant to special purpose foods, and for which the use of MFGM is consistent with their intent.

[1] http://www.fao.org/fao-who-codexalimentarius/en/

The 2023 revision of the Codex Standard for Follow-up Formula for Older Infants and Product for Young Children (CXS 156-1987)[2] specifically addresses products for young children (12-36 months); Section B: Drink for young children with added nutrients or product for young children with added nutrients or drink for young children or product for young children. The standard allows for the addition of optional ingredients provided the safety and suitability of the ingredient for a particular nutritional purpose, at the level of use, is evaluated by national and/or regional authorities and demonstrated by generally accepted scientific evidence. Furthermore, that the optional ingredient is present in sufficient amounts to achieve the intended effect (CODEX Alimentarius, 2023).

The proposed addition of MFGM-WPC to FSFYC under Standard 2.9.3 is aligned with the intent of the Codex follow-up formula standard. Additionally, the production and specifications set for MFGM are consistent with the Codex recommendations for raw materials and ingredients for use in infant and follow-up formula and formulae for special medical purposes for infants and young children (Codex Code of Hygienic Practice for Powdered Formulae for Infants and Young Children[3] (Codex Alimentarius, 2008).

The Codex Guidelines for Formulated Supplementary Foods for Older Infants and Young Children[4], allows for the use of other ingredient that may "improve the nutritional quality and/or acceptability of the Formulated Complementary Foods provided that they are readily available and have been proven to be suitable and safe for their intended purpose" (Codex Alimentarius, 2017). On this basis the addition of MFGM-WPC to Formulated Supplementary Foods for Older Infants and Young Children would be permissible, and indeed it is added under this standard in a number of countries.

Permission to add MFGM-WPC t FSFYC in ANZ will further align domestic regulation in ANZ with CODEX.

1.7.2 Other national standards

Formulated products for young children that contain added or mention of MFGM or SM are available in a number of countries. These include, but are not limited to: Greece, Great Britain, Ireland, Slovakia, Hungary, Poland, Spain, the Netherlands, Portugal, Czech Republic, Sweden, Singapore, Hong Kong, Peoples Republic of China, Thailand, Malaysia, Philippines, Indonesia, Vietnam, South Korea, Japan, Sri Lanka, South Africa, United Arab Emirates, Saudi Arabia, Egypt, Russia, Ukraine, United States of America, Canada, Colombia, Argentina, Peru and Mexico.

In many of these countries, the ingredient is listed simply as whey protein concentrate or whey protein concentrate (containing MFGM). In Mexico it is listed as milk solids.

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[2] (https://www.fao.org/fao-who-codexalimentarius/sh-proxy/zh/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B156-1987%252FCXS_156e.pdf

[3] https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B66-2008%252FCXP_066e.pdf

[4] https://www.fao.org/fao-who-codexalimentarius/sh-proxy/zh/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B8-1991%252FCXG_008e.pdf

Figure 1-1 Global overview of countries where FSFYC-type products containing MFGM-WPC are available

(created using https://www.mapchart.net/world.html)

1.7.2.1 European Union

In the European Union (EU) milk-derived ingredients enriched with MFGM components, such as MFGM-WPC, have been available and added to consumer products marketed prior to 15th of May 1997. This is the cut-off date for novel foods described in the Novel Food regulation, Regulation (EU) 2015/2283. Products and ingredients in the market prior to that date are exempt from the Regulation. MFGM-WPC is therefore not considered to be a novel food and for that reason the ingredient does not need a safety approval before marketing in the member states of the EU. There has been no negative feedback from the competent authorities in the individual member states on formulas for young children which contain Lacprodan® MFGM-10. In the EU, there is no specific regulation for formulated products for young children however, MFGM-WPC has been consumed in formulated products for young children for at least the past 15 years.

The EU commission has accepted a ruling by the competent authority of Denmark under an Article 4 Request (Regulation (EU) 2015/2283)[5] that MFGM-WPC is not a novel food (Appendix II).

1.7.2.2 United Kingdom

On exiting the European Union, the United Kingdom (UK) adopted the majority of the EU food laws, including approved novel foods, and those not deemed novel under the EU regulations. As MFGM-WPC falls into this latter category, it may be used in formulated products for young children the UK.

1.7.2.3 United States of America

In accordance with 21 CFR 170.30(b) and in conformance with the guidance issued by the Food and Drug Administration (FDA) under 81 Fed. Reg. 54960 (Aug. 17, 2016), the applicants MFGM-WPC

[5] https://food.ec.europa.eu/document/download/a897488b-126a-4105-8fb9-589dc7e2a783_en?filename=novel-food_consult-status_whey-protein.pdf

(Lacprodan® MFGM-10) is self-determined as GRAS based on scientific procedures for its intended use. Lacprodan® MFGM-10 is intended for use in foods other than infant formula, targeting the general population. This includes those foods intended for young children. Label information may or may not contain a comment about being a source of MFGM.

Accordingly, permission for use of MFGM-WPC in FSFYC in ANZ is aligned with policy guidelines as it will promote consistency between the domestic and international markets and support an internationally competitive food industry.

1.8 Checklists

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	☒	Pages sequentially numbered	Yes	
	☒	Electronic copy (searchable)	Yes	
	☒	All references provided	Yes	
☒		**B Applicant details**	Yes	
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	☒	Non-confidential summary provided	Yes	
☒		**H Other confidential information**	Section	18
	☒	Confidential material separated from other application material	Yes	
	☒	Formal request including reasons	Yes	
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2 Substances used for a nutritive purpose (3.3.3)

2.1 Information on the use of the nutritive substance

2.1.1 Purpose of the nutritive substance

The purpose of the use of MFGM-WPC in FSFYC is to support immune function in children aged 1 to 3 years. The addition of MFGM-WPC to formulated supplementary foods replenishes and enriches milk-based products with MFGM-derived lipid and protein components. The evidence to support on-going immune function benefits associated with MFGM intake in young children is discussed in Section 2.5.4.

The MFGM-lipid components of MFGM-WPC are primarily associated with its physiological benefits and are also the key characterising components, when added to FSFYC. The whey component contributes to the total protein content and nutritional function of protein in the FSFYC. Whey and ingredients containing whey are commonly used in FSFYC. The protein content (minimum) of FSFYC is regulated by the Food Standards Code in Australia and New Zealand (Standard 2.9.3 Division 4) and by other regulatory instruments around the world.

2.9.3—7 Compositional requirements for formulated supplementary foods for young children

2.9.3-7 (5) MFGM enriched whey protein concentrate (MFGM-WPC) may be used as a nutritive substance in a formulated supplementary food for young children at a maximum rate of 2.3 g/serving.

2.9.3-7 (6) When MFGM enriched whey protein concentrate (MFGM-WPC) is used as a nutritive substance in a formulated supplementary food for young children the total amount of sphingomyelin, both from added from MFGM-WPC, and naturally occurring, must not be more than 40 mg/serving.

2.9.3-8 Labelling of formulated supplementary foods for young children

2.9.3-8 (7) If MFGM-WPC is added, the label on a package of formulated supplementary food for young children may include words indicating that the product contains MFGM enriched whey protein concentrate (MFGM-WPC) and /or total SM.

The addition of MFGM-WPC to FSFYC can be characterised by the quantification of sphingomyelin in the final FSFYC product.

Bovine derived MFGM-WPC is a source of MFGM components, together with those from EV's. These components are naturally present in all mammalian milks. The immune function benefits of these components for young children have been identified, and the safety of these in products established in both human and animal studies.

2.1.2 General data requirements for supporting evidence

A literature search was undertaken to identify published studies supporting the safe use and tolerance of MFGM-WPC in FSFYC and studies that substantiate immune function and immune-related outcomes associated with the addition of MFGM enriched ingredients, such as MFGM-WPC, in products consumed by young children.

2.1.2.1 Clinical studies related to MFGM and immune function in young children

A literature search was undertaken on the 10th of July 2024 to identify clinical trials in young children which included measures of immune function and immune-related outcome measures following intervention with MFGM. The targeted search was completed using PubMed and an extensive EndNote databases.

Search strategy: ((MFGM) OR (Milk Fat Globule) OR (Phospholipid*)) AND ((immun*) or (diarrho*) or (fever) or (rotavirus)) AND ((infant) OR (child) OR (toddler)) AND (trial) AND ((formula) OR (complementary food))

Inclusion criteria: Included intervention in young children with formula, food or supplements containing MFGM, food or supplements containing specific MFGM components; children (inclusion of assessment at ≥ 12 months of age); assessment of a physiological effect relevant to immune function and immune-related outcomes;

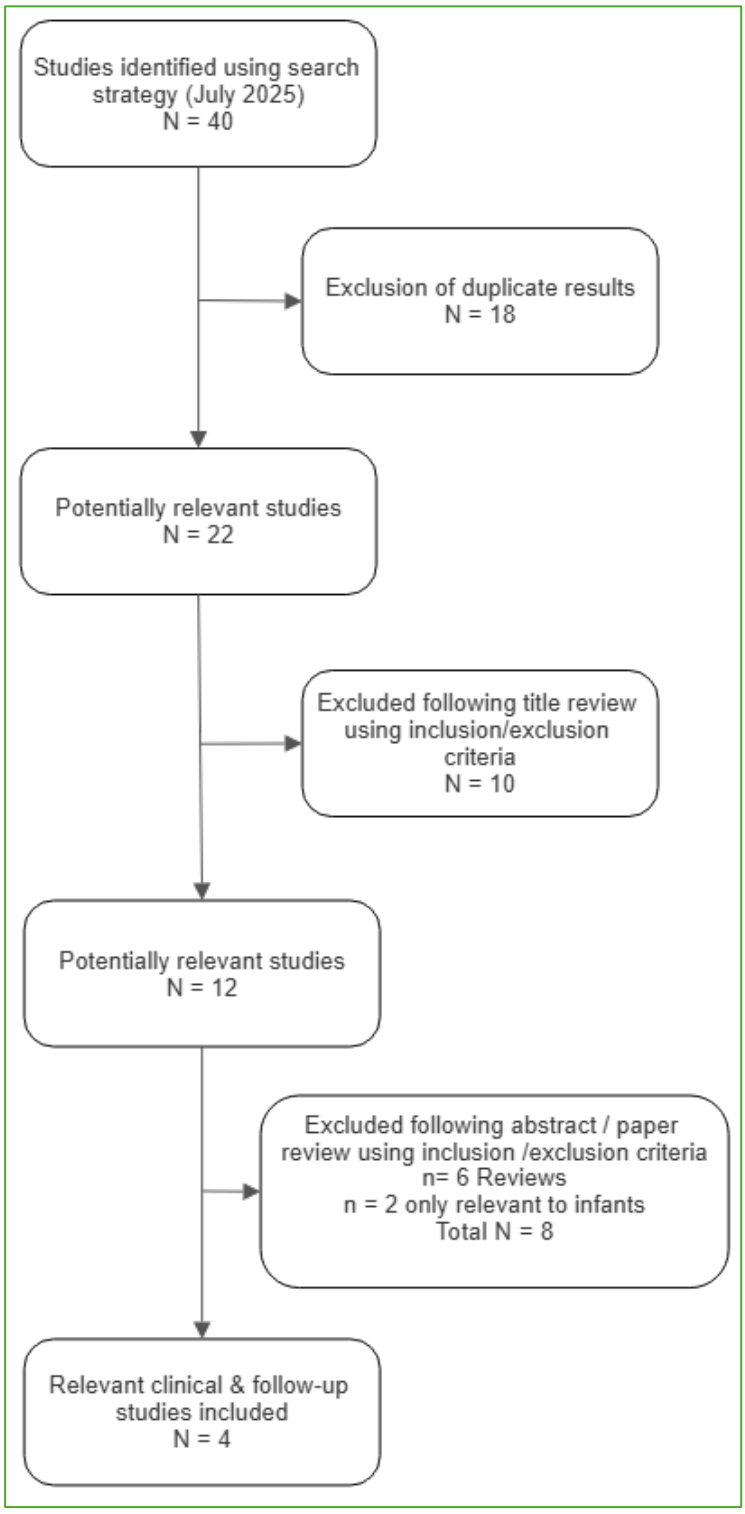
Exclusion criteria: infants only (<12 months) , older children only (>3 years) and adults; studies without immune or immune-related outcome measures; review articles; study protocols; abstracts only (conference proceedings).

The search resulted in a total of 40 citations identified as meeting the search criteria (Figure 2-1) with 18 duplicates immediately excluded. Using the inclusion/exclusion criteria a further 10 results were excluded based on title and another 8 following abstract or paper review. A total of 4 publications were identified as relevant to supporting immune function in young children.

Excluded studies, with reason for exclusion, are listed in Appendix I.

A review of the evidence from clinical studies investigating the benefits of MFGM on immune function in young children is discussed in 2.5.4.

Figure 2-1 Literature search for clinical trials in young children for the effect of MFGM on immune function and immune-related outcomes



2.1.2.1 Safety studies in young children

A literature search was undertaken (24th July 2024) using PubMed (n = 127), Scopus (n = 110) and the Cochrane Library (n = 157) databases to identify clinical trials in young children that address the safety and tolerance of MFGM-WPC and other MFGM-like ingredients. An updated search was completed in PubMed on 10th February 2025, no further references were identified.

- *Search strategy*: (("mfgm") OR ("MFGM") OR ("sphingomyelin") OR ("ganglioside") OR ("PLs") OR ("milk fat globule")) AND (("infant") OR ("toddler") OR ("child")) AND ("trial") NOT (("cancer") or ("disease"))
- *Inclusion criteria*: children (consumption of MFGM or MFGM-like ingredient at ≥ 12 months of age); reported measures of safety and tolerance.
- *Exclusion criteria*: non-intervention studies (e.g. observational); non-infant cohorts; studies without an MFGM-like ingredient; review articles; study protocols; abstracts only (conference proceedings).

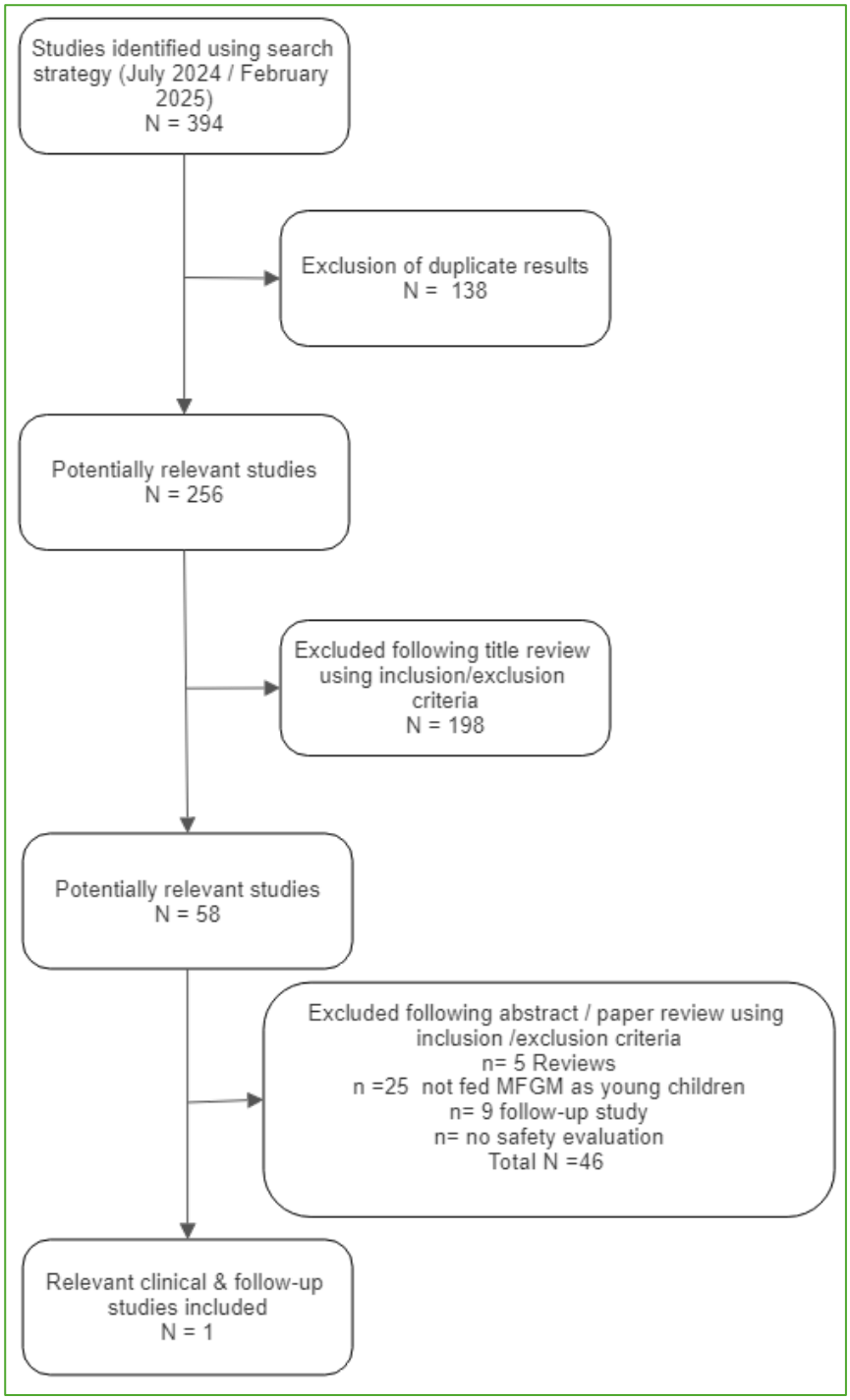
The search resulted in a total of 394 citations identified as meeting the criteria (

Excluded studies, with reason for exclusion, are listed in Appendix I.

Figure 2-2) with 138 excluded immediately based on duplication of results from the different databases. Using the inclusion/exclusion criteria a further 198 results were excluded based on titles, and another 46 following abstract or paper review. Only 3 independent clinical trials where young children consumed MFGM-WPC or MFGM-type ingredients were identified, only 1 of which included an evaluation of safety as part of the overall study objectives.

Excluded studies, with reason for exclusion, are listed in Appendix I.

Figure 2-2 Literature search of safety and tolerance studies of MFGM-WPC and other MFGM-enriched ingredients in young children



2.1.2.2 *Safety studies – preclinical*

A literature search was conducted between the 10th - 24th March 2024 by Saxocon A/S to identify publicly available non-clinical (animal models) data to support the assessment of the safety and tolerance of MFGM, and to identify any potential to cause adverse events or effects, or toxicity following oral administration of MFGM.

The Web of Science core collection and Scopus databases were used for this literature search. These sources were chosen as they contain the most peer-reviewed scientific journals with a notable impact factor.

To define the gross scope of available literature regarding MFGM the relevant fields (i.e., title, abstract, and author keywords) were searched using the following search terms:

MFGM OR (milk fat globule)

After removing non-original articles (e.g., reviews and textbook content), articles lacking sufficient abstract information and duplicates, 4596 articles were identified.

To identify references containing data about MFGM's potential to cause the standard toxicity endpoints (i.e., genotoxicity or systemic toxicity) or any other adverse effect(s) the following search string was used to further refine the search:

AND (genotox* OR mutagen*) OR (tox* NOT toxin) OR (adverse OR safe*)

However, this failed to identify any relevant articles. As Boolean search strings were not effective in this search to identify relevant studies, a cluster analysis was used, identifying clusters of terms and enabling screening based on the relevance of the cluster.

The literature search was then refined using exclusion terms associated with the unrelated clusters:

AND NOT (dairy AND process*) OR (cheese OR casein OR cream)

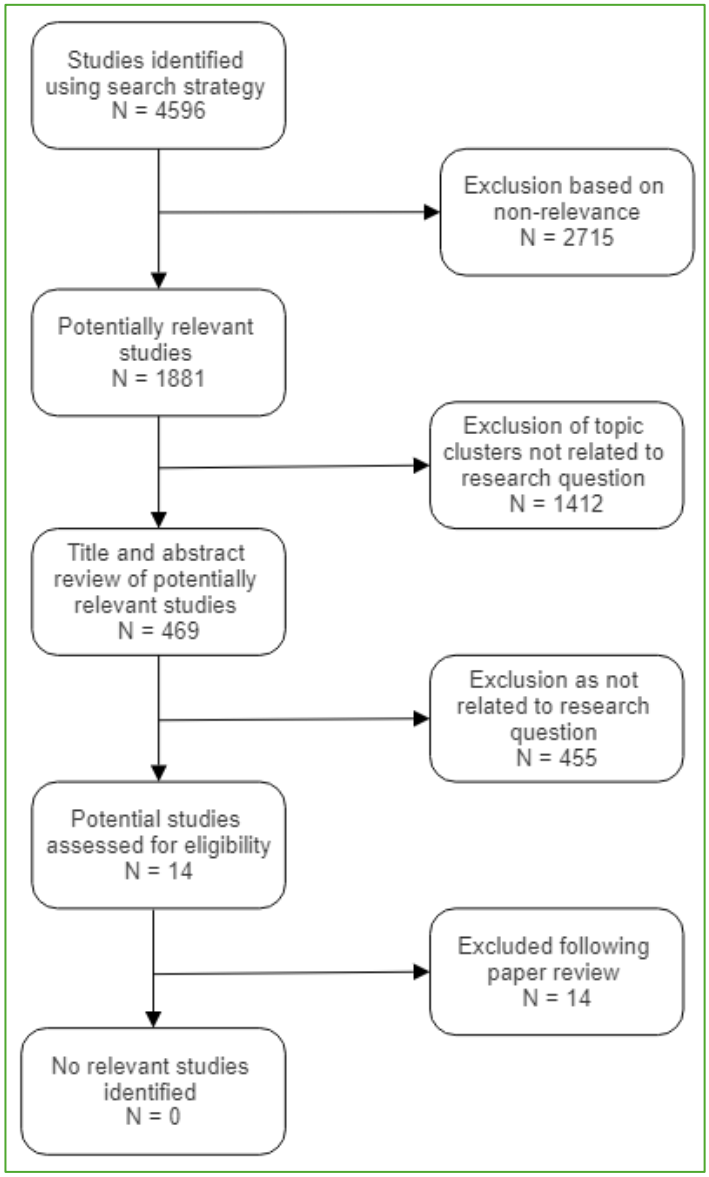
AND NOT e8 OR mfg-e8 OR mfge8 OR lactadherin

AND NOT human-breast OR (human AND breast) AND (cancer OR carc*)

The refined search identified 1881 papers written in English. Further cluster analyses were completed to refine the search based with 469 potentially relevant articles were identified. Titles and abstracts were screened for eligibility and 14 articles were identified for full review. None of the studies were found to specifically address safety aspects.

Figure 2-3 provides an overview of the search results.

Figure 2-3 Literature search for preclinical safety studies



2.2 Technical information on the use of MFGM-WPC

The nutritive substance, MFGM-WPC, is a complex whey dominant material derived from bovine milk. The manufacturing process (Section 2.2.5.1) results in a phospholipid enriched WPC. It is these phospholipid components that are the basis of this ingredient meeting the definition of a nutritive substance over and above the nutritional properties of standard whey protein concentrate (WPC) ingredients. Furthermore, the sphingomyelin component specifically allows for identification of added MFGM-WPC in FSFYC products.

The lactating mammary gland packages and releases lipids by a unique mechanism to be secreted in milk. Lipid molecules are present in milk primarily as milk fat globules (MFG) and EV's (Sprenger, Ostenfeld, Bjørnshave, Rasmussen, & Ejsing, 2023). The origin of MFGM is therefore unique to the mammary gland and thus MFGM is only found in mammalian milks (Heid & Keenan, 2005). Extracellular vesicles are actively secreted into milk and are composed of a single membrane bilayer, which originates from shredding off the apical plasma membrane (rich in sphingolipids and cholesterol, and devoid of triacylglycerols (TAGs)) as microvesicles or secretion of intracellularly formed exosomes (Blans et al., 2017; van Niel, D'Angelo, & Raposo, 2018). Both human and bovine milk contains EVs which can be absorbed by intestinal cells (Yung et al., 2024; Zempleni et al., 2017). The contribution and role of EVs is an emerging area of research contributing to the understanding of the phospholipid fractions present in milk and potential nutritional significance (Sprenger et al., 2024). In the production of bovine milk products, the ratio of EV:MFG derived material is consistent between skim milk and whey products (e.g. a standard WPC-80 and MFGM-WPC) showing the production process of MFGM-WPC does not change the ratio from that of standard milk (Midtgaard et al., 2025).

Collectively the components of the MFGM and EVs are enriched in MFGM-WPC. Until recently, research has focussed on the overall composition of the phospholipid enriched fraction of bovine milk, and this has collectively been referred to as MFGM. Accordingly, these contributing fractions are simply referred to as MFGM throughout this document.

Bovine milk contains about 3 to 5% fat, secreted as droplets or globules roughly 2 to 15 µm in diameter, surrounded by a membrane of polar lipids (phospholipids and sphingolipids) and proteins termed the MFGM (Lucey, Otter, & Horne, 2017).

In the cytoplasm of the mammary epithelial cells, droplets of triacylglycerol are surrounded by a coating consisting of a phospholipid/cholesterol monolayer with incorporated proteins. As the lipid droplets reach the apical cell membrane, an additional phospholipid bilayer encases the fat droplet before being extruded from the cell (Mather & Keenan, 1998). This tri-layer membrane is known as MFGM (Figure 2-4) and contains 60-70% of total milk phospholipids, as well as glycolipids, proteins, glycoproteins, cholesterol, and other lipids (H. Lee, Padhi, et al., 2018). The inner monolayer contains proteins and polar lipids derived from the endoplasmic reticulum. The outer double layer membrane also contains polar lipids and proteins that come from the apical plasma membrane of the mammary epithelial cells which surround the fat globules as they are secreted (Guerin, Burgain, Gomand, Scher, & Gaiani, 2019; Heid & Keenan, 2005).

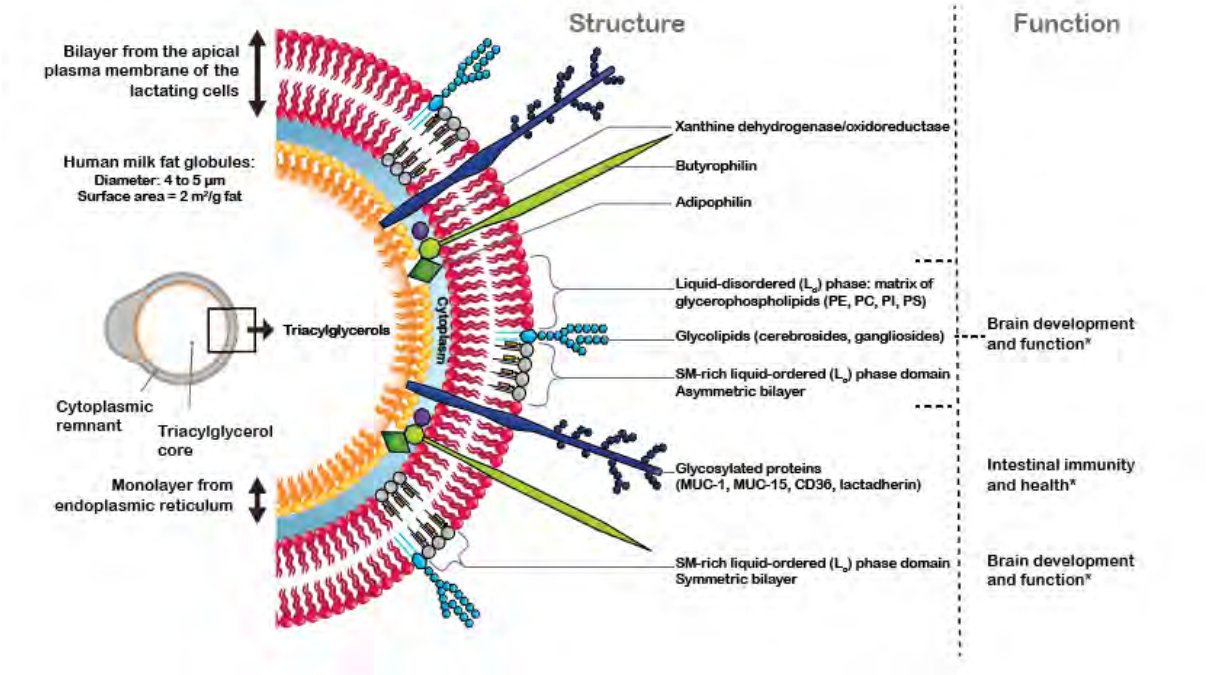
The MFGM consists of a variety of phospholipids, sphingolipids, membrane-specific proteins (mainly glycoproteins), triglycerides, cholesterol, enzymes and other minor components (Singh, 2006). A

large number of reviews are regularly published detailing the composition and potential health functions of the constituent molecular species (Dewettinck et al., 2008; El-Loly, 2011; Guerin et al., 2019; Kosmerl, Rocha-Mendoza, Ortega-Anaya, Jiménez-Flores, & García-Cano, 2021; H. Lee, Padhi, et al., 2018; Mather, 2000; Singh, 2006; Smoczyński, Staniewski, & Kiełczewska, 2012; Thum, Roy, Everett, & McNabb, 2023; Yao, Ranadheera, Shen, Wei, & Cheong, 2023).

The mass of the MFGM is estimated to be 2 – 6% of the total MFG, with the proteins and PL together accounting for up to 90% of the membrane dry weight (Singh, 2006). Typical ranges of bovine MFGM lipid and protein fractions based on data from a range of literature sources (Dewettinck et al., 2008; Bertram Y. Fong, Norris, & MacGibbon, 2007; Jiménez-Flores & Brisson, 2008; Sánchez-Juanes, Alonso, Zancada, & Hueso, 2009; Singh, 2006; Smoczyński et al., 2012) are summarised in Table 2-1.

Bovine MFGM proteins account for only 1 – 4% of the total bovine milk proteins (Cavaletto, Giuffrida, & Conti, 2008), but account for 25 – 70% of the MFGM depending on the source and method of extraction (Dewettinck et al., 2008; Singh, 2006), and other characteristics of the milk fat globule such as globule size (J. Lu et al., 2016; Thum et al., 2023). Similarly, reported fat content varies up to 80% of MFGM weight. Differences may be dependent on methodology and potential contamination of the triglycerides in the core of the MFG during isolation of the membrane for analysis (Singh, 2006).

Figure 2-4 Schematic of MFGM structure



from H. Lee, Padhi, et al. (2018)

Table 2-1 Typical lipid and protein fractions of bovine MFGM

MFGM component	% of MFGM	% of MFGM lipid	
Total MFGM lipid	50 - 80		
Triacylglycerols		56.0 - 62.0	
Diacylglycerols		2.1 - 9.0	
Monoacylglycerols		0.4	
Free fatty acids		0.6 - 6.0	
Sterols		0.2 - 2.0	
Phospholipids		26.0 - 46.0	**% of total PL**
Sphingomyelin			18.8 - 22.0
Phosphatidylcholine			27.4 - 36.0
Phosphatidylethanolamine			27.0 - 36.0
Phosphatidylinositol			11.0
Phosphatidylserine			4.0
Lysophosphatidylcholine			2.0
Total MFGM protein	25 - 70	**% MFGM protein**	**Molecular weight (kDa)**
BRCA1 and BRCA2			210
Mucin I (MUC1)			160 - 200
Xanthine oxidase (XO)		20	146 - 155
PAS III			94 - 100
CD36 or PAS IV		≤ 5	76 - 78
Butyrophilin (BTN)		20 - 43	66 - 67
Adipophilin (ADPH)			52
Lactadherin (PAS 6/7)			47 - 59
Proteose peptone (PP3)			18 - 34
Fatty acid binding protein (FABP)			13 - 15

adapted from Guerin et al. (2019), Mather (2000), Smoczyński et al. (2012), Singh (2006), Bertram Y. Fong et al. (2007)

2.2.1 MFGM components in bovine milks

2.2.1.1 The phospholipid content of bovine milk

The phospholipid content of bovine milk is approximately 0.8% of the total lipid content, with the majority being membrane bound, and the remainder in the aqueous phase (MacGibbon & Taylor, 2006; Sprenger et al., 2023). Patton and Keenan (1971) identified the phospholipids present in skim milk and the MFGM are the same, and present in similar proportions.

The fat globules in bovine milk vary in size from ~2 µm to ~15 µm in diameter. Distribution of individual phospholipid components were determined in two different sized bovine milk globule fractions (7.6- and 3.3-µmm average sizes) (J. Lu et al., 2016). The major phospholipids phosphatidyl choline (36-37%), sphingomyelin (23-26%) and phosphatidyl serine (8%) were not statistically different between the small and large bovine MFGM fractions. There was a statistically meaningful difference in phosphatidyl ethanolamine (PE) (19-27%) and phosphatidyl inositol (PI) levels (5-10%). Minor variations in phospholipid composition are attributed to fat globule size (J. Lu et al., 2016).

The typical proportions of PL reported in bovine milk are shown in Table 2-2.

Table 2-2 Relative proportion of phospholipids in bovine milk

Reference	**Method**	**PE (% total PL)**	**PI (% total PL)**	**PS (% total PL)**	**PC (% total PL)**	**SM (% total PL)**
Andreotti, Trivellone, and Motta (2006)	^{31}P NMR	23.5	12.0	3.6	24.0	24.2
Murgia, Mele, and Monduzzi (2003)	^{31}P NMR	25.8	14.0	1.5	26.8	26.8
MacKenzie, Vyssotski, and Nekrasov (2009)	^{31}P NMR TLC	26.1±0.1 22.1±0.3	7.5±0.1 7.0±0.2	11.7±0.2 10.4±0.3	26.5±0.3 28.1±0.3	20.8±0.5
C. Garcia et al. (2012)	^{31}P NMR	31.8±3.0	3.7±1.9	10.0±2.8	28.7±2.7	20.0±1.4
Zou et al. (2013)	HPLC-ELSD	30.23±2.69	9.89±0.87	7.32±0.99	25.20±1.88	27.36±1.07

In a review of the MFG, Cyrielle Garcia and Innis (2013) provided the average proportions of PL in bovine milk (Table 2-3).

Table 2-3 Average proportions of major phospholipids in bovine milk

Analyte	**Bovine milk (% of total PL)**
Sphingomyelin	22
Phosphatidyl ethanolamine	33
Phosphatidyl choline	29
Phosphatidyl serine	10
Phosphatidyl inositol	4

from Cyrielle Garcia and Innis (2013)

2.2.1.2 Ganglioside content of MFGM

Gangliosides (GGs) are complex lipids composed of a ceramide (Cer) and an oligosaccharide that contains one or more sialic acid (SA) residues such as N-acetylneuraminic acid (Neu5Ac). The structural diversity of gangliosides is due to variation in the oligosaccharide and ceramide parts, such as different sequences of monosaccharides as well as different lengths and saturation levels of the sphingoid base and N-fatty acyl (N-FA) substituents (Hewelt-Belka, Młynarczyk, Garwolińska, & Kot-Wasik, 2023).

In addition to phospholipids and sphingolipids, milk bovine MFGM also contains specific gangliosides; the main ganglioside in bovine milk is disialoganglioside (GD3). Although much of the focus on gangliosides has been on the carbohydrate and sialic acid, the sphingoid base fatty acids also show unexpected specificity and species-specific differences. GD3 is the predominant form of GGs in bovine milk (Laegreid, Otnaess, & Fuglesang, 1986; H. Lee, German, Kjelden, Lebrilla, & Barile, 2013; Pan & Izumi, 2000), amounting to 80% of total bovine milk GGs (Laegreid et al., 1986). In another study, GD3 represented 61.0% of cow's milk GGs compared to 31.8% of human milk GGs (Pan & Izumi, 2000). Typical total levels of GGs reported in bovine milk are shown in Table 2-4.

Table 2-4 Average ganglioside concentrations in bovine milk (mg/L)

Reference	Bovine (mg/L)
Laegreid et al. (1986)	11
Cyrielle Garcia and Innis (2013)	4
Pan and Izumi (2000)	3.98

There is relatively limited data available on GG in FSFYC, or more specifically toddler or growing up milks (Tan et al., 2020). However, in a study in Malaysian toddlers (aged 12 -24 months) investigating the potential correlation between dietary GG intake and serum GG levels, Khor et al. (2016) reported total GG contents ranging between 0.5 -11.4 mg/100 g of 33 bovine-based growing up milk powders, with GD3 the dominant GG class.

2.2.1.3 Cholesterol in MFGM

Cholesterol is a minor component of the MFGM typically located in the SM rich domains of the lipid raft structure of the outer MFGM bilayer (Lopez, Madec, & Jimenez-Flores, 2010). In mature bovine milk cholesterol and cholesterol esters account for approximately 0.30 ± 0.03 % and 0.04 ± 0.01 % respectively of the total lipids (Bitman & Wood, 1990), and is predominantly associated with the MFGM.

Cholesterol is necessary for synthesis of lipoproteins, bile acids, hormones and calciferols, therefore, essential to early growth (Delplanque, Gibson, Koletzko, Lapillonne, & Strandvik, 2015), neurological tissues and during the neuroplasticity period (Hussain et al., 2019; F. Lu, Ferriero, & Jiang, 2022). Brain cholesterol however is dependent on de novo synthesis by brain cells, as circulating cholesterol-containing lipoproteins cannot cross the blood-brain barrier (F. Lu et al., 2022).

2.2.1.4 Proteins in MFGM

The protein composition of the MFGM varies as a function of stage of lactation, environmental factors, particularly immune assaults, and species. Bovine MFGM proteins account for approximately 1 – 4 % of total bovine milk protein (Cavaletto et al., 2008). MFGM proteins constitute a significant proportion (25 – 70%) of the MFGM (Guerin et al., 2019; Singh, 2006). Proteins found in MFGM range from 15 to 240 kDa in size (Dewettinck et al., 2008; Mather, 2000).

A comprehensive review article summarised the nomenclature and identification of major proteins in bovine milkfat globule membranes (Mather, 2000). MFGM proteins were mainly identified based on comparison of electrophoretic mobilities, staining of the gels, molecular cloning techniques, reaction with specific antibodies, and identification by N-terminal amino acid sequencing. Polyacrylamide gel electrophoresis (PAGE) had been used to elucidate the protein composition of the MFGM and major MFGM proteins were designated according to their relative mobility in sodium dodecyl sulphate (SDS)-PAGE and their ability to stain with Coomassie blue or the glycoprotein-specific periodic acid/Schiff (PAS) reagent. Bovine MFGM is resolved into 7 to 8 major bands of protein when separated by SDS-PAGE. Major MFGM proteins are mucin 1, xanthine dehydrogenase/oxidase, PAS III, PAS IV or CD (Cluster of Differentiation) 36, butyrophilin, lactadherin, and fatty acid binding protein (Mather, 2000). The reversible phosphorylation of proteins is central to the regulation of most aspects of cell function biological processes (Cohen, 2002). Characterization of phosphoproteins in bovine MFG using label-free phosphoproteomics was investigated and identified differences in phosphoproteins present across lactation (colostrum and mature milk) (Bai et al., 2024). In mature bovine milk, as used for the production of Lacprodan® MFGM-10, 136 phosphorylation sites from 91 MFGM phosphoproteins were identified with adipophilin (ADPH; also known as perilipin-2) the protein with the greatest number of phosphorylation sites. Proteins in the perilipin family regulate lipolysis by controlling the access of proteins to lipid droplet surfaces (Beller et al., 2010). The phosphoproteins of mature bovine milk MFGM were mainly associated with metabolic function (Bai et al., 2024). The properties and putative physiological functions of the major and minor proteins of the MFGM have been reviewed by H. Lee, Padhi, et al. (2018).

Figure 2-5 adapted from Singh (2006), shows major MFGM proteins separated by electrophoresis.

Proteomic approaches, including mass spectrometric (MS) analysis, provided rapid, unambiguous information on protein identity and further identification of minor proteins of bovine MFGM (Cavaletto et al., 2008; Manoni, Di Lorenzo, Ottoboni, Tretola, & Pinotti, 2020; Reinhardt & Lippolis, 2006).

Functions of the 120 MFGM proteins identified by Reinhardt and Lippolis (2006) in bovine MFGM were associated with membrane/protein trafficking (23%), cell signalling (23%), unknown functions (21 %), fat transport/metabolism (11 %), transport (9 %), protein synthesis/folding (7 %), immune proteins (4%) and milk proteins (2%).

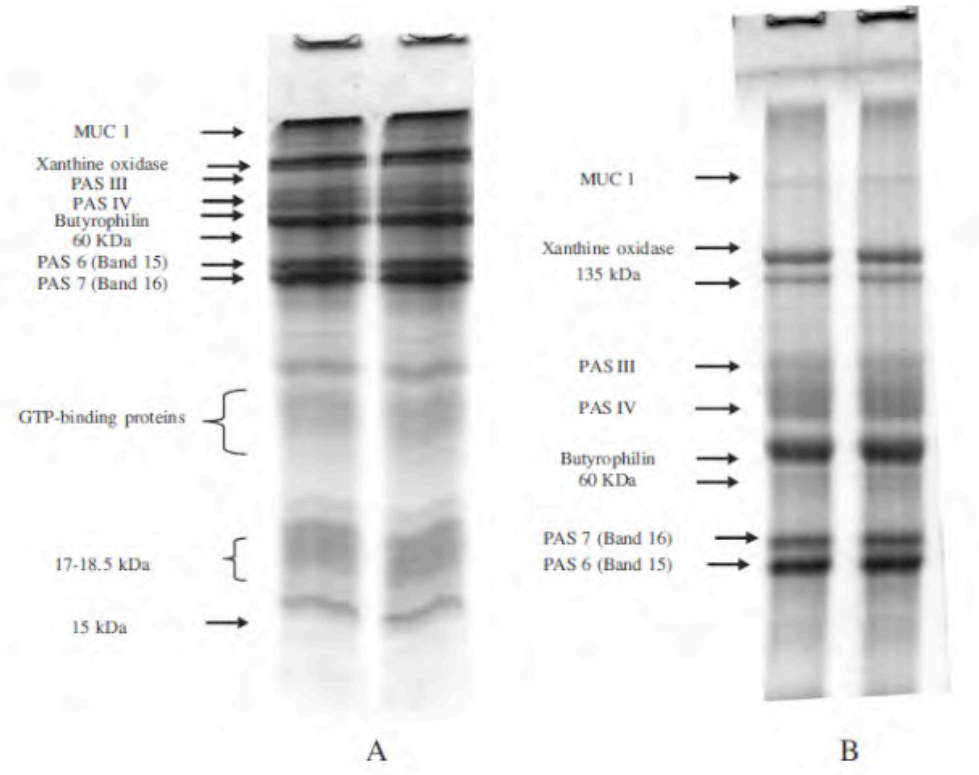
In a further study of bovine MFGM, 345 proteins are either uniquely presented or were significantly higher in abundance. These proteins were mainly involved in actin organization, vesicle mediate transport, carbohydrate catabolic process and response to bacterium (Lu et al. 2016b). In multiple species, 520 MFGM-enriched proteins in milk from the Holstein, buffalo, Jersey, yak, goat, camel, horse, and human were inferred through peptide identification and iTRAQ proteomic approach (Yang et al., 2016; Yang et al., 2015). In the MFGM-enriched protein compartment, a wide variety of proteins

were identified from abundant fraction to minor fraction of milk protein, like several proteins such as alpha-S1-casein, alpha-S2-casein, kappa-casein, beta-casein, albumin, and alpha-lactalbumin.

Table 2-5 lists some of the major proteins identified in bovine MFGM. The main protein of the MFGM is the glycoprotein butyrophilin (about 40% of the total proteins of the MFGM), with xanthine oxidase, which comprises 12 to 13% of the total MFGM protein content, being the second most abundant. Other proteins are present in MFGM at concentrations of 5% or less. Almost 50% of the MFGM proteins have membrane/protein trafficking or cell signalling functions (Reinhardt and Lippolis 2006) and may play a role in synthesis and secretion of MFGM in milk.

The reversible phosphorylation of proteins is central to the regulation of most aspects of cell function biological processes (Cohen, 2002). Characterization of phosphoproteins in bovine MFG using label-free phosphoproteomics was investigated and identified differences in phosphoproteins present across lactation (colostrum and mature milk) (Bai et al., 2024). In mature bovine milk, as used for the production of Lacprodan® MFGM-10, 136 phosphorylation sites from 91 MFGM phosphoproteins were identified with adipophilin (ADPH; also known as perilipin-2) the protein with the greatest number of phosphorylation sites. Proteins in the perilipin family regulate lipolysis by controlling the access of proteins to lipid droplet surfaces (Beller et al., 2010). The phosphoproteins of mature bovine milk MFGM were mainly associated with metabolic function (Bai et al., 2024). The properties and putative physiological functions of the major and minor proteins of the MFGM have been reviewed by H. Lee, Padhi, et al. (2018).

Figure 2-5 Separation of bovine MFGM proteins by SDS-PAGE.



from Singh (2006)

Table 2-5 Major proteins from bovine MFGM as detected by SDS-PAGE.

Protein	Type	Molecular weight (Da)	Reference
Mucin1	Glycoprotein	>160,000	Schroten et al. (1992)
Xanthine oxidase		150,000	Mather (2000), Vorbach, Scriven, and Capecchi (2002)
PAS III	Glycoprotein	~100,000	Mather (2000)
PAS IV or CD36	Glycoprotein	78,000	Greenwalt et al. (1992), Mather (2000),
Butyrophilin	Glycoprotein	66,000	Mather (2000)
Lactadherin	Glycoprotein	~50,000	Honan, Fahey, Fischer-Tlustos, Steele, and Greenwood (2020)
Fatty acid binding protein (FABP)		15,000	Spitsberg (2005)

2.2.2 Information to enable the identification of MFGM-WPC

MFGM-WPC is a bovine milk derived WPC developed and marketed globally by Arla Foods Ingredients P/S under various brand names such as Lacprodan® MFGM-10. The unique feature is the processing enrichment of the WPC with bovine MFGM achieved through processing. Specifically, the phospholipid, sphingolipid, and membrane protein components are present at higher levels compared to typical WPC. As MFGM is a collection of components, it does not have a chemical name (according to Chemical Abstracts and the International Union for Pure and Applied Chemistry), a Chemical Abstracts Service (CAS) registry number or a structural formula. In some jurisdictions MFGM-WPC has been considered as a normal WPC and does not require separate identification of its component parts.

The major protein components in MFGM-WPC are normal whey proteins, typical of regular whey-protein ingredients (e.g. WPC 80), they account for approximately 70 % of the MFGM-WPC and are not unique or novel to MFGM-WPC. Thus, the routine quantification of MFGM proteins is challenging given their relatively low proportion of total protein in the product. MFGM-WPC is differentiated from regular whey protein products, in that the major lipid and protein components of the MFGM are enriched and present at two-to-four times the quantity present in typical WPCs (such as AFI's Lacprodan® WPC-80). When used in FSFYC, MFGM-WPC not only provides protein but results in elevated levels of MFGM components.

For the purposes of identification, the major lipid components of the MFGM can provide measurable differences to typical WPCs that may be added to FSFYC. This can be complicated by the presence of phospholipid species from other ingredients such as lecithin. However, as sphingomyelin and gangliosides are unique to milk these are suitable markers to determine the addition of MFGM components to FSFYC.

Detailed information of the applicants MFGM-WPC (Lacprodan® MFGM – 10) are used in the following sections to provide technical details of the proposed nutritive substance. Where necessary for comparative purposes technical details of AFI ingredients such as Lacprodan® WPC 80 are used.

2.2.3 Information on the chemical and physical properties of MFGM-WPC

2.2.3.1 Incorporation of MFGM-WPC into FSFYC

MFGM-WPC, such as Lacprodan® MFGM-10, is typically a spray dried powder of homogenous composition. It is suitable for addition into FSFYC during either the wet mix phase of production of FSFYC powders or may be used in FSFYC manufactured by the dry blending process. MFGM-WPC is essentially a whey powder, with a particle size distribution the same as that of standard WPC products. It is free flowing and easily added into the wet mix of FSFYC production where it is solubilised and distributed homogenously throughout the mix. Standard dairy processes such as homogenisation and turbulence are used in wet mix production to ensure homogeneity of spray-dried powders. When used in dry blend manufacturing applications, the physical and physicochemical properties of MFGM-WPC mean it blends homogenously with other dairy powders and ingredients. On reconstitution it readily disperses and dissolves during formula mixing. Accordingly, the particle size of MFGM-WPC is not important in the context of considering any differences in nutritional status or toxicology when comparing to other WPCs and components of WPCs.

2.2.3.2 Chemical properties of MFGM-WPC

Comparison of the composition of a typical MFGM-WPC (Lacprodan® MFGM-10) to a standard WPC (Lacprodan® WPC 80) provides context of the key parameters that make the MFGM-WPC unique but also demonstrate compositional commonality. The following composition and chemical properties of MFGM-WPC are based on the applicant's product Lacprodan® MFGM-10.

2.2.3.2.1 MFGM-WPC lipids

The total phospholipid content in Lacprodan® MFGM-10 amounts to approximately 6.7 g/100 g of powder (Table 2-6). The two to four-fold higher level of fat content found in Lacprodan® MFGM-10 compared to a standard WPC such as Lacprodan WPC80 results in enrichment of key phospholipids (PLs), sphingomyelin and gangliosides (Table 2-6). The composition of these complex lipids is constant across whey dominant streams.

[redacted]

Table 2-6 Typical compositional comparison of Lacprodan® MFGM-10 to WPC-80

Analyte	**Lacprodan® MFGM-10 (n=5) (%)**	**Lacprodan® WPC 80* (n=4) (%)**	**Typical ratio**
Total fat	18.6	5.5	3.4
Total PL	6.7	1.85	3.6
Phosphatidyl choline (PC)	1.86	0.50	3.8
Phosphatidyl inositol (PI)	0.38	0.136	2.7
Phosphatidyl serine (PS)	0.65	0.180	3.6
Phosphatidyl ethanolamine (PE)	2.0	0.46	4.3
Sphingomyelin (SM)	1.69	0.46	3.7

** Commercial fractions sold by Arla Foods ingredients P/S for food applications including FSFYC*

Key phospho- and sphingolipids that are present in typical WPC-80 versus MFGM-WPC as a percentage of total fat are listed in Table 2-7. The phospholipid values reported for standard WPC-80 and MFGM-WPC are quite similar when reported as a percentage of total fat. Greater than 98% of total phospholipid contribution is from these five major PL components.

Composition is monitored by AFI with the total and major individual phospholipid components listed in Table 2-7 measured in every lot of Lacprodan® MFGM-10. Total gangliosides content is monitored periodically and measured at least 4 times a year.

Table 2-7 Typical phospholipid, sphingolipid and gangliosides levels in Lacprodan® MFGM-10 versus standard WPC-80 expressed as average of the batches in percentage of total fat

Analyte	Lacprodan® MFGM-10 (g/100 g fat) n=4	Lacprodan® WPC 80 (g/100g fat) n=5
Total phospholipids (PL)	35	32
Phosphatidyl choline (PC)	10.1	8.8
Phosphatidyl ethanolamine (PE)	9.1	8.1
Phosphatidyl inositol (PI)	2.0	2.4
Phosphatidyl serine (PS)	3.5	3.2
Sphingomyelin (SM)	10.9	8.1
Gangliosides	1.43	1.25

2.2.3.2.2 MFGM-WPC proteins

The major protein and lipids components in MFGM-WPC are also present in typical whey-protein ingredients, albeit at lower levels, and are not unique or novel to Lacprodan® MFGM-10 (Figure 2-6).

Quantification of the levels of all MFGM-associated proteins in milk is relatively challenging. The relative abundance of major MFGM-associated proteins can be determined using semi-quantitative methods such as SDS-PAGE. As seen below, SDS-PAGE analysis of 7.5 µg of protein samples, from WPC-35, WPC-80, and Lacprodan® MFGM-10 ingredients, shows similar profiles between ingredients but higher levels of MFGM-associated proteins in the Lacprodan® MFGM-10. Lactadherin, butyrophilin, and xanthine oxidase (XDH/XO) are reported to be three of the most prominent MFGM-associated proteins (Demmelmair, Prell, Timby, & Lonnerdal, 2017). The presence of these proteins in other WPC ingredients (not deliberately enriched in MFGM) illustrates that MFGM-associated proteins are not novel to MFGM-WPC.

Furthermore, the overall amino acid composition of Lacprodan® MFGM-10 is comparable with standard Lacprodan® WPC-80 (DI-8090) that is typically sold for nutritional applications (Table 2-8). As for all milk products, amino acid composition varies slightly due to natural conditions such as cow's feed, season, and lactation. Typically, there are minor variations in amino acid contents across the whey dominant streams.

There is similar distribution of essential amino acids between Lacprodan® MFGM-10 and Lacprodan® WPC-80 (used as protein source in FSYFYC). These data and other calculations show that replacement or addition of a portion of MFGM-WPC for standard WPC does not result in an increase in total protein or changes in protein quality as the amino acid profiles are similar.

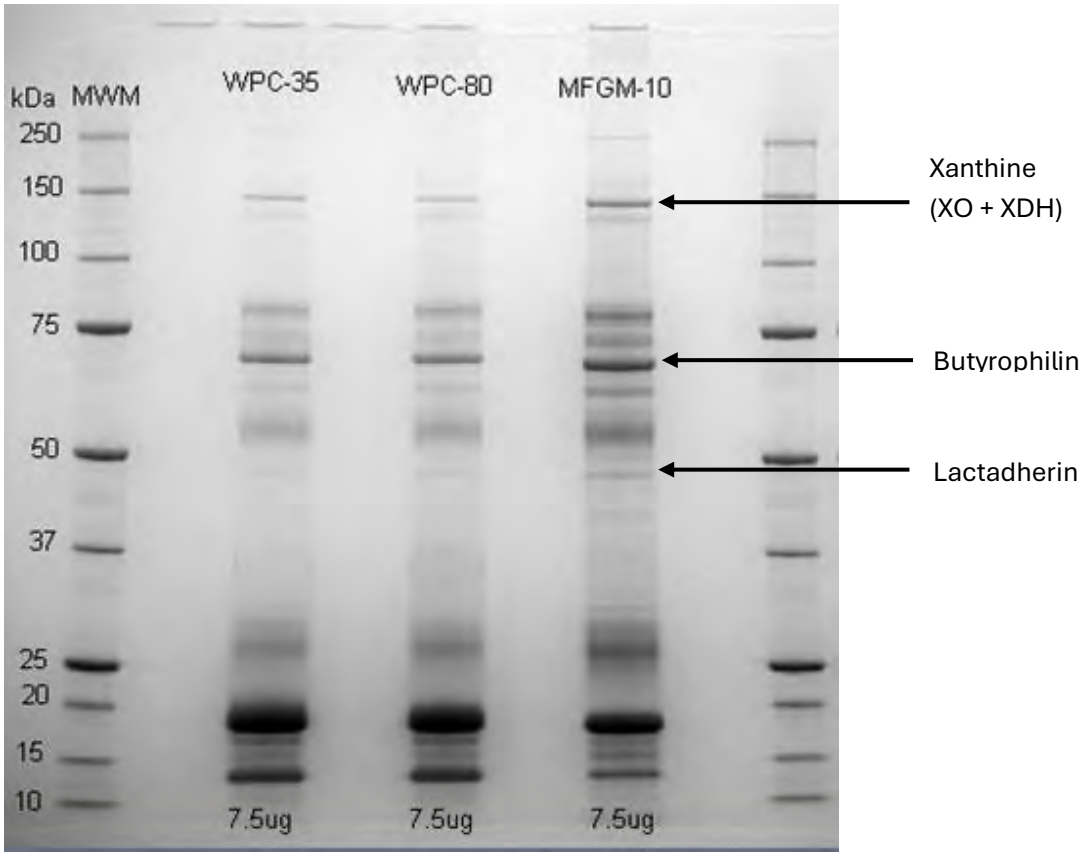
Table 2-8 Typical amino acid composition of Lacprodan® MFGM-10 versus standard WPC 80

Amino acid	#Lacprodan® MFGM-10 (%)	#Standard WPC 80 – Lacprodan® DI-8090 (%)
Alanine	4.9	5.5
Arginine	3.7	2.7
Aspartic acid (asparagine)	10.3	11.3
Cysteine (cystine)	2.1	2.4
Glutamic acid (glutamine)	16.5	18.4
Glycine	2.2	2.0
Histidine*	2.4	1.9
Isoleucine*	5.6	6.6
Leucine*	10.8	11.4
Lysine*	9.1	9.9
Methionine*	2.1	2.3

Amino acid	#Lacprodan® MFGM-10 (%)	#Standard WPC 80 – Lacprodan® DI-8090 (%)
Phenylalanine*	3.7	3.5
Proline	5.8	6.6
Serine	6.4	5.7
Threonine*	6.7	7.5
Tryptophan*	1.6	1.9
Tyrosine	3.8	3.2
Valine*	5.6	6.6

#Commercial fractions sold by Arla Foods Ingredients P/S for nutritional applications
**Essential amino acids*

Figure 2-6 SDS-PAGE protein analysis of Lacprodan® MFGM-10 WPC compared to WPC



Source: AFI

2.2.4 Information on the impurity profile

The production of Lacprodan® MFGM-10 as a MFGM enriched WPC is subject to regular monitoring for pesticides (Regulation (EC) No 396/2005) and contaminants (Commission Regulation (EU) 2023/915) under the EU regulatory framework, and as part of AFI's global monitoring program. Lacprodan® MFGM-10 is monitored for heavy metals, pesticide residues, and contaminants biannually by an external laboratory using validated analytical methods. The resulting analyses for pesticide residues and contaminants are within the regulatory limits established for these substances. Additionally, as Lacprodan® MFGM-10 is a powdered ingredient intended to be added to formulated nutritional products, the levels of arsenic, cadmium, lead, and mercury are regularly determined. Data from 4 representative non-consecutive batches manufactured in 2018/2019 are provided in Table 2-9. Within Australia and New Zealand no regulatory limits for heavy metal contaminants are set for FSFYC (Schedule 19 of the FSC).

Table 2-9 Heavy metal analyses for 4 representative batches of Lacprodan® MFGM-10

Analysis	**Unit**	**Tested batches**			
		MFGM-1 2018	**MFGM-2 2018**	**MFGM-3 2018**	**MFGM-1 2019**
Arsenic	mg/kg	<0.01	<0.01	<0.01	<0.01
Cadmium	mg/kg	<0.01	<0.01	<0.01	<0.01
Lead	mg/kg	No data	<0.003	<0.003	0.0045
Mercury	mg/kg	<0.005	<0.005	<0.005	<0.005

Microbiological safety and potential contamination are assessed by the routine analysis of every production batch of Lacprodan® MFGM-10. Microbiological analysis includes; total plate count following incubation at 30°C, aerobic thermophilic count, and levels of yeasts and moulds, *Bacillus cereus*, sulphur-reducing clostridia, coagulase-positive *staphylococci*, *Enterobacteriaceae*, and *Salmonella* in accordance with methods established by the International Organization for Standardization. Results from the analysis of 4 representative non-consecutive batches are presented in Table 2-10. No microbiological limits are specifically set for FSFYC (Schedule 27) in the FSC.

Table 2-10 Microbiological results of 4 representative batches of Lacprodan®MFGM-10

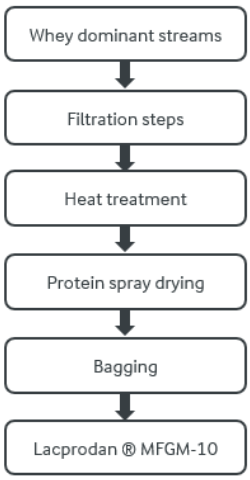
Parameter	**Unit**	**Manufacturing batch numbers**			
		MFGM-1 2024	**MFGM-2 2024**	**MFGM-3 2024**	**MFGM-4 2024**
Total plate count (30°C)	cfu/g	100	100	<100	300
Total plate count(55°C)	cfu/g	<100	<100	100	200
Bacillus cereus	cfu/g	10	<10	<10	<10
Sulfite-reducing *Clostridia*	cfu/g	<10	<10	<10	<10
Enterobacteriaceae	cfu/g	<10	<10	<10	<10
Coagulase-positive staphylococci	cfu/g	Absent	Absent	Absent	Absent
Yeast and mold	cfu/g	<10	<10	<10	<10
Salmonella	cfu/g	Absent	Absent	Absent	Absent

CFU = colony forming units.

2.2.5 Manufacturing process for MFGM-WPC

The manufacture of MFGM-WPC is suitably described based on the AFI production process for Lacprodan® MFGM-10. Lacprodan® MFGM-10 is produced from bovine whey dominant streams produced using well-accepted dairy processing methodologies to separate an enriched fraction of MFGM components in the whey (Figure 2-7). The raw material feed for Lacprodan® MFGM-10 production is whey dominant streams conforming to the European Union Food Hygienic Guidelines and EU Regulation 853/2004.

Figure 2-7 Generic process flow diagram for Lacprodan® MFGM-10



The raw material (whey dominant streams) comes from authorised dairy product facilities that have implemented Hazard Analysis Critical Control Point (HACCP) methods and comply with current Good Manufacturing Practices (cGMP). They are regulated in the European Union under Regulation (EC) No 852/2004 on hygiene of foodstuffs.

The AFI production sites that manufacture MGFM-WPC have implemented quality control systems in accordance with cGMP and HACCP principles pursuant to Regulation (EU) No 852/2004, raw material analytical control, and physicochemical and microbiological final product controls.

MFGM-WPC, including Lacprodan® MFGM-10, is produced at Danmark Protein in Videbæk, Denmark. Due to capacity of the spray dryer at Danmark Protein, some Lacprodan® MFGM-10 liquid may be transported to the AFI factory Arinco, a plant 7 km from Danmark Protein, to be spray dried and bagged (Figure 2-8). Danmark Protein is certified under DS/EN ISO 50001: 2011, ISO 22000: 2005 / TS 22002-1: 2009 and FSSC 22000, and Arinco is certified under ISO 14001: 2015, ISO 22000: 2005 / TS 22002-1: 2009 and FSSC 22000.

All processing equipment is suitable for food applications and in compliance with processing and production requirements set by the European Union legislation (Čapla, Zajác, Ševcová, Čurlej, & Fikselová, 2023) and Danish Veterinary and Food Administration (DVFA) (https://en.foedevarestyrelsen.dk). AFI is certified for the development, production, and sale of products based on whey protein and lactose by DVFA. Arla Foods Ingredients P/S is a member of the

Danish Agriculture and Food Council (https://agricultureandfood.dk/danish-agriculture/) and adheres to the practices set by the organisation.

2.2.5.1 General description of the manufacturing process for MFGM-WPC

All process operations used in the manufacture of MFGM-WPC, including Lacprodan® MFGM-10, are standard dairy processing operations.

2.2.5.1.1 Feed material for Lacprodan® MFGM-10 manufacture

MFGM-WPC is produced from whey dominant by-product streams from raw skim milk used to produce cheese or casein.

All raw milk is pasteurised (72°C for 15 seconds) prior to processing. This is a critical control point (CCP) at the dairy facilities, eliminating the risk of pathogens. The pasteurisers, all operate with divert valves, which are tested at the start of every production lot. The pasteurised milk is then processed to manufacture cheese or casein, yielding whey dominant streams that contain the components that are to be enriched in the MFGM-WPC production process. The whey dominant streams are clarified, separated, and undergo additional pasteurisation (72°C for 15 seconds) or microfiltration (ceramic membrane <1.4 µm) prior to cooling. The whey dominant streams are transported under hygienic conditions at 5°C to Danmark Protein for further processing.
(https://www.arlafoodsingredients.com/about/contact/locations/danmark-protein/)

Quality control parameters for the whey dominant streams include temperature, pH, and nitrate level as release parameters. Nitrate is controlled as an indicator of HNO3, a clean-in-place (CIP) cleaning residue. Gross composition is assessed through protein quantification, and selected microbial analyses are carried out.

In order to standardize the whey dominant streams for MFGM-WPC production, the whey dominant streams pass through an ultrafiltration step to increase the concentration of the whey protein components, and reduce water and components such as lactose and minerals. If required, this step can alternatively be performed before the transportation to Danmark Protein.

The whey dominant streams (after ultrafiltration) must comply with the below specification on the parameters: dry matter, protein and fat contents as well as pH (Table 2-11). If pH adjustment is required, NaOH, KOH or a combination of NaOH and KOH are used.

Table 2-11 Specification of whey dominant streams entering Lacprodan® MFGM-10 production

Parameter	Min (%)	Max (%)	Analytical method
Dry matter	8.4	10.3	ISO 6731:2010(E)/IDF 21:2010(E) mod. "Milk, cream and evaporated milk. Determination of the total solids content"
Protein as is	5.5	8.5	ISO 8968-3:2004(E)/IDF 20-3:2004: Determination of nitrogen content. Block digestion method (semi-micro rapid routine method)
Protein in dry matter	65	83	
Fat as is	0.31	0.78	ISO 7208/IDF 22: 2008 Skimmed Milk, whey and buttermilk. Determination of Fat Content. Röse-Gottlieb Gravimetric Method (Reference Method)
Fat in dry matter	3.7	7.6	
pH	5.6	6.8	IDF 115/ISO 5546 2nd ed 2010-06-01 (modified)

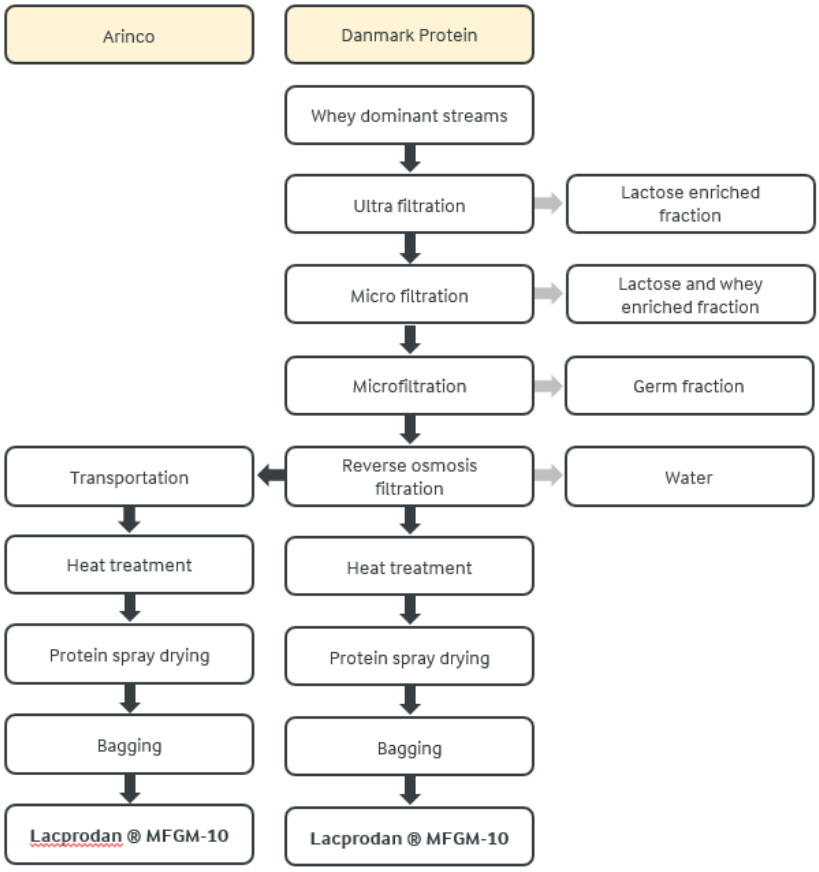
2.2.5.1.2 MFGM-WPC production

On receipt to the whey processing factory, the whey dominant streams are stored at 5°C prior to entering the MFGM-WPC manufacturing process. Maximum holding time is 72 hours prior to manufacturing.

A series of specific ultra- and micro-filtration process operations are completed that separates the whey dominant stream into 4 retentate and permeate streams (Figure 2-8):

i. Ultrafiltration (UF) retentate containing the MFGM components. This continues through the process;
ii. UF permeate – a high lactose enriched fraction containing minor amounts of components like non-protein nitrogen and minerals that is used for further processing;
iii. Microfiltration (MF) permeate – lactose and whey enriched fraction e.g. beta-lactoglobulin and alpha-lactalbumin that is used for further processing;
iv. MF retentate (germ fraction) containing most of the microorganisms from the raw material, which is discarded.

Figure 2-8 Detailed process flow diagram for Lacprodan® MFGM-10



Following the selective separation by filtration, the MFGM enriched whey stream is concentrated using reverse osmosis (RO). Dependent on plant processing capacity, the concentrate stream may

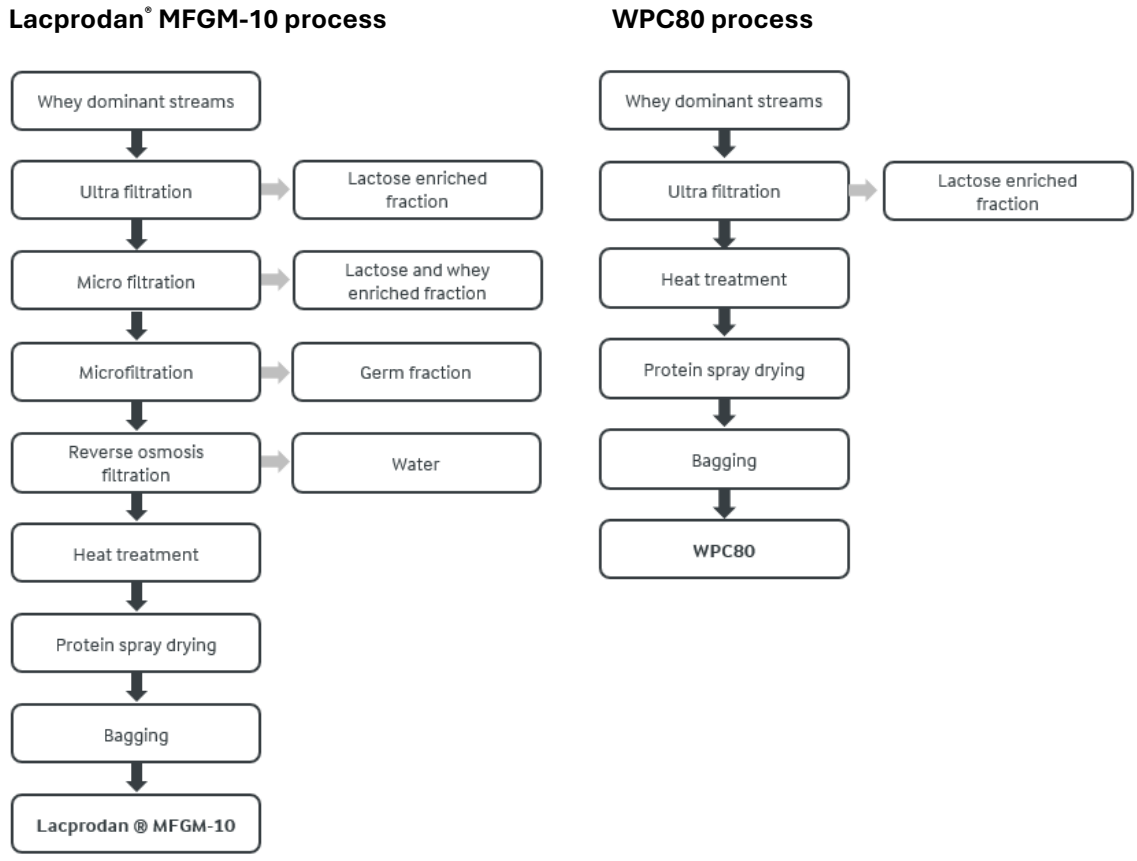
be sent to the nearby AFI Arinco plant where it is processed in the same way as at the main Danmark Protein plant. The product is transported liquid and is kept below 8 °C during transport.

At both manufacturing sites the product material is heat treated at 60-70 °C for approximately 20 s prior to being sprayed dried as part of the standard operational procedure. Following drying the powder passes through a sieve with a nominal mesh size of either 4 mm (Danmark Protein) or 2 mm (Arinco) and rotating magnet. The product is packed after drying, passing through a metal detector prior to packing into bags. There may be an intermediate bulk holding time of up to 2-3 weeks before packing into bags for customer supply.

Finished product analysis (compositional, functional, and physicochemical, and microbiological) is completed prior to release of the powder for customer supply.

For comparative purposes (Figure 2-9) shows the key differences between the manufacture of the MFGM enriched Lacprodan® MFGM-10 and an AFI WPC80.

Figure 2-9 Comparison of production methods for Lacprodan® MFGM-10 and WPC-80



2.2.5.2 Impact of processing on MFGM-WPC

All processes used for the manufacture of MFGM-WPC are standard dairy processes and conditions and are not expected to impact the product any differently to other conventional dairy products.

Specifically the filtration steps used to manufacture AFI's MFGM-WPC, such as Lacprodan® MFGM-10, concentrate lipids and MFGM components from whey to a level higher than that normally found

in standard WPC. These filtration steps are the only difference between the AFI manufacturing processes for WPC and Lacprodan® MFGM-10 (Figure 2-9).

The filtration is not known to have any effects on secondary or tertiary protein structures that differ from commodity WPC manufacturing. The main causes for conformational changes and/or post-translational modifications in protein are heat treatments, enzymatic activity, shear, pressure, or ultra-violet (UV). AFI has designed and optimized its production processes in order to avoid these processing influencers:

- Heat treatments: The raw material (whey dominant stream) is transported cold to our production plant, and it is kept cold until spray drying.
- Enzymatic activity: The whey dominant stream is pasteurized before processing at our plant, and bacterial growth is controlled during processing since everything is kept cold.
- Shear/pressure/UV: The production process for Lacprodan® MFGM-10 is not adding unusual shear or pressure, compared to standard processes for whey proteins. UV is not used in production.

2.2.5.3 Materials and processing aids

Raw materials used in the manufacture of Lacprodan® MFGM-10 include:

a. Whey dominant streams from the manufacture of cheese or casein in AFI production facilities (Section 2.2.5.1.1, Table 2-11).
b. If a pH adjustment is required of the incoming whey dominant stream, sodium hydroxide (NaOH), potassium hydroxide (KOH) or a combination of these are used.

No other materials or processing aids are used in the production of Lacprodan® MFGM-10.

2.2.6 Specification for identity and purity of MFGM-WPC

Schedule 3 Identity and purity (specifically S3-53) of the FSC contains a specification for "Bovine milk fat globule membrane-enriched whey protein concentrate". For that section, bovine milk fat globule membrane-enriched whey protein concentrate is a preparation of cow's milk consisting of lipids and proteins.

The specification and purity of MFGM-WPC as per S3-53 is shown in Table 2-12.

Table 2-12 Schedule 3 specification for bovine milk fat globule membrane-enriched whey protein concentrate

Parameter	Unit	Specification
Description		Off white powder
Total protein	%	69.0 - 76.0
Lactose	%	≤2.0
Fat	%	16.0 - 22.0
Phospholipids	%	6.0 - 10.0
Sphingomyelin	%	1.3 - 2.3
Ash	%	<3.0
Moisture	%	<5.0

Parameter	Unit	Specification
Arsenic (As)	mg/kg	≤0.2
Cadmium (Cd)	mg/kg	≤0.1
Lead (Pb)	mg/kg	≤0.05
Mercury (Hg)	mg/kg	≤0.02
Total plate count (30°C)	cfu/g	≤10000
Total plate count (55°C)	cfu/g	≤1000
Bacillus cereus	cfu/g	<50
Sulfite-reducing *Clostridia*	cfu/g	<10
Enterobacteriaceae	cfu/g	<10
Coagulase-positive staphylococci		Absent/1 g
Yeast and mould s	cfu/g	<10

[redacted]

2.2.7 Stability of Lacprodan® MFGM-10

Lacprodan® MFGM-10 is a WPC that contains proteins typically found in other WPC with enriched levels of some MFGM-related proteins and lipids. Whey protein concentrates are common food ingredients with a long history of incorporation into a wide variety of food matrices, including infant formula products. Lacprodan® MFGM-10 has similar properties to other WPCs in relation to its incorporation and stability as a bulk ingredient and as an ingredient in food matrices. The stability of Lacprodan® MFGM-10 powder, across a variety of parameters is demonstrated in Section 2.2.7.1. The stability of Lacprodan® MFGM-10 in infant formula powders, is a suitable proxy for FSFYC and is shown in Section 2.2.7.2.

2.2.7.1 Stability of Lacprodan® MFGM-10 powder

Four batches of Lacprodan® MFGM-10 were stored at ambient conditions representative of climate zone 1 (21°C, 45% relative humidity (±5%)) to document the stability of the ingredient for 18 months as listed in the specification (Section 2.2.6). Lacprodan® MFGM-10 bags from commercial production were used; 15 kg powder packed in a paper bag with a polyethylene inner liner. This packaging material is light-proof and partially air-permeable.

The study report for commercially packed Lacprodan® MFGM-10 is provided in Appendix II.

2.2.7.1.1 Methodology

Stability was evaluated based on the following parameters: colour, water activity, water content, pH, protein content, fat content, ganglioside GD3, phospholipids, peroxide value, major whey proteins, immunoglobulin G (IgG), lactoferrin, microbiology and sensory properties (

Table 2-13). Samples were analysed at baseline and at 3, 6, 9, 12, 15 and 18 months. An intact bag was analysed at each time point.

Table 2-13 Methods for Stability Study

Analysis	Method	Laboratory
Colour (L*, a*, b*)[1]	Minolta colour system	Internal
Water activity, a_w	Internal	Internal
Water content	ISO 6731	Internal
pH	ISO 5546	Internal
Protein content	ISO 8968-3	Internal
Fat content	ISO 1736	Internal
Gangliosides	GANGLIO-r – LC-MS/MS	External, NIZO
Phospholipid content	Phosphorus-31 NMR method	External, Spectral Service
Peroxide value	AOCS Cd 8b-90	External, Eurofins Steins
Major whey proteins	HPLC	Internal
IgG	Radial immunodiffusion (RID)	External, Eurofins Steins
Lactoferrin	ELISA method	External, Eurofins Steins
Microbiological analyses		Internal and external
Sensory properties	Descriptive evaluation	External, Aarhus University

[1] *Commission Internationale de l'Eclairage color space (L* indicates lightness, a* is the red/green coordinate, and b* is the yellow/blue coordinate)*

The characteristics that are prone to change during storage and are likely to affect the quality and use of Lacprodan® MFGM-10 are listed below (see Table 2-2) in the shelf-life specification, which has been created specifically for the shelf-life study.

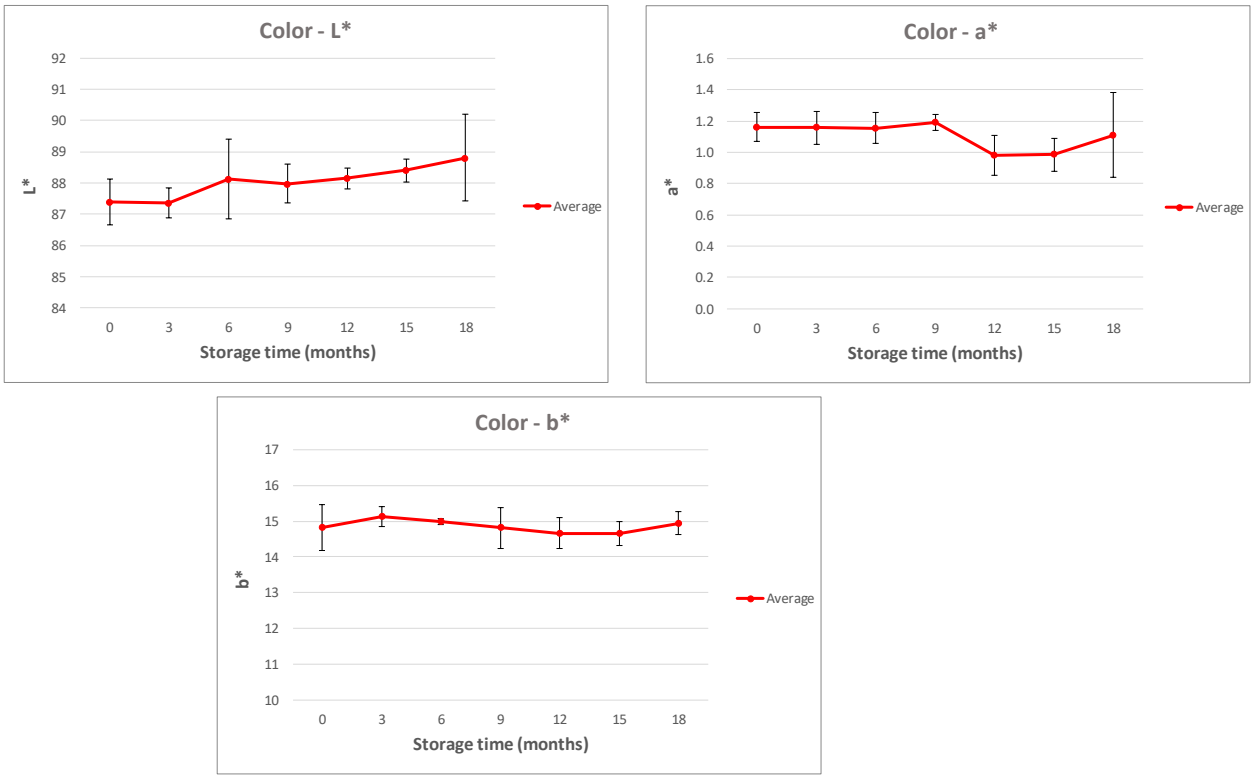
Table 2-14 Shelf-life specification for Lacprodan® MFGM-10

Shelf-life specification	Acceptance level
Colour change (ΔE*ab)	< 3
Water activity, a_w	≤ 0.5
Water content	≤ 6%
Total fat	≥ 16%
Protein content	≥ 73%
Phospholipids	≥ 6%
Gangliosides	N/A
Sensory analysis	No discrimination against fresh sample
Microbiology	Within release specification

2.2.7.1.2 Colour

Colour development in the 4 batches over 18 months is shown in Figure 2-10. The average L* value increased 1.4 unit from 0 to 18 months. No net change in the average a* and b* values were observed. In summary, the powder maintained its colour throughout 18 months of storage.

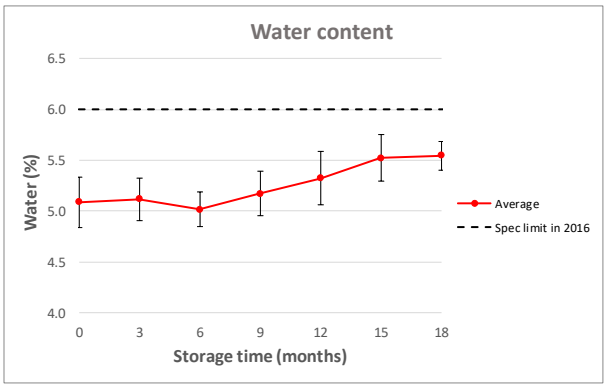
Figure 2-10 Colour changes during stability study



2.2.7.1.3 Water content

An increase in water content was expected due to the hygroscopic nature of the powder that was stored in a partially air-permeable packaging material, and such an increase was observed (Figure 2-11)[6].

Figure 2-11 Changes in water content over stability study

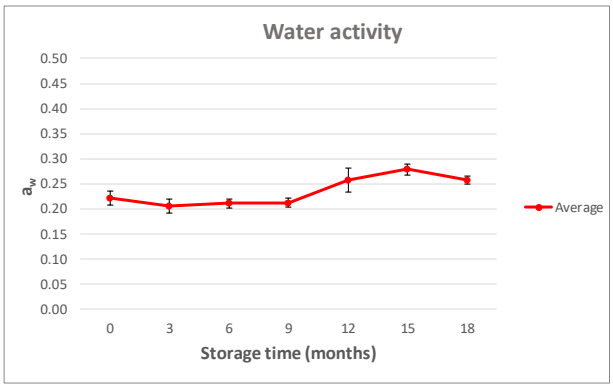


[6] At the time the stability study was performed, the specification for water content was ≤6%; it has since been reduced to ≤5%.

2.2.7.1.4 Water activity

There was no net change in the average a_w from 0 to 9 months (Figure 2-12). From 9 to 18 months, a small increase was observed. The water activity was still well below 0.5, sufficiently low to be incompatible with microbial growth (Labuza & Dugan Jr, 1971). The increase in a_w is attributed to a partially air-permeable barrier of the packaging material.

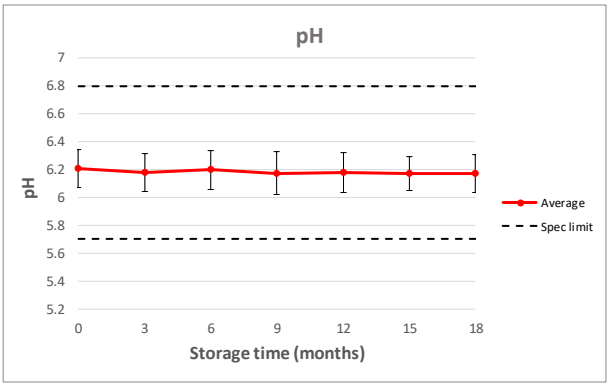
Figure 2-12 Changes in water activity over stability study



2.2.7.1.5 pH

As shown in Figure 2-13, there was no change in pH over 18 months.

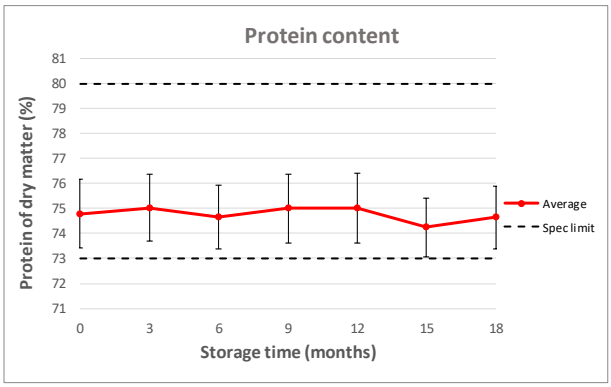
Figure 2-13 Changes in pH over stability study



2.2.7.1.6 Protein

There was no change in the protein content of the dry matter over 18 months (Figure 2-14).

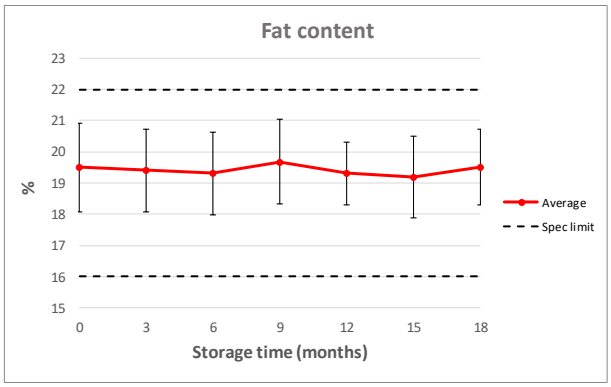
Figure 2-14 Changes in protein in dry matter over stability study



2.2.7.1.7 Fat

Total fat content did not change over 18 months (Figure 2-15**)**.

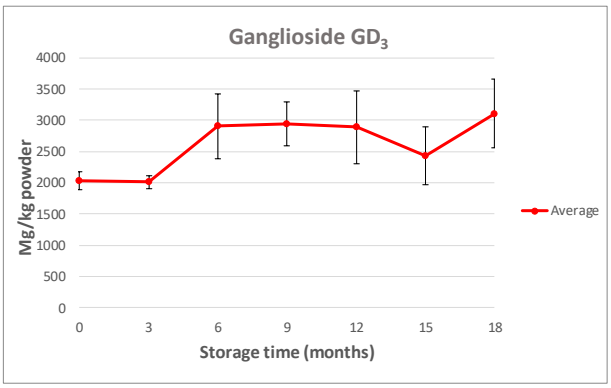
Figure 2-15 Changes in fat content over stability study



2.2.7.1.8 Ganglioside GD3

No net decrease in the average content of ganglioside GD3 was observed over 18 months (Figure 2-16).

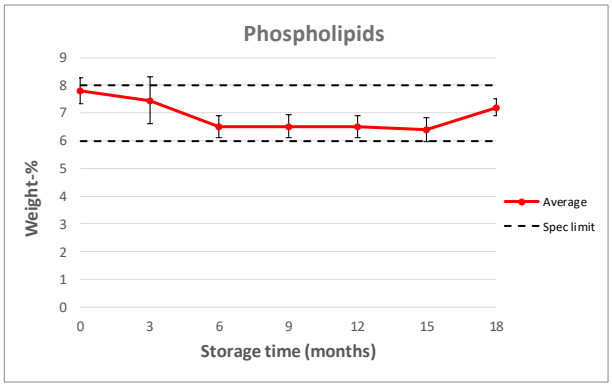
Figure 2-16 Changes in ganglioside GD3 over stability study



2.2.7.1.9 Phospholipids

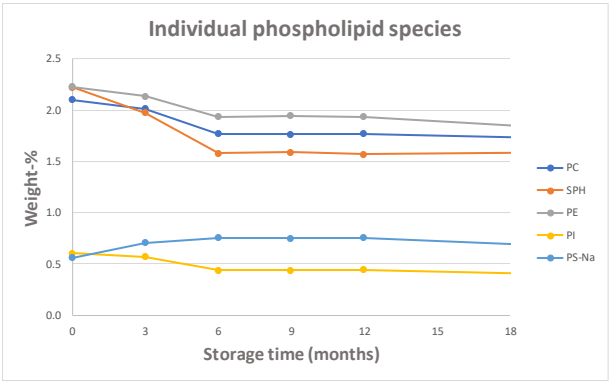
The average content of phospholipids remains above the minimum specification limit during 18 months of storage (Figure 2-17).

Figure 2-17 Changes in phospholipids over stability study



The content of individual species of phospholipids except phosphatidylserine decreased during the first 6 months of storage and were henceforth mostly stable, as shown in Figure 2-18. Data are from one representative batch.

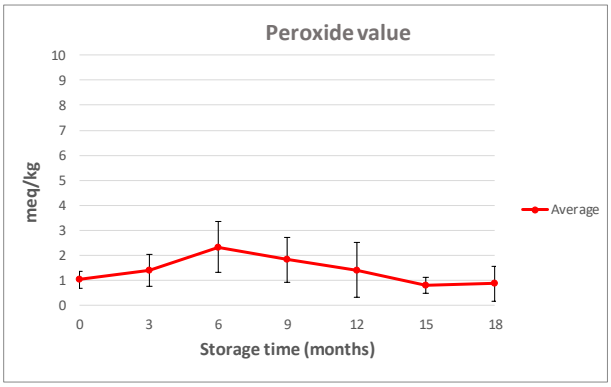
Figure 2-18 Changes in phospholipid species over stability study



2.2.7.1.10 Peroxide value

Despite fluctuations of analysis results for peroxide value throughout the storage period, no significant net change in the average peroxide value was observed at 18 months of storage (Figure 2-19).

Figure 2-19 Changes in peroxide value over stability study



2.2.7.1.11 Major whey proteins

No significant change in measured native alpha-lactalbumin (α-LA), casein glycomacropeptide (CGMP) or beta-lactoglobulin (β-LG) was detected over 18 months (Figure 2-20, Figure 2-21 and Figure 2-22 respectively).

Figure 2-20 Changes in alpha-lactalbumin over stability study

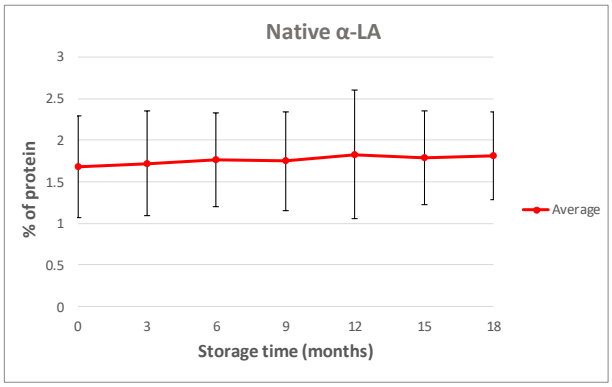


Figure 2-21 Changes in casein glycomacropeptide over stability study

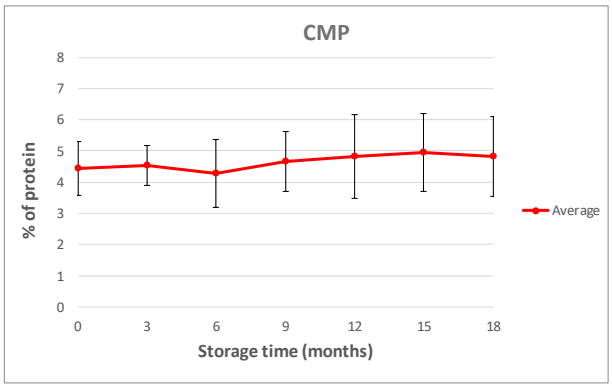
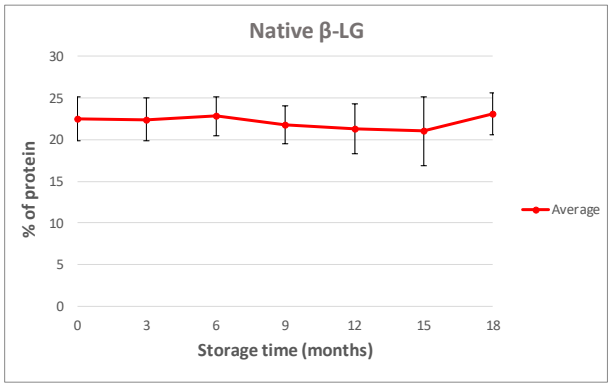


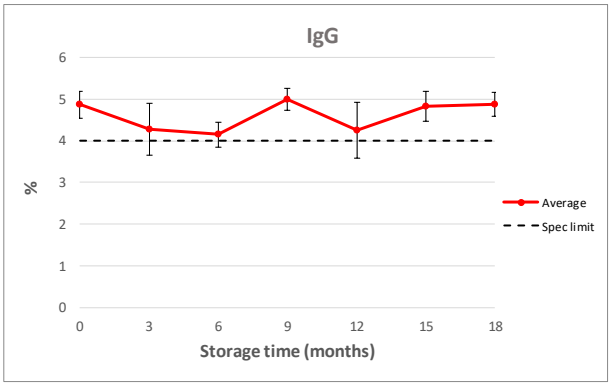
Figure 2-22 Changes in β-lactoglobulin over stability study



2.2.7.1.12 IgG

As shown in Figure 2-23, the IgG level showed some fluctuation, but overall did not change over the 18-month storage period.

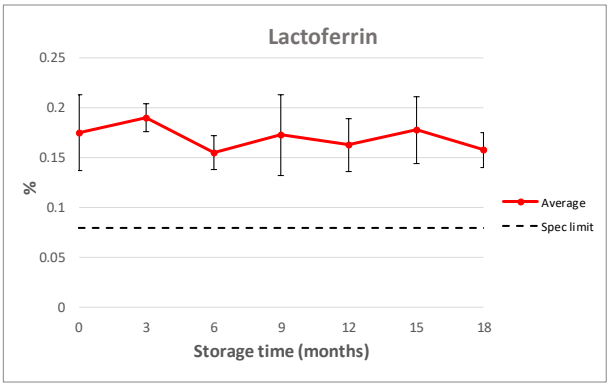
Figure 2-23 Changes in IgG over stability study



2.2.7.1.13 Lactoferrin

The lactoferrin content remained above the minimum specification limit during 18 months of storage and no significant decrease in the average content was observed (Figure 2-24).

Figure 2-24 Changes in lactoferrin over stability study



2.2.7.1.14 Microbiology

Microbiological compliance was assessed a end of the 18-month stability period (Table 2-15).

Table 2-15 Microbiological compliance over 18 month storage

Analysis	Acceptance Level	Compliant through 18 Months?
Total plate count	≤10,000 cfu/g	Yes
Enterobacteriaceae	≤10 cfu/g	Yes
Bacillus cereus	≤50 cfu/g	Yes
Staphylococcus aureus coagulase + a	Absent in 1 g	Yes
Yeast/mold	≤10 cfu/g	Yes
Salmonella spp.	Absent in 125 g	Yes
Cronobacter sakazakii	No standard	N/A (result: negative)
Listeria monocytogenes	No standard	N/A (result: negative)
Sulfite reducing bacteria	No standard	N/A (result: <10 cfu)
Thermophilic count	No standard	N/A

2.2.7.1.15 Sensory properties

Differences in sensory characteristics among samples were not significant, but some trends were evident to experienced tasters: an increase in cardboard, bitterness, stale flavour, teeth-coating, and drying-out mouthfeel were indicated with increasing age of samples. A trend towards a decrease in whey aroma and whey flavour was also noted. However, it was concluded that samples stored for up to 18 months cannot be distinguished from fresh samples based on taste, aroma, or mouthfeel.

2.2.7.1.16 Conclusion

All parameters assessed over the duration of the stability study remained within acceptance limits up to 18 months of storage. In conclusion, the stability data set documents a shelf life of 18 months for commercial packaged Lacprodan® MFGM-10 powder.

2.2.7.2 Stability of Lacprodan® MFGM-10 in nutritional products

A systematic series of stability studies of Lacprodan® MFGM-10 in formulated nutritional powders were performed to assess the nutrient stability and sensory characteristics. This stability work was initiated in a variety of formulas targeted for infants (infant formula, 0-6 months; and follow-on formula, 6-12 months). Three independent stability trials were conducted to assess the overall stability of Lacprodan® MFGM-10 in finished powders. Although these products are not FSFYC, they are a suitable proxy as are formulated oil-filled, whey and micronutrient fortified dairy-based

powders. The stability of SM in a FSFYC (toddler milk) products was assessed and is reported in Section 2.2.7.2.3.

2.2.7.2.1 Macronutrient stability in finished powders

The stability studies of Lacprodan® MFGM-10 were conducted in commercially available nutritional powders. [redacted]

The first study assessed macronutrient stability. In addition, key fatty acids and vitamins that are prone to lose stability over time in powders were also assessed. This trial was undertaken in formulas fortified with Lacprodan® MFGM-10 in addition to other nutrients. The fortification of Lacprodan® MFGM-10 was 5-6 g/L in infant formula products and approximately 2.5 g/L in follow-on products (Table 2-16). These formulas were packaged in conventional cans used for commercial sales.

Table 2-16 Products used in stability study 1

Formula Type	Package
Infant formula	Metal Can
Follow-on formula	Metal Can

Samples of stage 1 and 2 products with added Lacprodan® MFGM-10 were tested for stability of macronutrients, key fatty acids, and vitamins at two different temperature conditions, 25ºC and 60% relative humidity (Climate Zone II) and 30ºC and 65% relative humidity (Zone IV), for at least 17 to 24 months (Table 2-17). The tested nutrients were chosen as those most prone to stability issues. For expediency, some stable nutrients were not measured at 24 months under the Zone II storage condition as Zone IV is the worst case. Analytical methods are shown in Table 2-18.

Table 2-17 Stability conditions

Storage Conditions	Temperature / Relative Humidity	Analysis Time Points (Months)
Climate Zone II	25ºC/ 60% RH	0, 17, 24
Climate Zone IV	30º C/ 65% RH	0, 17, 24

Table 2-18 Assay methodologies

Parameter	Instrument	Reference
Carbohydrates	Calculated by difference as 100 g minus the sum of grams moisture, protein, fat, and ash.	FSANZ - available CHO by difference (S11—3)
Fat	Mojonnier Model D Tester for Butterfat	AOAC 989.05
Loss on Drying	Vacuum Oven, Fisher Isotemp Model 281, or equivalent	AOAC 927.05
Protein (N * 6.25)	Erba Instruments Model 1500 ANA or equivalent Thermo Flash 2000 ANA	In-house method based on Dumas Method AOAC 968.06
C18:2n6 Linoleic Acid	Agilent Technologies 7890A Gas Chromatograph	AOAC 991.39
C18:3n3 Linolenic Acid	Agilent Technologies 7890A Gas Chromatograph	AOAC 991.39
C22:6n3 DHA	Agilent Technologies 7890A Gas Chromatograph	AOAC 991.39
Vitamin E	Agilent 1100 or equivalent Liquid Chromatograph	In-house method
Vitamin K	Hewlett Packard 1050 or equivalent Liquid Chromatograph + UV Wavelength Detector	In-house method

Parameter	Instrument	Reference
Vitamin C, Ascorbic Acid	Hewlett Packard 1100 or equivalent Liquid Chromatograph + UV Wavelength Detector	In-house method

Analytical results for each of the formulas are presented below (Table 2-19;Table 2-20).

Table 2-19 Stability of macronutrients, key fatty acids, and vitamins in infant formula fortified with Lacprodan® MFGM-10.

Parameter	Unit of Measure	Storage Conditions	Time (Months)		
			Zero	1	24
Carbohydrate	% w/w	Zone II	56.9	57.6	N/A
		Zone IV	56.9	57.4	57.4
Fat	% w/w	Zone II	26.7	26.5	N/A
		Zone IV	26.7	26.4	26.6
Loss on Drying	% w/w	Zone II	2.04	1.74	N/A
		Zone IV	2.04	1.89	1.79
Protein (N * 6.25)	%w/w	Zone II	11.33	11.32	N/A
		Zone IV	11.33	11.38	11.23
C18:2n6 Linoleic Acid	mg/100kcal	Zone II	877	851	N/A
		Zone IV	877	851	862
C18:3n3 Linolenic Acid	mg/100kcal	Zone II	75.8	74.2	N/A
		Zone IV	75.8	74.2	74.8
C22:6n3 DHA	mg/100kcal	Zone II	16.2	16.6	N/A
		Zone IV	16.2	16.5	16.2
Vitamin E	IU/100kcal	Zone II	3.35	3.49	3.31
		Zone IV	3.35	3.33	3.78
Vitamin K	mcg/100kcal	Zone II	10.98	10.05	9.87
		Zone IV	10.98	10.59	9.81
Vitamin C, Ascorbic Acid	mg/100kcal	Zone II	23.9	22.5	22.1
		Zone IV	23.9	22.5	21.9

M = months, Zone II = 25^{0}C/60% relative humidity, Zone IV = 30^{0}C/65% relative humidity

Table 2-20 Stability of macronutrients, key fatty acids, and vitamins in follow-on formula fortified with Lacprodan® MFGM-10.

Parameter	Unit of Measure	Storage Conditions	Time (Months)		
			Zero	17	24
Carbohydrate	%w/w	Zone II	60.5	60.6	N/A
		Zone IV	60.5	60.5	60.8
Fat	%w/w	Zone II	18.5	18.5	N/A
		Zone IV	18.5	18.6	18.6
Loss on Drying	%w/w	Zone II	1.72	1.65	N/A
		Zone IV	1.72	1.74	1.58
Protein (N * 6.25)	%w/w	Zone II	15.63	15.63	N/A
		Zone IV	15.63	15.66	15.51
C18:2n6 Linoleic Acid	mg/100cal	Zone II	662	648	N/A
		Zone IV	662	635	649
C18:3n3 Linolenic Acid	mg/100cal	Zone II	57.4	56.7	N/A
		Zone IV	57.4	55.6	56.0
C22:6n3 DHA	mg/100cal	Zone I	17.4	17.7	N/A
		Zone IV	17.4	17.2	16.8
Vitamin E	IU/100cal	Zone II	2.85	3.73	3.28
		Zone IV	2.85	3.22	3.03
Vitamin K	mcg/100cal	Zone II	10.25	9.76	10.68
		Zone IV	10.25	9.96	13.43
Vitamin C, Ascorbic Acid	mg/100cal	Zone II	24.9	24.4	22.4
		Zone IV	24.9	24.3	23.7

The stability data presented in this study shows that the macronutrients, fatty acids, and vitamins studied are stable through the label claim period of 24 months at climatic conditions where these products are intended to be distributed. This study assured that addition of Lacprodan® MFGM-10 had no impact on macronutrient, fatty acid, or vitamin stability in infant and follow-on formula matrices for at least 24 months.

2.2.7.2.2 Oxidative stability in finished powder

Lipid molecules are prone to oxidation, infant and follow-on formulas containing Lacprodan® MFGM-10 were assessed for oxidative stability. The infant formula products (infant formula and follow-on formula) were studied in 3 different storage conditions (Climate Zones II and IV and an accelerated condition at 40ºC and 75% relative humidity) at multiple time points up to 24 months (Table 2-21). Infant formula and follow-on formula powders were packaged in two different commercial grade metal cans (one existing and one under development) (Table 2-22).

Table 2-21 Stability conditions

Storage Conditions	Temperature/ Relative Humidity	Analysis Time Points* (Months)
Zone II	25ºC/60% RH	0, 2, 3, 4, 6, 8, 9, 12, 15, 17, 18, 21, 24
Zone IV	30º C/65% RH	0, 2, 3, 4, 6, 8, 9, 12, 15, 17, 18, 21, 24
Accelerated	40ºC/75% RH	0, 2, 3, 4, 6, 8

**Results reported for highlighted time points, RH = relative humidity*

Table 2-22 Packaging used in stability trials

Formula Type	Package
Infant formula	Can 1
	Can 2
Follow-on formula	Can 1
	Can 2

To determine the oxidative status of infant formula containing Lacprodan® MFGM-10, free fat, propanol, hexanal, and free oxygen were measured in these products. Table 2-23 describes the assay methodologies used for each analyte.

Table 2-23 Assay methodologies

Analyte	Instrument	Reference
Free Fat	Mojonnier oven/Air Oven and Mojonnier Hotplate/Steambath	In-house method
Propanol	Static Headspace/Gas Chromato-graphy/Flame IonizationDetector (SHS/GC/FID)	In-house method
Hexanal	Static Headspace/Gas Chromato-graphy/Flame IonizationDetector (SHS/GC/FID)	In-house method
Oxygen	PBI-Dansensor Checkmate 9900	In-house method

Concentrations of the key analytes, free fat, propanol, hexanal, and oxygen, chosen to monitor oxidative stability for the three different formulas under the two climatic conditions and the accelerated condition are reported below in Table 2-24 through Table 2-27. These data reveal that little oxidation occurs over the time periods studied.

Table 2-24 Oxidative stability in infant formula, Can1

Analyte	Unit	Storage conditions	Time (Months)							
			Zero	2	3	4	6	8	17	24
Free Fat	% w/w	Zone II	0.62	N/A	0.69	0.35	0.71	0.73	0.75	0.82
		Zone IV	0.62	N/A	0.75	0.72	0.79	0.82	0.86	0.91
		Accelerated	0.62	0.90	0.99	1.27	1.36	1.80	N/A	N/A
Propanol	ppm	Zone II	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
Hexanal	ppm	Zone II	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
O2	%	Zone II	2.29	2.18	2.01	4.72	3.51	1.07	3.35	0.178
		Zone IV	2.29	2.17	1.75	4.65	3.43	1.09	3.24	0.124
		Accelerated	2.29	2.12	1.22	4.65	3.05	0.801	N/A	N/A

Table 2-25 Oxidative stability in infant formula, Can2

Analyte	Unit	Storage conditions	Time (Months)							
			Zero	2	3	4	6	8	17	24
Free Fat	%w/w	Zone II	0.52	N/A	0.43	0.58	0.53	0.57	0.6	0.54
		Zone IV	0.52	N/A	0.34	0.54	0.56	0.58	0.7	0.66
		Accelerated	0.52	0.68	0.49	0.78	0.94	1.32	N/A	N/A
Propanol	ppm	Zone II	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
Hexanal	ppm	Zone II	<0.05	<0.05	<0.05	0.05	<0.05	<0.05	<0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
O_2	%	Zone II	2.07	2.85	1.83	3.83	2.83	0.684	3.00	0.292
		Zone IV	2.07	4.76	1.27	3.40	2.50	1.39	2.54	0.169
		Accelerated	2.07	2.85	1.23	3.35	2.23	0.811	N/A	N/A

Table 2-26 Oxidative stability in follow-on formula, Can1

Analyte	Unit	Storage conditions	Time (Months)							
			Zero	2	3	4	6	8	17	24
Free Fat	% w/w	Zone II	0.28	N/A	0.33	0.32	0.34	0.30	0.32	0.25
		Zone IV	0.28	N/A	0.37	0.31	0.35	0.30	0.35	0.34
		Accelerated	0.28	0.38	0.41	0.38	0.40	0.41	N/A	N/A
Propanol	ppm	Zone II	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
Hexanal	ppm	Zone II	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
O_2	%	Zone II	2.16	1.99	1.51	4.10	2.78	0.943	3.14	0.220
		Zone IV	2.16	2.11	1.64	3.74	3.02	0.827	3.19	0.157
		Accelerated	2.16	2.57	1.11	4.21	2.86	0.785	N/A	N/A

Table 2-27 Oxidative stability in follow-on formula, Can2

Analyte	Unit	Storage conditions	Time (Months)							
			Zero	2	3	4	6	8	17	24
Free Fat	% w/w	Zone II	0.27	N/A	0.37	0.40	0.39	0.37	0.59	0.39
		Zone IV	0.27	N/A	0.29	0.39	0.35	0.36	0.61	0.38
		Accelerated	0.27	0.44	0.25	0.46	0.49	0.41	N/A	N/A
Propanol	ppm	Zone II	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
Hexanal	ppm	Zone II	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	<0.05
		Zone IV	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	<0.05
		Accelerated	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	N/A	N/A
O_2	%	Zone II	2.99	2.17	1.53	4.18	3.30	1.31	2.38	0.306
		Zone IV	2.99	8.82	1.72	3.43	2.75	1.49	2.73	0.213
		Accelerated	2.99	1.59	1.60	3.27	2.69	0.744	N/A	N/A

2.2.7.2.3 Stability of sphingomyelin in finished formula powders

Lacprodan® MFGM-10 contains higher phospholipid and sphingomyelin content compared to typical WPC (Section 2.2.3.2.1; Table 2-6). Addition of Lacprodan® MFGM-10 results in distinctly higher levels of sphingomyelin compared to non-fortified formulas and can be used as one of the markers to assure Lacprodan® MFGM-10 addition. Moreover, sphingomyelin levels can be quantitatively measured using established methods in finished infant formula.

To assess sphingomyelin stability in Lacprodan® MFGM-10-fortified products, two infant formulas with Lacprodan® MFGM-10 at 6 g/L and one toddler formula Lacprodan® MFGM-10 at 2.5 g/L were analysed at 0, 6, and 18 months. During the stability study period the formulas were stored at Zone II conditions (25ºC and 60% relative humidity). Sphingomyelin analysis was carried out using quantitative 31P-NMR spectroscopy according to SAA-MET002-02.

Table 2-28 summarises the results of sphingomyelin content assayed at various time points. The different values in the table clearly reflect only analytic variability, not loss / gain of sphingomyelin content.

Table 2-28 Sphingomyelin content (mg/g) in finished formula powders

Product	Months		
	0	6	18
Infant formula	1.10	1.05	1.10
Follow-on formula	1.05	1.10	1.20
Toddler formula	0.45	N/A	0.55

2.2.7.2.4 Conclusion

In conclusion, in a variety of stability testing protocols of analytes (macronutrients, susceptible fatty acids, and vitamins; oxidation; and sphingomyelin content), at different packaging and storage conditions, infant and follow-on formulas containing MFGM-WPC (Lacprodan® MFGM-10) showed acceptable stability of all parameters assessed.

2.2.8 Analytical method for detection

It is the presence of the elevated levels of MFGM phospholipids and sphingolipids that assigns MFGM-WPC its primary identity.

Several methods for the analysis of phospholipids are available. Arla Foods Ingredients P/S uses and recommends the use of $_{31}$P NMR (MacKenzie et al., 2009; Murgia et al., 2003). Phospholipid analysis is completed for Lacprodan® MFGM-10 by Spectral Services (Spectral Service, Köln, Germany). The $_{31}$P NMR method is recommended as the preferred method to measure total PLs including SM in milk-based matrices.

Spectral Service uses the soxhlet extraction procedure prior to $_{31}$P NMR analysis, which appears to have a better recovery for individual PL components, specifically SM (Figure 2-25).

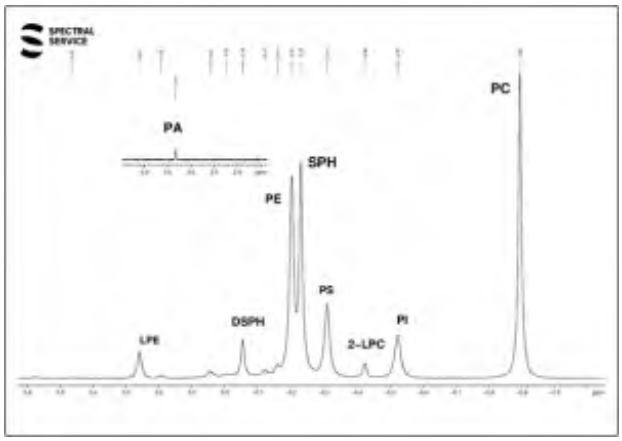
https://www.spectralservice.de/phospholipid-analysis-by-31p-nmr-spectroscopy/

In New Zealand, Callaghan Innovation routinely uses $_{31}$P NMR for the analysis of phospholipids in dairy products based on MacKenzie et al. (2009) on a contract laboratory basis.

It is noted the recently published Chinese Light Industry Standard (QB/T 5805-2023) uses HPLC-ELS for the identification and quantification of phospholipids including SM, and HPLC MS/MS for the quantification of gangliosides.

Quantification of PL by HPLC MS/MS has also been used.

Figure 2-25 Typical 31P-NMR spectrum of a milk sample, detail (phospholipids)(Spectral Services)



AOAC has established Standard Method Performance Requirements (SMPRs®) for the determination of phospholipids in infant and adult/paediatric nutritional formula (AOAC SMPR® 2021.017) (AOAC International, 2022). The scope of these requirements covers the quantitative determination of nutritionally relevant PL including PC, PE, PI, PS and SM. Any analytical method that meets the performance requirements is acceptable for the determination of PL in infant and adult nutritional products.

Given the complexity of MFGM-WPC composition, it is important that a marker protein or lipid is designated to assure the consistency of raw material from batch to batch and to assure incorporation

into infant formula. Sphingomyelin is proposed as the identifying component for MFGM-WPC for the following reasons:

i. Sphingomyelin is one of the major phospholipids present in MFGM fractions.
ii. Standardised analytical methods are available to assay SM in both the ingredient (MFGM-WPC) and finished FSFYC matrices.
iii. Sphingomyelin is present in very low amounts in conventional protein fractions (WPC), not present in vegetable oils or lecithin and hence easily quantifiable in finished FSFYC.
iv. Sphingomyelin levels of FSFYC made with whole milk or skim milk are markedly less than the levels of SM found in FSFYC with added MFGM-WPC (see Section 3.2.1).

2.2.9 Information on the proposed food label

2.2.9.1 Ingredient listing

A number of options are proposed as to how MFGM-WPC may be listed in the ingredients list of FSFYC:

- Milk fat globule membrane enriched whey protein concentrate (**milk**)
- Phospholipid enriched whey protein concentrate (**milk**)
- Whey protein phospholipid concentrate (**milk**)
- MFGM enriched whey protein concentrate (**milk**)
- Whey protein concentrate (containing milk fat globule membrane) (**milk**)
- Whey protein concentrate (containing MFGM) (**milk**)

These options accurately reflect the nature of MFGM-WPC.

2.2.9.2 Quantification in Nutrition Information Panel (NIP)

The identification of added MFGM-WPC and / or sphingomyelin in the nutrition information panel (NIP) is proposed as optional. Currently Standard 2.9.3-8 (1) only requires mandatory labelling of vitamins and minerals used as nutritive substances. There is no mandatory requirement for the NIP to include and quantify the optional nutritive substance lutein if added. However for lutein (as an optional nutritive substance) as per Standard 2.9.3-8 (6) the label on a package of formulated supplementary food for young children must not include any words indicating, or any other indication, that the product contains lutein unless the total amount of lutein is no less than 30 µg/serving. Typically, this level would require the addition of lutein or lutein containing ingredients to meet this threshold.

Other foods e.g. Formulated supplementary sports foods (Standard 2.9.4 -5) requires mandatory declaration of added nutritive substances, and the amount which may be declared either immediately after the statement referring to the presence of the substance, or in the ingredient list following the substance listing, or in the NIP.

By way of example, a sample of a proposed NIP format is provided in
Table 2-29.

2.2.9.3 Plain English Allergen Labelling (PEAL)

MFGM-WPC is a dairy ingredient therefore is subject to the requirements of mandatory allergen labelling (Standard 1.2.3 Information requirements – warning statements, advisory statements and declarations).

MFGM-WPC must be labelled as the allergen **milk** in the ingredients list as shown above (Section 2.2.9.1)**.** Plain English allergen labelling (PEAL) requirements also require a separate summary statement: **Contains: milk**.

Table 2-29 Proposed NIP format

NUTRITION INFORMATION			
Serving size: Serves per can:			
	Average Quantity per Serving	% RDI per Serve*	Average Quantity Per 100 mL
Energy	kJ kcal		kJ kcal
Protein	g		g
Fats – total	g		g
- saturated	g		g
- trans	g		g
- monounsaturated	g		g
- polyunsaturated	g		g
-			
Carbohydrates	g		g
- Sugars	g		g
-			
Sodium	mg		mg
Vitamins			
Vitamin A	µg-RE	%	µg-RE
Vitamin D	µg	%	µg
Vitamin E	µg-αTE	%	µg-αTE
Thiamin	mg	%	mg
Riboflavin	mg	%	mg
Vitamin B_6	µg	%	µg
Vitamin B_{12}	µg	%	µg
Vitamin C	mg	%	mg
Vitamin D	µg	%	µg
Vitamin E	µg	%	µg
Niacin	mg	%	mg
Folate	µg	%	µg
Vitamin B_6	mg	%	mg
Vitamin B_{12}	µg	%	µg
Vitamin C	mg	%	mg
Minerals			
Calcium	mg	%	mg
Phosphorus	mg	%	mg
Magnesium	mg	%	mg
Iron	mg	%	mg
Zinc	mg	%	mg
Iodine	µg	%	µg
Other			
Lutein	µg		µg
MFGM-WPC[1]	g		g
- Sphingomyelin	mg		mg

*%

Recommended Dietary Intake for children aged 1 – 3 years. [1]Milkfat Globule Membrane enriched Whey Protein Concentrate.

2.3 Data related to the safety of Lacprodan® MFGM-WPC

2.3.1 Toxicokinetics and metabolism of MFGM-WPC

Specific studies on the toxicokinetics of MFGM-WPC have not been undertaken. The ingredient is predominantly WPC with the inclusion of a complex mixture of components, of which the major groups of components (lipids and proteins) are discussed below. Whey protein concentrate is a common ingredient in many foods and has a long history of safe use.

The lipid fraction of MFGM contains polar lipids such as glycerophospholipids, sphingolipids (including sphingomyelin) and glycolipids (including gangliosides), cholesterol and triglycerides (Singh, 2006).

2.3.1.1 Absorption, distribution, metabolism and excretion of specific MFGM components

2.3.1.1.1 Phospholipids

Phospholipids comprise approximately 6% of the mass of MFGM; however, the majority of the phospholipid in milk is in the MFGM (H. Lee, Padhi, et al., 2018). Milk phospholipid digestion and absorption have been studied, with several studies on the digestion and absorption of isolated phospholipid components.

Milk phospholipids are cleaved into monoglycerides, free fatty acids, and lyso-phospholipids by a variety of lipases, including lingual lipase, gastric lipase, pancreatic lipase, colipase, phospholipase A2, and BSSL (Å Nilsson & Duan, 2019). The main products of glycerophospholipid digestion in the luminal phase of digestion are the 1-acyle-phosphatidyl (lyso-phospholipid) compounds and these are the primary forms in which the glycerophospholipids are absorbed from the intestinal tract (Borgström, 1974).

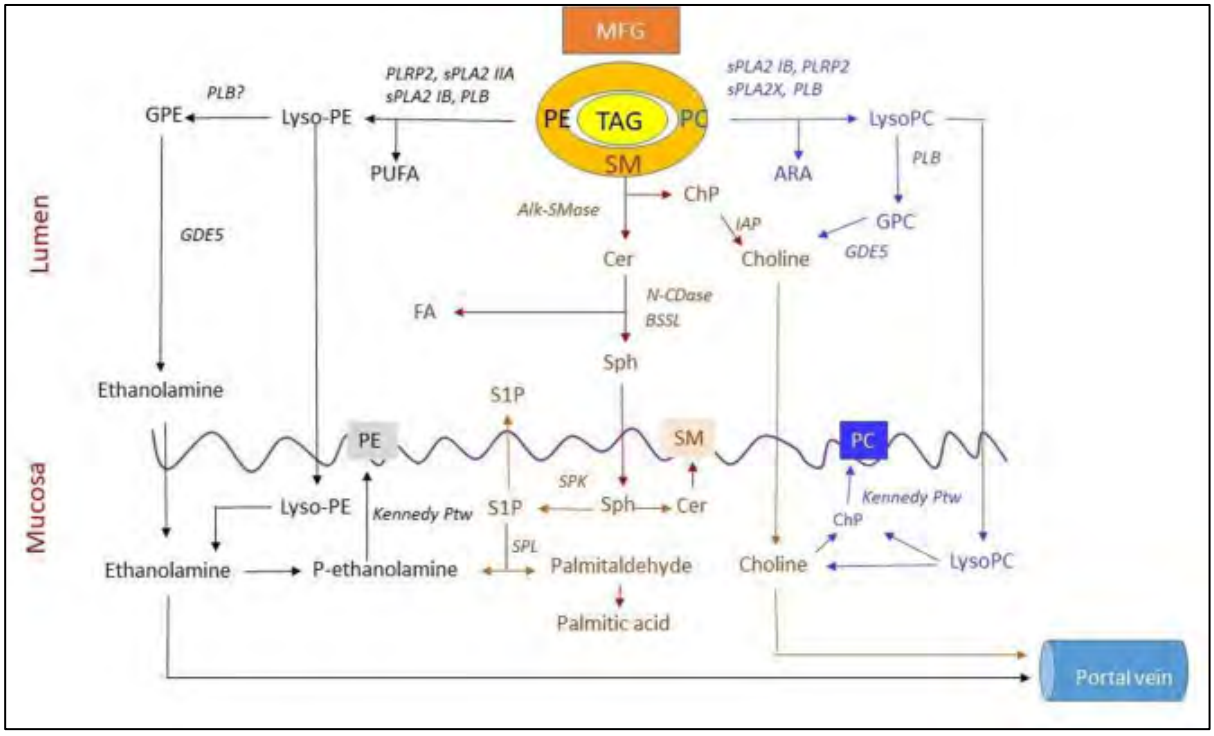
The digestion and absorption of phosphatidylcholine have been extensively studied (Borgström, 1974; Å Nilsson & Duan, 2019; Parthasarathy, Subbaiah, & Ganguly, 1974). In the proximal small intestine, phosphatidylcholine is initially hydrolysed to 1-lyso-phosphatidylcholine and free fatty acids by the pancreatic phospholipase A2 Ib. Lyso-phosphatidylcholine can be absorbed into mucosal cells or hydrolysed further by jejunoileal brush border phospholipase B/lipase and mucosal-secreted phospholipase A2. Both pancreatic and mucosal phases of phosphatidylcholine digestion are mediated by the combined action of secretory pancreatic phospholipase A2 IB (sPLA2 IB) and secretory pancreatic phospholipase A2 IB (sPLA2 IB) (Åke Nilsson, Duan, & Ohlsson, 2021; Å Nilsson & Duan, 2019). Absorbed lyso-phosphatidylcholine is partitioned in the mucosal cells between degradation and re-acylation into chylomicron phosphatidylcholine (Å Nilsson & Duan, 2019). There is little or no absorption of intact phosphatidylcholine (Parthasarathy et al., 1974).

Åke Nilsson et al. (2021) have reviewed the digestion and absorption of milk phospholipids, demonstrating that the fate of the different phospholipids follow similar digestion and absorption processes to phosphatidylcholine, albeit producing different metabolites (Figure 2-26). The dialogue to Figure 2-26 of Åke Nilsson et al. (2021) provides an excellent overview of the digestion and absorption process:

"*Lipids in the milk are organized in the milk fat globule (MFG) with polar lipids in the membrane and triacylglycerol (TAG) in the core. The secretory pancreatic phospholipase A2 IB (sPLA2 IB), sPLA2, and sPLA2X hydrolyze phosphatidylcholine (PC) to lysoPC and may release arachidonic acid (ARA) for*

both phospholipid (PL) and prostaglandin (PGD) synthesis. Phospholipase B (PLB) can degrade both PC and lysoPC to glycerophosphocholine (GPC) and then by GDE5 to choline. Both lysoPC and choline are absorbed. Absorbed lysoPC may release choline that can, in turn, be phosphorylated to choline phosphate (ChP) in mucosal cells. Sphingomyelin (SM) is sequentially hydrolyzed by alkaline SMase (alk-SMase), neutral ceramidase (N-CDase), and bile salt-stimulated lipase (BSSL) to ceramide (Cer), sphingosine (Sph), and fatty acid (FA). The cleaved ChP is degraded to choline by intestinal alkaline phosphatase (IAP). Then, Sph, choline, and FA are absorbed. The Sph in mucosa may be phosphorylated by sphingosine kinase (SPK) to S1P, most of which is degraded by sphingosine lyase (SPL) to palmitaldehyde and further converted into palmitic acid. This reaction generates ethanolamine phosphate (ethanolamine-P). Both ethanolamine and choline are substrate for synthesis of phosphatidylethanolamine (PE) and PC, respectively, via the Kennedy pathway or transported via portal vein. PE is degraded by sPLA2 IB, sPLA2 IIA, PLRP2, and PLB to lysoPE for absorption. LysoPE can be degraded by PLB to glycerophosphoethanolamine (GPE) and then to ethanolamine likely by GDE5 for absorption and resynthesis of PE. Meanwhile, polyunsaturated fatty acid (PUFA) can be formed during PE hydrolysis."

Figure 2-26 Digestion and absorption of the major polar lipids in milk



from Åke Nilsson et al. (2021)

2.3.1.1.1.1 Effect of MFGM phospholipids on lipid digestion and absorption

Because MFGM acts as an emulsifier of milk fat (He et al. 2017), it may increase digestion of triacylglycerols (Luo et al., 2019). Berton et al. (2012) found that bovine MFGM improves the efficiency of lipid digestion by human pancreatic lipase *in vitro*. Pancreatic lipase was more active against small milk fat globules than large ones, and the 25-fold increase in globule surface area brought about by

homogenization resulted in a 2-fold increase in lipid digestion. The investigators attributed the enhancement of lipid digestion to the MFGM proteins (Berton et al., 2012).

Lecomte et al. (2015) compared the effects milk polar lipids (MPLs) to soy polar lipids (SPLs) on lipid digestion in an *in vitro* model and on lipid absorption in an *in* vivo murine model. They found an emulsion stabilised by MPLs enhanced lipid hydrolysis resulting in more rapid postprandial fat absorption in mice compared to an emulsion stabilised by SPLs. Mice fed with MPLs showed higher plasma TGs and NEFAs at 1 hour compared to mice fed with SPLs, indicating faster gastric emptying and intestinal absorption for MPL-fed mice (Lecomte et al., 2015). Similarly, milk phospholipids enhanced lipid intestinal hydrolysis and promoted more rapid intestinal lipid absorption and sharper kinetics of lipemia than soy phospholipids (Mathiassen et al., 2015).

Bach Korsholm Knudsen et al. (2021) investigated if emulsification of the lipid components of a diet in neonatal piglets differed between bovine milk derived emulsifiers containing various MFGM components and soy lecithin (SL), and any difference in lipid digestion occurred. Initially, lipid digestibility was determined *in vitro* in oil-in-water emulsions using four different milk-derived emulsifiers or SL and by measuring the degree of hydrolysis. Electron microscopy was used to assess the ultrastructural appearance of the emulsions. Milk-derived emulsifiers (containing protein and phospholipids from milk fat globule membranes and extracellular vesicles) showed increased effects on fat digestion compared to SL in an *in vitro* digestion model. Ultra-structural images indicated a more regular and smoother surface of fat droplets emulsified with milk-derived emulsifiers relative to SL. Relative to SL, milk-derived emulsifiers resulted in a different surface ultrastructure on the lipid droplets, and increased lipid digestion (Bach Korsholm Knudsen et al., 2021).

The role of the MFGM and its components on the digestion and absorption kinetics of lipids within the milk fat globule is complex, influencing not only the size and structure of milk fat globules, but also providing a number of components that participate in the digestive processes (Bourlieu & Michalski, 2015; Singh & Gallier, 2017).

2.3.1.1.1.2 Effect of MFGM phospholipids digestion products on microbiome and intestinal development

Feeding milk-based phospholipids (H. Lee, Zavaleta, Chen, Lonnerdal, & Slupsky, 2018; Nejrup, Licht, & Hellgren, 2017; Ortega-Anaya & Jiménez-Flores, 2019) enriched preparations of gangliosides (Rueda, Maldonado, Narbona, & Gil, 1998), or sphingomyelin (Norris, Jiang, Ryan, Porter, & Blesso, 2016) has been reported to normalize the gastrointestinal microbiome to resemble that of naturally fed animals, a finding which has been confirmed in clinical studies (He et al., 2019; Rueda, Sabatel, Maldonado, Molina-Font, & Gil, 1998). Microbiome effects of feeding MFGM preparations may be related to the glycoprotein moiety of MFGM (Guerin et al. 2019) or the persistence of sphingolipids throughout the GI tract (Larson, Falk, Hynsjö, Midtvedt, & Midtvedt, 1990). MFGM components, digested or incompletely digested may promote GI development directly and indirectly, thereby normalising intestinal development and function (Anderson, MacGibbon, Haggarty, Armstrong, & Roy, 2018; Bhinder et al., 2017; Huërou-Luron, Lemaire, & Blat, 2018; Norris, Milard, Michalski, & Blesso, 2019). He et al. (2019) provided further clinical evidence that the MFGM may have a role in the modulation of microbiota activity and function. As discussed in Section 2.5.4 the microbiota and gut health continue to play central roles in supporting healthy immune function in young children.

2.3.1.1.2 Sphingomyelin

Unlike the phospholipids, sphingomyelin (SM) is hydrolysed by brush border alkaline sphingomyelinase (SMase) cleaving the phosphocholine head from SM to produce ceramide (Duan & Nilsson, 2000; Åke Nilsson et al., 2021). The ceramide is then hydrolysed by neutral ceramidase to sphingosine and free fatty acids, which are well absorbed (Nilsson and Duan 2018) (Figure 2-26). The key SMase responsible for digestion of SM in the gut is alkaline SMase (alk-SMase), which is attached to the surface of the intestinal brush border by a short intracellular domain with its active catalytic site exposed in the intestinal lumen (Cheng, Nilsson, Tömquist, & Duan, 2002; Liu, Nilsson, & Duan, 2000; Zhang et al., 2011).

In the intestinal tract, the total amount of glycerophospholipids is much higher than that of SM. These phospholipids may function as inhibitors of SM hydrolysis induced by alkaline SMase, delaying the digestion of SM until most of the phospholipids have been hydrolysed by phospholipases and their products such as fatty acids, diacylglycerols, and lysophospholipids have been absorbed. Liu et al. (2000) suggested this may explain why hydrolysis of SM occurs mainly at the distal part of the jejunum and intact SM can be found in the colon and faeces even when fed in small amounts. Larson, Watsfeldt, Falk, Leffler, and Koprowski (1987) showed that faecal excretion of sphingolipids including sphingomyelin, may persist in neonates and young children up to 2 years of age. Later studies suggested a relationship between the persistence these compounds and the establishment of the microbiota. In particular, non-pathogenic species functionally specialised in degrading oligosaccharide chains.

Duan and Nilsson (2000) concluded there is little or no absorption of intact sphingomyelin. Recently however, in-depth lipidomic analyses of the tissues of mice fed a MFGM-WPC supplemented diet showed the accretion of milk-derived very long odd-chain fatty acids from sphingomyelin and ceramides in blood plasma, liver, kidney and skeletal muscle, together with subtle level changes in the cerebral cortex and cerebellum (Sprenger et al., 2024). The basic premise of the study is that sphingolipids and glycerophospholipids have varying but significant levels of odd numbered acyl chains of their fatty acids. In comparison animal and human tissue have very low levels of lipids with odd numbered acyl chains. The trial diets included levels of all branched-chain amino acids, which thereby make it highly unlikely that any differences in levels of lipids with odd-numbered hydrocarbon chains are due to the use of these amino acids as substrates for endogenous production of odd-chain fatty acids. Therefore, changes in the levels of tissues with odd-numbered chains serve as a proxy for whether the intake of MFGM promotes uptake and incorporation of intake milk lipids or structural components of the same (Sprenger et al., 2024). The study could not determine whether the identified odd chain sphingoloids are disassembled by intestinal enzymes, absorbed and ultimately reassembled, or whether intact dietary sphingomyelin(s) and or ceramides can be selectively absorbed and transported.

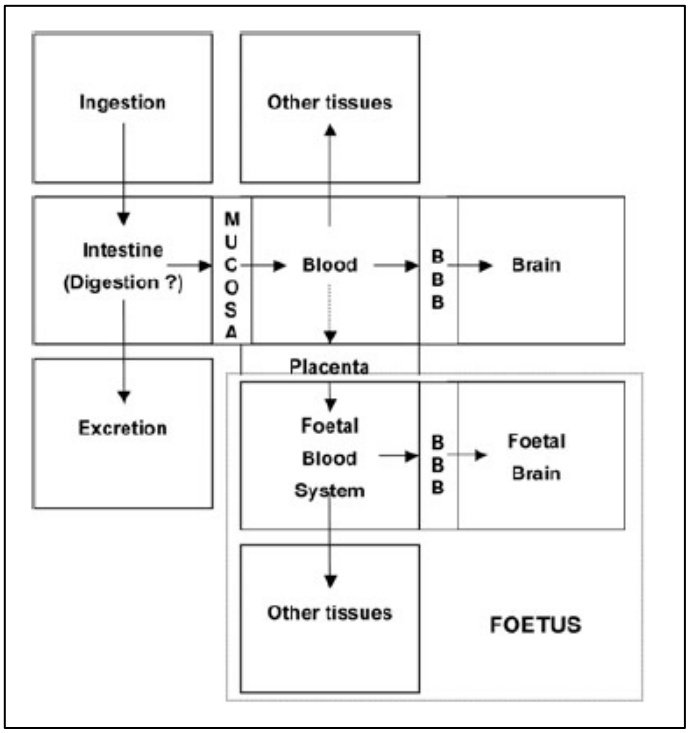
2.3.1.1.3 Gangliosides

The most abundant ganglioside in bovine milk is GD3 (Laegreid et al., 1986). Digestion studies indicate gangliosides are not digested during the gastric phase, reaching the intestinal region of the gastrointestinal tract intact where they are able to be absorbed in the small intestine for transport to different membrane sites in the body (McJarrow et al., 2009). The ability of gangliosides to be taken up by human intestinal cells and its metabolic fate determined by the site of uptake was shown by

Schnabl, Larcelet, Thomson, and Clandinin (2009), using a human cell line. Saqr, Pearl, and Yates (1993) showed *in vitro* exogenous intact gangliosides could be taken up by a range of biological cells including blood cells enabling transport throughout the body to different tissues.

In rats fed a GD3 enriched diet for 2-weeks, Eek Joong Park, Miyoung Suh, Kal Ramanujam, et al. (2005) showed dietary gangliosides were absorbed in the small intestine and transported to various membrane sites. Changes in ganglioside levels were observed in the intestinal mucosa plasma and brain. Furthermore, dietary gangliosides were shown to increase total retinal ganglioside and GD3 content during retinal development (Park, Suh, & Clandinin, 2005). Based on this work the research team concluded that the dietary availability of gangliosides may impact on the lipid composition of developing tissues and hence they may be biologically important (Eek Joong Park, Miyoung Suh, & M. Thomas Clandinin, 2005; Eek Joong Park, Miyoung Suh, Kal Ramanujam, et al., 2005). The study demonstrated that dietary gangliosides can be absorbed and distributed to tissues intact. McJarrow et al. (2009) summarised the uptake of dietary gangliosides for all life-stages as shown in Figure 2-27.

Figure 2-27 Uptake of dietary gangliosides by the body



from McJarrow, Schnell, Jumpsen, and Clandinin (2009)

Two metabolic fates are known for exogenous gangliosides (McJarrow et al., 2009):

i. In the Golgi body direct glycosylation resulting in higher homologous gangliosides or modified gangliosides may occur.
ii. In the lysozymes gangliosides may be catabolised to intermediates of both the saccharide and lipid components, and available for further distribution and metabolism.

From the Golgi body gangliosides enter the plasma membrane where they are assembled into microdomains consisting of cholesterol and sphingolipid-rich caveolae and rafts (McJarrow et al., 2009). E. J. Park et al. (2005) showed dietary gangliosides may be directly incorporated into the

microdomains. The relative amounts of dietary gangliosides that are excreted intact, to those that are absorbed or metabolised is unknown (McJarrow et al., 2009).

2.3.1.1.4 MFGM proteins

Kobylka and Carraway (1973) were among the first to study *in vitro* digestion of proteins in MFGM preparations. They showed comparable susceptibility to proteolysis of proteins in MFGM and proteins in fresh cream. The study used both trypsin and pronase to probe whether the membrane was intact with 'sidedness'; that is, whether membranes were intact with an inside that was distinct from an outside. There was no reduction of activity in any proteins in fresh cream compared to MFGM, which the investigators interpreted to mean that there is no barrier to proteolysis of MFGM proteins in the intact fat globule. Kobylka and Carraway (1973) contrasted these results to red-cell membranes, which remain intact through similar purification steps, limiting proteolysis of internal facing membrane proteins. While trypsin-mediated digestion appeared less active toward MFGM glycoproteins, pronase (which contains phospholipase A), showed activity against glycoproteins comparable to that against non-glycosylated proteins. These results indicate that there is no alteration in digestibility of MFGM proteins resulting from preparation of the MFGM material.

As information accumulated about the composition and structure of MFGM, methods also became more precise. Vanderghem et al. (2011)) used an enzymatic approach similar to that employed by Kobylka and Carraway (1973), extending the study to individual proteins of MFGM with two-dimensional gel electrophoresis and mass spectrometry (Vanderghem et al., 2011). These investigators determined that lactadherin, butyrophilin, and adipophilin were surface proteins readily digested (though a portion of lactadherin resisted digestion, possibly because of surface carbohydrate). Xanthine dehydrogenase/xanthine oxidase was distributed in two pools, some on the surface and some deeply embedded in the membrane (resistant to proteolytic attack), and the fatty-acid binding protein was also embedded. As these five proteins are among the six most abundant proteins in MFGM, they give a good first-order representation of the digestion of the large number of proteins that have been detected in MFGM in bovine milk (Affolter, Grass, Vanrobaeys, Casado, & Kussmann, 2010; Cavaletto et al., 2008; B. Y. Fong & Norris, 2009; Liao, Alvarado, Phinney, & Lönnerdal, 2011; Reinhardt & Lippolis, 2006) and caprine milk (Juvarajah et al., 2018).

Aiqian Ye, Cui, and Singh (2010) and A. Ye, Cui, and Singh (2011) also used a proteolytic approach and two-dimensional electrophoresis to identify MFGM proteins but used pepsin to simulate gastric digestion (A. Ye et al., 2011) and pancreatic lipase to simulate upper intestinal tract digestion (Aiqian Ye et al., 2010). Xanthine oxidase was more readily lost to gastric digestion than butyrophilin or lactadherin, though comparable amounts of each were digested over the *in vitro* incubation period (A. Ye et al., 2011).

A further application of enzymatic study with two-dimensional gel electrophoresis for identification of individual MFGM proteins (Le et al., 2012) used a mix of enzymes (pepsin, trypsin, α-chymotrypsin, and pancreatin) to simulate human digestion. Mass spectrometry was used to identify resistant proteins. This study found that fatty acids were needed to protect PAS6/7 (lactadherin) from digestion, and that MUC-1 resisted initial digestion by pepsin and some proteins also resisted subsequent digestion by trypsin (Le et al., 2012).

Chatterton, Rasmussen, Heegaard, Sørensen, and Petersen (2004) reported bovine lactadherin was stable to digestion at pH 4 and above, with bovine lactoferrin unhydrolyzed at pH 5.0 but hydrolyzed below pH 5.0. Typically results indicate similar vulnerability to digestion of MFGM proteins and other milk proteins.

The excretory products of protein metabolites are well documented, and the main metabolites of MFGM-WPC will be the same as for WPC.

2.3.2 Information on the toxicity of MFGM

The essential nature of MFGM in all mammalian milks, together with a long history of safe consumption of milk indicates a lack of toxicological concern. Safety studies identified by AFI in the scientific literature relate to the safety and tolerance of MFGM in formulations fed to animals and humans. Arla Foods Ingredients P/S is not aware of studies on MFGM that have investigated acute, short-term, and long-term toxicity and carcinogenicity, nor studies on reproductive toxicity, developmental toxicity, genotoxicity and immunotoxicity. Arla Foods Ingredients P/S does not consider these studies are required to establish the safety of MFGM in the context of incorporating the ingredient into FSFYC.

2.3.3 Potential allergenicity of MFGM

No new dairy proteins or components are introduced to FSFYC with the use of MFGM-WPC. All are present in bovine milk.

MFGM-WPC is manufactured from dairy streams (e.g. whey) that are already extensively and normally used for the manufacture of FSFYC. Additionally, the use of whole milk is common in the production of FSFYC. The manufacturing process for MFGM-WPC does not include any processing technologies or processing aids that change the chemical or physical properties of the protein components beyond that of standard dairy processing technologies. On this basis the potential allergenicity of MFGM is within the existing set of potential allergens from bovine milk to which young children consuming foods containing bovine milk derived ingredients will already be exposed to.

2.3.4 Safety of MFGM-WPC

2.3.4.1 Safety studies – preclinical

The literature search (Section2.1.2.2) to identify safety studies of MFGM-WPC in preclinical (animal) models failed to identify any studies specifically investigating the safety of MFGM in preclinical models. However, safety measures are routinely assessed in preclinical studies as a part of the good laboratory / clinical practice requirements to monitor growth, development, health and potential adverse outcomes related to interventions. A number of studies have reported safety outcomes in neonatal models; in rats (Brink, Gueniot, & Lonnerdal, 2019; Collins et al., 2022; Jiang, Du, Brink, & Lönnerdal, 2022; Moukarzel et al., 2018) and pigs (Berding et al., 2016; Fil et al., 2019; Zhang et al., 2023), no issues have been found for body weight gain, and no studies have noted any problems associated with toxicity or illness.

Studies of MFGM in preclinical models relevant to young children have not reported any safety outcomes, adverse effects on growth or safety concerns (Kodde et al., 2017; Teller et al., 2018).

2.3.4.2 *Safety studies – clinical*

Studies investigating the safety of MFGM-WPC have predominantly focussed on safety and tolerance of MFGM-WPC in infant populations representing a wide range of ethnicities (Billeaud et al., 2014; Hari, Ochiai, Shioya, & Katsuragi, 2015; Jaramillo-Ospina et al., 2023; Jaramillo-Ospina et al., 2022; B. Jiang et al., 2022). Those studies, together with a large number of other efficacy focussed studies in infants who consumed MFGM-WPC or other MFGM ingredients, some with follow-up studies through to up to 14 years of age, provide extensive evidence for the safety of MFGM-WPC as an ingredient in nutritional products (Abrahamse-Berkeveld et al., 2024; Colombo et al., 2023; Diéguez et al., 2022; Lazarte et al., 2021; Lazarte et al., 2023; Nieto-Ruiz et al., 2021; Timby et al., 2021).

Hari et al. (2015) evaluated the safety of a high dose of MFGM in a Japanese adult cohort and found no adverse effects of the intervention or safety concerns.

Studies in young children presented in Section 2.1.2.1 also report no safety concerns. No specific safety studies in young children were identified, safety and tolerance reported outcomes are a part of the overall study.

The significant body of evidence of no safety concerns from clinical trials across the lifespan (infant through adult) and ethnicities using MFGM-ingredients as the intervention provides direct evidence for the safety of MFGM-WPC.

2.3.5 Safety assessment reports prepared by international or national government agencies

Arla Foods Ingredients P/ S is not aware of any safety assessment reports by international or national government agencies.

In China the Chinese Society of Food Science and Technology produced a Scientific Consensus on Milk Fat Globule Membrane and Its Ingredients (Chinese Institute of Food Science and Technology, 2022) based on a literature review (1965 to January 2022). The review concluded that based on the extensive international use of MFGM ingredients globally, together with clinical trials reporting safety and tolerability there was no concern for the use of MFGM ingredients, and that MFGM ingredients are safe and well tolerated.

2.4 Information on the dietary intake of the nutritive substance

2.4.1 Food and food groups proposed to contain MFGM-WPC

This application is for the addition of MFGM-WPC to FSFYC as regulated under Part 2.9 Special purpose foods, of the Food Standards Code, specifically Standard 2.9.3 Formulated meal replacements and formulated supplementary foods Division 4 Formulated supplementary foods for young children.

2.4.2 Proposed levels permitted in FSFYC

The proposed permissible optional addition rate of MFGM-WPC in FSFYC is a maximum of 2.3 g per serve.

As the addition of MFGM-WPC as an ingredient cannot be quantified by analysis, sphingomyelin is proposed as the quantifying analyte by which to verify addition. Although there potentially is a low baseline level of SM in dairy-based FSFYC products, the levels proposed in the specification can only be achieved with the addition of MFGM-WPC as permitted. Sphingomyelin is the analytical metric proposed to quantify and regulate MFGM-WPC addition rates.

2.9.3—7 Compositional requirements for formulated supplementary foods for young children

2.9.3-7 (5) MFGM enriched whey protein concentrate (MFGM-WPC) may be used as a nutritive substance in a formulated supplementary food for young children at a maximum rate of 2.3 g/serving.

2.9.3-7 (6) When MFGM enriched whey protein concentrate (MFGM-WPC) is used as a nutritive substance in a formulated supplementary food for young children the total amount of sphingomyelin, both from added from MFGM-WPC, and naturally occurring, must not be more than 40 mg/serving.

2.9.3-8 Labelling of formulated supplementary foods for young children

2.9.3-8 (7) If MFGM-WPC is added, the label on a package of formulated supplementary food for young children may include words indicating that the product contains MFGM enriched whey protein concentrate (MFGM-WPC) and /or total SM.

2.4.3 Information on the likely levels of consumption of FSFYC

There is little data on the proportion of toddlers in ANZ consuming toddler milks (FSFYC) however a study of 12 -16 months old Australian children estimated 32% of the participants surveyed (n = 551) consumed a formula, median intake 441 g/day (IQR 258 – 618 g/day) and 78% consumed cow's milk (370 g / day [IQR 129 – 577]) (Byrne, Magarey, & Daniels, 2014).

Previous estimates of dietary intakes of FSFYC for determining the intake of new ingredients in FSFYC (Food Standards Australia New Zealand (FSANZ), 2009), have assumed FSFYC intake replaces all

milk, infant formula and follow-on formula consumed by children in the age range. This approach resulted in an estimated consumption of 423 g/day for Australian children aged one year, and 281 g/day for New Zealand children aged 1-3 years. The average consumption of FSFYC by Australian children aged 2-3 years was estimated to be 403 g/day. These amounts approximate one to two 200 mL serves of FSFYC per day (Food Standards Australia New Zealand (FSANZ), 2009). This data drew on national dietary studies in Australia (Australian Bureau of Statistics, 2011) and New Zealand[7].

The 2016 NZ Total Diet Survey (Ministry for Primary Industries, 2018) estimated the whole milk (3.25% fat) milk intake of NZ toddlers (1 – 3 years) to be 307 g/day, skim milk 18 g/day, flavoured milk 9 g/day, yoghurt 64 g/day, butter 4 g/day and cheese 10 g/day. Milk intake may be replaced with formula-type FSFYC, but it is unlikely other dairy foods would be substituted.

On this basis the approximation of up to 2 x 200 mL serves per day (Food Standards Australia New Zealand (FSANZ), 2009) remains a suitable model to estimate the intake of FSFYC in ANZ toddlers.

More recently Szymlek-Gay, Heath, Gibson, and Ferguson (2024) considered the role of toddler milks (FSFYC) in the diets of young NZ children to ensure adequate intakes of essential nutrients. Their recommendation was 10 serves of FSFYC per week (or about 2 cups (500 g per day)) of iron fortified toddler milk. Similarly, the 2021 Australian Feeding Infants and Toddlers Study (OzFITS 2021) which looked at nationwide survey of the feeding practices of children under 2 years, found 25% of toddlers had inadequate iron intakes (Netting, Moumin, Makrides, & Green, 2022), but did not report or consider the potential role of FSFYC.

2.4.4 Percentage of food group to which MFGM-WPC is proposed or the percentage of the market likely to use the nutritive substance

Products manufactured in ANZ under Standard 2.9.2 Division 4 FSFYC are normally “toddler milks”, however the standard does allow for other formulated foods for this age group.

The inclusion of MFGM-WPC in non-formula products under FSFYC in Australia and New Zealand is dependent on new product development initiatives of manufacturers and brand owners in the region. This may be aligned product development for export markets also. Possible products to which the addition of MFGM-WPC may be considered are more likely to be other dairy-based foods for children such as yoghurts. However, as this category outside of milk-based formula is practically non-existent. It is difficult to predict new products that may be developed in the future. Any NPD in this area would as a part of the project be expected to standardise the product and any MFGM=WPC addition within the constraints of this application, thoroughly understand the overall composition and compliance with the Code. Shelf life studies ar typically completed on new NPD products prior to commercialisation.

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[7] *New Zealand Food Safety Authority (2006) Bread and Toddler Milk Consumption - Omnibus Results [September/October/November 2006] DRAFT REPORT (not accessible).*

Several manufacturers have expressed an interest in providing MFGM-enriched FSFYC formula options for Australasian infants.

Introduced in June 2025 proposal P1066[8] – review of young child formula is in process, with a planned completion date of end 2027. This proposal is intended to revise and clarify Code provisions as they apply to young child formula (1-3 years) including regulatory definitions, composition, labelling and representation of products. Future potential recategorisation of products based in on an updated regulatory framework is not expected to increase the total number or volume of products available to or consume by young children in ANZ, rather just better define the categories.

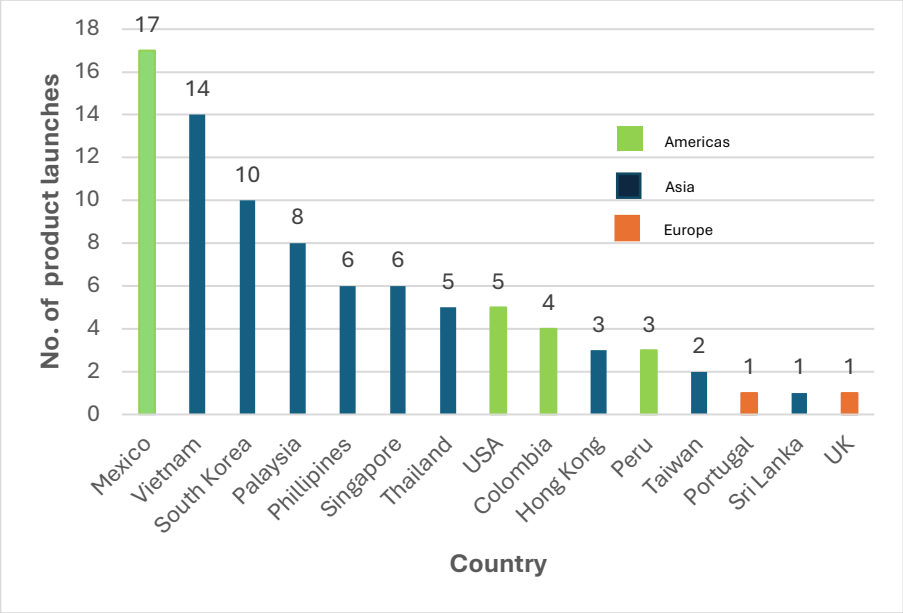
Arla Foods Ingredients P/S proposes that up to 25% of available FSFYC and if regulated separately Young Child Formula, in Australia and New Zealand may be MFGM enriched using MFGM-WPC within the next 5 to 10 years.

2.4.5 Information relating to the use of MFGM in other countries

The use of MFGM-WPC in FSFYC has been widely adopted in a large number of countries around the world (Section 1.7.2), and in addition MFGM in some FSFYC naturally elevated due milk source is highlighted in some products.

An independent market analysis (Innova Market Insights) was completed in April 2024 with data relating specifically to FSFYC equivalent product launches extracted in August 2024 (Appendix II). Between 2019 to April 2024 there were 78 product launches identified as having reference on pack to the presence of MFGM.

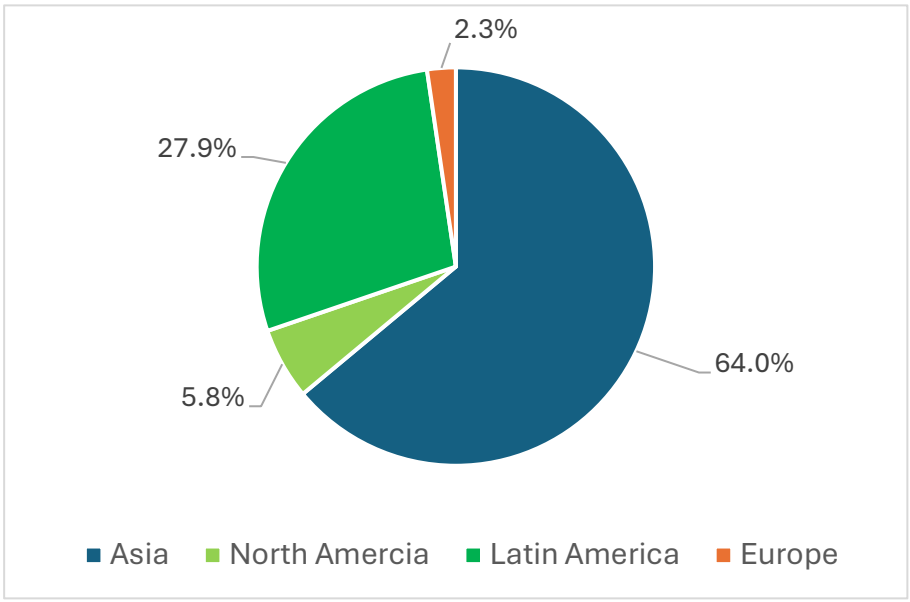
Figure 2-28 Annual number of FSFYC with MFGM reference launched (2019- Apr 2024)



[8] *https://www.foodstandards.gov.au/sites/default/files/2025-07/P1066_Administrative%20Assessment%20Report_0.pdf*

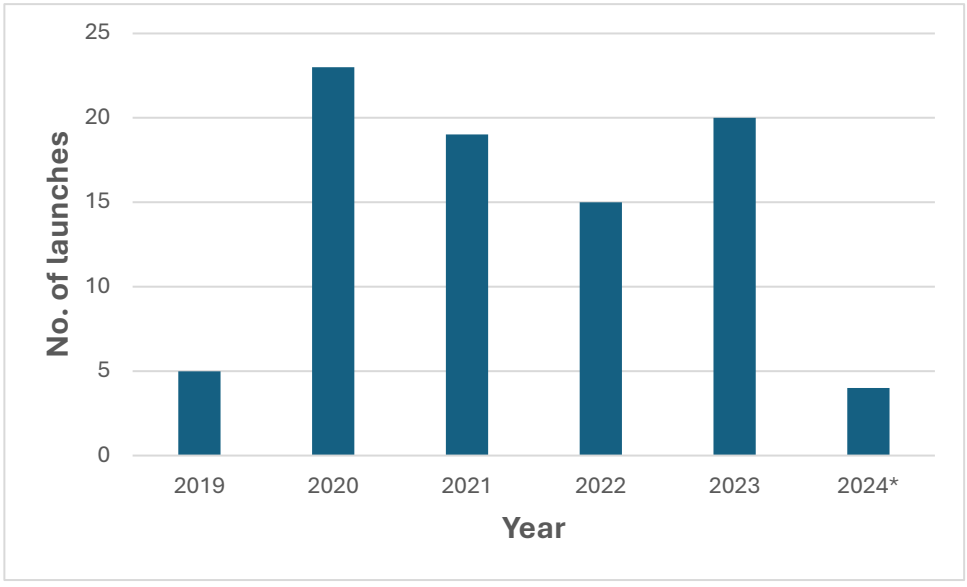
The regional distribution shows the Asia having the largest number of launches (64%) followed by the Americas (33.7%) (Figure 2-29).

Figure 2-29 Proportion of FSFYC products launched in global regions (2019 -2014) with reference to MFGM



Between 2020 and 2023 the number of product launches of FSFYC equivalent products globally was relatively static (Figure 2-30).

Figure 2-30 Annual number of FSFYC with MFGM launched globally



** to April 2024*

2.4.6 Information on likely current food consumption for foods where consumption has changed in current years

There have been no reported or observed significant changes in intakes of FSFYC in recent years. Dietary intake data is only collected in ANZ infrequently. Recent estimated intakes and recommendations are aligned with previous estimated intakes.

2.5 Information related to the nutritional impact of MFGM-WPC

2.5.1 Information related to the nutritional purpose of the use of MFGM-WPC

The nutritional purposes of the addition of MFGM-WPC to FSFYC include the contribution of protein together with providing a source of MFGM to restore levels of MFGM closer to that of whole milk. Based on the available evidence MFGM provides continued nutritional support for immune function in young children. This nutritional purpose constitutes the central justification for this application.

Other reported benefits of MFGM across the lifespan include neurodevelopment and cognition, improved neuromuscular function and physical performance, improved cardiometabolic health, encapsulation and delivery of bioactive substances (including control of fat globule size) and improved gastrointestinal health (O'Callaghan, McCarthy, & Carey, 2025; Wilmot, Miller, Patil, Kelly, & Jimenez-Flores, 2025).

2.5.2 Immune function in young children

Immune function is dependent on a number of factors including genetics, age, environmental factors, exposure to infection agents and allergens, the gut microbiota and diet. The influence of food on health and vulnerability to infection is well known and particularly critical for infants, young children and the elderly (Fragkou, Karaviti, Zemlin, & Skevaki, 2021; van Neerven, 2025), with food affecting the immune system via a number of mechanisms which include its influence on intestinal microbiota and the subsequent metabolites (Palmer, Bedsaul-Fryer, & Stephensen, 2024) (Figure 2-31). Food components interact with the intestinal epithelium and its critical barrier properties, directly with immune system cells in intestinal tissue which may initiate immune responses, and also systemically influence immune function throughout the body following the uptake and distribution via the bloodstream of small nutritional components (Wells et al., 2022). During early childhood, the gradual maturation of innate defence mechanisms and immune responses influence susceptibility to illness, effectiveness of vaccinations, potential development of allergies and overall child health (Pieren, Boer, & de Wit, 2022).

Following infancy, the major shifts in immune function in young children (1 to 3 years) involve the transition from an innate-like and tolerogenic immune system to a more mature, memory-generating adaptive immune system (Pieren et al., 2022). Key changes from infancy over this age range include (Pieren et al., 2022; Simon, Hollander, & McMichael, 2015):

- *The transition of innate-like features to adaptive memory*. In infancy, the immune system is dominated by innate-like T and B cells, which provide rapid but short-term protection against pathogens. These cells express toll-like receptors (TLRs) and produce cytokines like IL-8.

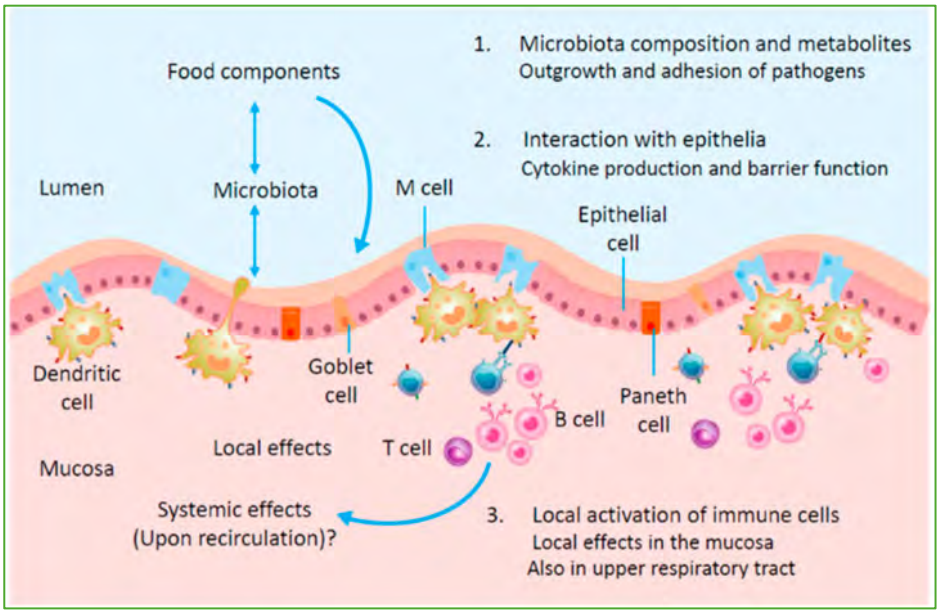
Over time, the immune system shifts towards generating memory T and B cells, which are essential for long-term immunity and vaccine efficacy.

- *T-cell Development*. Whilst neonatal T cells are skewed towards Th2 responses, which are less effective against certain pathogens, the Th2 bias diminishes with age, allowing for more balanced Th1/Th2 responses. CD8+ T cells in neonates are more effector-like and have a high turnover, but their ability to form stable memory populations improves with age.
- *Regulatory T Cells (Tregs)*. Infants have higher levels of Tregs to maintain immune tolerance and prevent excessive inflammation. Tregs levels decline as the immune system matures, allowing for stronger inflammatory responses when needed.
- *B-cell responses*. In infancy, B cells produce lower levels of antibodies with lower affinity, and these antibodies wane rapidly. By 12–48 months, B cells show improved class-switch recombination, somatic hypermutation, and germinal centre formation, leading to higher-affinity and longer-lasting antibodies.
- *Follicular Helper T Cells (TFH)*. TFH cells, which are critical for supporting B-cell maturation and antibody production, are less functional in neonates. Their functionality improves with age, enhancing the ability to generate robust antibody responses.
- *Impact of Maternal Antibodies*. Maternal antibodies provide passive immunity in infancy but can interfere with the infant's own immune responses. As maternal antibodies wane, the child's immune system becomes more independent and capable of responding to vaccines and infections.
- *Microbiome Influence*. Neonatal establishment of the microbiome plays a significant role in shaping the immune system. By 12–48 months, the microbiome is more stable, contributing to the maturation of the immune system.

These shifts collectively enhance the ability of young children to generate long-term immunity, respond effectively to vaccinations, and combat infections more robustly compared to infants. However the immune system continues to develop though the 1 to 3 year old age range and it remains immature relative to that of adulthood. The innate immune system provides immediate, general protection but is relatively immature in early childhood, leading to less effective initial responses to pathogens (Simon et al., 2015). The adaptive immune system, which creates targeted responses and immune memory, gradually improves with age. However, children's antibody responses after vaccines and infections may be weaker and wane more rapidly than those of older children and adults (Pieren et al., 2022).

Recent reviews (Fragkou et al., 2021; Palmer et al., 2024; van Daal, Folkerts, Garssen, & Braber, 2021; van Neerven, 2025; Wells et al., 2022) of the critical role of nutrition and food components in the development and maintenance of immune system function in young children have identified a range of nutrients and their specific functions, including MFGM (van Neerven, 2025; Wells et al., 2022) and its components.

Figure 2-31 Effects of food components on immune system function



(from Palmer et al. (2024))

2.5.3 MFGM and immune function across the lifespan

Clinical and nutritional studies across infancy, childhood, adulthood, through late adulthood have investigated the potential benefits of milk fat globule membrane-based ingredients, including effects on immune function (Raza, Herzig, & Leppäluoto, 2021; Sprenger et al., 2023). Clinical studies show immune-relevant effects of consuming MFGM across the lifespan – from infancy to old age.

The following is an overview of the immune-relevant effects of MFGM in infants and adults – age groups both prior to and following that of young children. No studies have been identified in older children (6 years and over) however the available evidence from studies in infants, as summarised below, would suggest an ongoing beneficial impact throughout childhood, regarding anti-inflammatory and immune-modulating properties. Normal immune function is a continuum across the life span.

2.5.3.1 Infants

In infant clinical studies, bovine MFGM—most often as MFGM-WPC or within multi-ingredient formulas—shows three convergent, immune-relevant effects: (1) a shift in gut ecology toward features more typical of breastfed infants (especially bifidobacteria-linked functions), (2) reduced pathogen adhesion/colonisation and lower infection burden, and (3) modulation of mucosal and cellular immune responses, including epithelial barrier maturation. Together these outcomes have been shown in clinical settings to shift formula-fed infants towards immune-relevant outcomes of breast-fed infants.

The recent review of evidence to support immune-relevant health effects in infants (Food Standards Australia New Zealand (FSANZ), 2025) concluded there was sufficient evidence to immune-relevant benefits for formula-fed infants—particularly via microbiome functional changes, pathogen-adhesion interference, and barrier/immune modulation.

2.5.3.1.1 Effect of MFGM on microbiota composition and function in infants

Infant studies that contributed to the evidence of microbiota composition and function included RCT's (He et al., 2019; Hanna Lee et al., 2021) comparing standard formula, MFGM-WPC formula, and a breastfed reference group. Formula containing MFGM produced modest compositional changes but clear functional shifts in the faecal metabolome (e.g., higher 1,2-propanediol; lower diversity akin to breastfed infants), suggesting microbiota activity moved toward a breastfed-like state despite limited taxonomic change. Chen et al. (2024) found that a formula containing MFGM plus a structured fat (1,3-dioleoyl-2-palmitoylglcerol (OPO)) showed greater early *Bifidobacterium* abundance compared to a standard formula, this outcome being more similar to that of the breast-fed infants. The observed abundance differences were no longer apparent between groups by 6 months of age, and the effect could not be solely attributed to the MFGM as the test formula also contained OPO. Chichlowski et al. (2021) found that compared to a standard formula, formula with added MFGM and lactoferrin (bLf) did not alter microbiome alpha diversity through to 4 months of age, however subtle but significant (p = 0.042) differences in beta diversity occurred. Specifically, *Bacteroides* species were more abundant in the MFGM + bLf group at 4 months of age. These changes, together with stool metabolite profiles observed are consistent with early-life coloniser activity and altered microbial metabolism. Zhao et al. (2022) analysed stool microbiota composition of breast fed and formula fed infants using high-throughput DNA sequencing and evaluated urinary

metabolome by NMR. Stool samples from the infants who were breastfed had a higher relative abundance of *Bifidobacterium* and a lower relative abundance of *Escherichia* than the formula-fed infants. The stool microbiome shifts were associated with urine metabolites changes. Three substances (lactadherin, sialic acid and phospholipid) which are key components of MFGM were significantly positively correlated to *Bifidobacterium* of stool samples from infants and stimulated the growth rate of *Bifidobacterium* significantly in a subsequent *in vitro* growth experiment with RNA analysis. Zhao et al. (2022) concluded MFGM modulated the infant gut microbiome and provided a plausible mechanism for supporting bifidobacterial growth.

2.5.3.1.2 Reduced infection in infants

Consistent with preclinical observations findings (Fuller, Kuhlenschmidt, Kuhlenschmidt, Jiménez-Flores, & Donovan, 2013; Sprong, Hulstein, Lambers, & van der Meer, 2012; Tellez et al., 2012; Zanabria, Tellez, Griffiths, Sharif, & Corredig, 2014), Timby et al. (2015) demonstrated a direct clinical signal for fewer common infections in early infancy with MFMG-WPC supplemented formula, compared to regular formula, and provided mechanistic plausibility via adhesion interference and microbiome changes. Through to 6 months, infants consuming MFGM-WPC supplemented formula showed reduced occurrence of acute otitis media and days using antipyretics, with rates approaching those of breastfed infants.

2.5.3.1.3 Modulation of mucosal and cellular immune responses in infants

Evidence to support the role of MFGM-WPC in direct immunomodulation and gut mucosal maturation is provided by preclinical studies. R. Jiang et al. (2022) showed orally ingested MFGM-WPC is partly resistant to *in vitro* (upregulated tight-junction genes and increased TEER) and *in vivo* digestion, activating multiple cellular signalling pathways to promote intestinal differentiation, increase expression of tight junction proteins, and strengthen the intestinal barrier. Using a neonatal rat model, Bhinder et al. (2017) showed MFGM-WPC formula normalised villus/crypt measures and increased Paneth/goblet cells toward breast milk controls—markers of innate defence and mucin production. Brønnum, Seested, Hellgren, Brix, and Frøkiaer (2005) showed human-milk–predominant gangliosides GD3/GM3 suppressed dendritic-cell cytokines (IL-6, IL-10, IL-12, TNF-α; GM3 narrower), a potential mechanism to prevent excessive immune activation in early life. In infants, Timby et al. (2015) observed lower levels of anti-pneumococcal IgG concentrations to some serotypes at 6 months in the MFGM-WPC group vs standard formula, providing *in vivo* support for MFGM-WPC having an immunomodulatory effect. Together the studies suggest MFGM-WPC may promote epithelial barrier integrity and temper antigen-presenting cell activity, consistent with supporting tolerance-oriented early-life immunity while maintaining host defence.

For infants, the evidence suggests MFGM-WPC supplementation provides plausible and increasingly substantiated immune-relevant benefits for formula-fed infants—particularly via microbiome functional changes, pathogen-adhesion interference, and barrier/immune modulation—though effects remain supplementary and do not replicate breastfeeding.

2.5.3.2 Adults

The potential for MFGM to have immunodulatory effects in adulthood, particularly older adulthood, has been evaluated in several clinical studies. Several studies investigated the immune relevant effect of burden of pathogen adhesion and infection, whilst others focussed on less direct immune-

specific measures, but none-the-less potentially immune function related. These include anti-inflammatory effects, physical function and the potential to positively influence gut microbiota, which is closely linked to immune function (Raza et al., 2021).

2.5.3.2.1 Studies investigating reduction in pathogen adhesion and lower infection burden in adults

Based on consistent preclinical findings (Fuller et al., 2013; Sprong et al., 2012; Tellez et al., 2012; Zanabria et al., 2014), Ten Bruggencate et al. (2016) investigated the effect of MFGM on resistance to diarrheagenic *E. coli* in healthy adults. Participants enrolled in the randomised, placebo-controlled, double-blind, parallel trial were randomly assigned to either the MFGM intervention (10 g of a MFGM rich milk protein concentrate, taken 2x daily; n = 30) or the placebo (10 g of sodium caseinate, taken 2x daily; n = 28) for 4-weeks. After 2 weeks all participants were challenged with live, attenuated diarrheagenic *E. coli* (10^{10} colony forming units (CFU)). Primary outcomes were infection-induced diarrhoea and faecal diarrheagenic *E. coli* excretion. Secondary outcomes were gastrointestinal symptoms [Gastrointestinal Symptom Rating Scale (GSRS)], stool frequency, stool consistency (Bristol Stool Scale (BSS)), and immune markers (Ten Bruggencate et al., 2016). Day 1, following the diarrheagenic *E. coli* challenge, the daily faecal weight of both groups increased significantly ($p < 0.001$), together with a significant reduction in faecal dry weight (< 0.001), with no differences between groups. Both faecal measures returned to baseline levels at Day 2 post challenge, with a non-significant ($p = 0.08$) trend for lower faecal output observed in the MFGM group. Faecal diarrheagenic *E. coli* was detected in all participants of both groups at Day 1 post challenge, with faecal loads reducing thereafter. There was no significant difference in the excretion of diarrheagenic *E. coli* between groups (Ten Bruggencate et al., 2016). Faecal calprotectin, a marker of intestinal inflammation, was measured to assess the immune response. Baseline, daily through to Day 4 post challenge and again at Day 14 assessments were completed. In both the control and MFGM groups a significant increase from baseline ($p < 0.001$) was observed, with levels returning to baseline by Day 14. No significant difference between groups was observed. Potential participants with evidence of previous diarrheagenic *E. coli* infection based on colonisation factor antigen II (CFAII) IgGor detectable faecal diarrheagenic *E. coli* counts were excluded because of likely resistance to the *E. coli* strain used in the study, and as CFAII IgG was used to evaluate the immune response to the challenge. At 14-days post challenge there was a significant ($p < 0.001$) increase in CFAII-specific IgG in both group (control group 354 ± 61 to 6279 ± 2068 $\log_2$ dilutions; MGFM group 375 ± 70 to 2912 ± 489 $\log_2$ dilutions), with no significant of difference between the groups. Following the challenge other secondary measures of faecal properties included a significant ($p< 0.001$) increase in Bristol Stool Score (no difference between groups); a significant ($p < 0.001$) increase in stool frequency in both groups at Day 1. By Day 2, the MFGM group had a significantly lower stool frequency (1.1 ± 0.1 stools/d MFGM group; 1.6 ± 0.2 stools/d in the control group; $p = 0.04$) than the control group; and a significant ($P < 0.001$) increase in diarrhoea in both groups at Day 1 post challenge. Although the number of participants reporting diarrhoea was less in the MFGM group (39%) than control (53%) it was not statistically significant. Consistent with this outcome, the Day 2 post treatment the GSRS diarrhoea scores of the MFGM group (1.1 ± 0.1 stools /day) were significantly lower ($p = 0.05$) than those of the control group (1.6 ± 0.2stools /day). Taken together the study outcomes suggest MFGM may have a small effect on improving resistance to diarrheagenic *E. coli* (Ten Bruggencate et al., 2016).

Ulfman et al. (2022) also investigated the effect of MFGM WPC on the effects of *E. coli* induced diarrhoea in otherwise healthy adults. In a double-blind, randomised, placebo-controlled study, n = 121 healthy male participants were randomly assigned to one of 3 treatments (control (a partially hydrolysed whey protein, n = 41); a MFGM rich WPC containing 7.0% PL (WPC high, n = 40); or a mix of the 2 containing 4.4% PL (WPC low, n = 41)), standardised to a similar calcium content and differing principally in fat and phospholipid (MFGM) content. Participants consumed the intervention (28 g in 180 mL water) twice per day (breakfast and evening meal) for 4 weeks. On day 14 of the study all participants consumed a dose (1 x 10^{10} CFU) of the attenuated diarrheagenic *E. coli* E392/75-2A. Primary outcomes were the rate of reduction in stool frequency between the 2 days following the *E. coli* challenge (days 15 and 16), and gastrointestinal diarrhoea symptoms (GSRS). Secondary outcomes evaluating stool consistency (BSS), gut comfort complaints, and further gastrointestinal symptoms (GSRS). Stool samples were collected at baseline and prior to the *E. coli* challenge to determine if the intervention products had any influence on gut microbiota as measured by 16S rRNA gene sequencing. Results of the study were analysed both by intent-to-treat (ITT) (n = 121) and per protocol (PP) (n = 110). Outcomes for the primary measures showed no statistically significant differences between the placebo, low-dose WPC, and high-dose WPC groups for stool frequency or diarrhoea scores. Although an overall significant effect of treatment dose on stool frequency ($p < 0.01$) was observed, the difference between the high-dose WPC and placebo groups suggested a somewhat delayed recovery in the high-dose group. However, this difference was not significant after correction for multiple testing ($p > 0.025$). Post hoc analysis of high responders (>1 stool on day 15) also did not show significant differences between the groups. Based on these results the dietary intervention with MFGM-WPC did not lead to faster recovery from *E. coli*-induced diarrhoea compared to the control. Outcomes for the secondary outcomes showed no significant effect in the PP analysis for any stool frequency-related secondary outcomes. In the ITT analysis, the percentage change in stool frequency between days 15 and 16 was statistically significant ($p = 0.035$) between intervention groups. However, sensitivity analysis did not confirm this result ($p = 0.112$). No significant effect was observed in the PP analysis for changes in GSRS diarrhoea scores, whilst the ITT analysis, the percentage change in GSRS diarrhoea score between days 15 and 16 was statistically significant ($p = 0.035$). Again, sensitivity analysis did not confirm this result ($p = 0.249$). No significant differences were found in stool consistency measures, including changes in maximum BSS scores or percentage changes in BSS scores between days 15 and 16. No robust or consistent effects were observed for secondary outcomes in the PP population, and the significant findings in the ITT analysis were not confirmed by sensitivity analysis. No safety concerns were identified with the study or interventions, no serious adverse events (SAE) were reported. Adverse events reported (n = 124 in total) primarily related to E. coli inoculation (n = 49); those unrelated to study (n = 53); those possibly related to investigational products (n = 22). Overall, the study supported the safe consumption of the MFGM-WPC intervention, however it did not yield significant benefits in reducing diarrhoea or altering gut microbiota in the context of *E. coli* infection.

2.5.3.2.2 Effect on MFGM on inflammatory responses in adults

Postprandial inflammatory responses are influenced by the fat content, particularly saturated fat, of meals (Deopurkar et al., 2010; Erridge, Attina, Spickett, & Webb, 2007), and preclinical studies have provided evidence MFGM can reduce inflammation (Dalbeth et al., 2010; Snow, Ward, Olsen, Jimenez-Flores, & Hintze, 2011).

Various effects of MFGM on the suppression of a range of postprandial inflammatory responses after consumption of high fat interventions was reported across a series of publications (Beals et al., 2019; Demmer et al., 2016; Rogers et al., 2017). Thirty-six (17 men and 19 women) overweight participants with 2 or more traits of the metabolic syndrome (overweight, high waist circumference, elevated blood pressure, plasma triglycerides, HDL-C, or fasting plasma glucose) completed a randomised, double-blind, four-way cross-over trial. Interventions included a high fat meal with and without MFGM and a whipping cream with and without MFGM. Washout periods of 1 -2 weeks occurred between test days, with each participant consuming each of the 4 interventions as randomised.

Demmer et al. (2016) reported on the acute postprandial inflammatory response of the high-saturated fat (palm oil) meal with or without MFGM. Participants consumed two isoenergetic high-fat test meals: one containing palm oil (PO) and the other containing palm oil with an added dairy fraction rich in milk fat globule membrane (PO + MFGM). The test meals were consumed in random order on separate test days, with a washout period of 1–2 weeks between meals to avoid carry-over effects. Blood samples and clinical measurements were taken at fasting and at 1, 3, and 6 hours postprandially. Beneficial immune-related effects of MFGM when added to a high-fat meal included a significant ($p = 0.011$) increase in the concentration of IL-10, an anti-inflammatory cytokine known for its atheroprotective effects, suggesting MFGM may help reduce inflammation in overweight and obese individuals. A significant ($p = 0.013$) reduction in Soluble Intracellular Adhesion Molecule (sICAM), which is associated with early events in atherosclerosis development was shown with the MFGM intervention. Lower sICAM levels indicate reduced vascular inflammation and atherogenic risk. Furthermore, in individuals with high baseline C-reactive protein (CRP) levels (≥3 mg/l), MFGM suppressed the insulin response and normalized inflammatory markers, reducing the negative effects of a high-fat meal.

Beals et al. (2019) reported on the effects of the high-fat whipping cream (WC) (with and without MFGM) aspects of the trial, providing additional immune-relevant measures to those previously reported by Demmer et al. (2016). In contrast to the findings of Demmer et al. (2016) no significant difference differ between the WC treatments (WC vs. WC+MFGM) were found for IL-10, sICAM, and along with a number of other inflammatory markers IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α, monocyte chemoattractant protein-1, and serum amyloid A (SAA). However, Beals et al. (2019) reported on additional immune-relevant measured. The study found that baseline cholesterol:HDL-cholesterol ratio (Chol:HDL) significantly modified the effect of MFGM on lymphocyte gene expression, particularly for CD14 ($p < 0.05$) and lymphotoxin beta receptor (LTBR) ($p < 0.005$). Postprandial IL-18 levels were significantly ($p < 0.05$) reduced, and lipopolysaccharide binding protein (LBP) levels were slightly higher following the WC+MFGM meal after adjustment for BMI, sex, baseline SAA, and baseline CRP. More complex measures of lymphocyte gene expression showed fold change in gene expression of 92 selected genes involved in inflammation and lipid metabolism as measured using TaqMan real-time RT-PCR. Specific genes such as soluble epoxide hydrolase (EPHX2), cluster of differentiation 14 (CD14), and lymphotoxin β receptor (LTBR) showed significant changes in expression. Taken together these outcomes suggest a potential protective role of MFGM in mitigating inflammation and atherosclerosis in high-risk individual through immune-mediated mechanisms.

Rogers et al. (2017) reported on the complete study design exploring the potential for postprandial changes in markers of bone turnover following the high fat interventions with and without MFGM. No significant effect of MFGM on bone turnover markers was observed.

Together these findings of this large study (Beals et al., 2019; Demmer et al., 2016; Rogers et al., 2017) suggest that MFGM has anti-inflammatory properties that may mitigate the harmful immune-related effects of high-saturated fat meals, particularly in individuals with elevated inflammation.

The effect of milk phospholipids (milk-PL) on lipid metabolism and cardiovascular disease risk factors, including immune-relevant markers, in overweight or obese men was investigated by Weiland, Bub, Barth, Schrezenmeir, and Pfeuffer (2016). Briefly, Weiland et al. (2016) conducted 2 double-blind parallel-group intervention trials in overweight or obese male subjects. In the first trial (trial 1), sixty-two men consumed milk enriched with either 2 g milk-PL or 2 g milk fat (control) for 8 weeks. In trial 2, fifty-seven men consumed 250 mL of milk containing either 3 g milk-PL or 2.8 g soya-PL daily for 7 weeks. The study found that milk-PL did not significantly affect immune-related parameters such as C-reactive protein (CRP), interleukin-6 (IL-6), soluble intracellular adhesion molecule (sICAM), or total homocysteine (tHcy) concentrations. Milk-PL supplementation showed modest benefits, including reduced waist circumference and lower γ-glutamyl transferase (GGT) activity, which may indicate improved liver function. However, it did not significantly impact lipid profiles, CVD risk factors and did not demonstrate beneficial effects on inflammatory immune-related parameters.

2.5.3.2.3 The impact of MFGM on physical function in adult populations

Studies in older adult populations have investigated the effect of an MFGM intervention on physical fitness and motor function, physical function, strength and frailty (H. Kim et al., 2015; Hunkyung Kim et al., 2019; Yoshinaka et al., 2018), with the studies showing positive physical benefits, along with safety and tolerance. Whilst these studies are not conventional immune-modulatory (infection) models, physical activity can impact (Lesnak, Berardi, & Sluka, 2023; Shao et al., 2021) and be impacted by immune factors (Nieman & Wentz, 2019).

H. Kim et al. (2015) included a range of haematological measures in a randomised, double-blind, placebo-controlled study in frail Japanese women (> 75 years), of which a range of immune-related factors associated with inflammation and muscle function were measured. Participants (n = 131) were randomly assigned to 1 of 4 groups (exercise + MFGM; exercise + placebo; MFGM only; placebo only). The MFGM was provided in supplement form (1 g pills, 6 per day for 3 months), the daily MFGM intake approximately 1 g per day (H. Kim et al., 2015). The Brain-Derived Neurotrophic Factor (BDNF) is a recognised immune-related protein and a key factor in maintaining neurological health. In this study MFGM alone did not significantly increase BDNF levels. However *post hoc* analysis showed exercise combined with MFGM significantly ($p < 0.05$) increased within-group BDNF levels from baseline to follow-up. Increases in BDNF may contribute to improved cognitive function, mood, and overall quality of life in frail elderly individuals (H. Kim et al., 2015).

Similarly, while MFGM alone did not result in any significant changes to myostatin levels, MFGM in combination with exercise significantly decreased myostatin levels within the group from baseline to post-intervention and follow-up. As a negative regulator of muscle growth, elevated levels of myostatin are associated with muscle atrophy and reduced muscle mass, which are key contributors

to frailty in older adult. A reduction in myostatin may help improve muscle growth and physical function contributing to a reversal of frailty and sarcopenia (H. Kim et al., 2015). Also related to muscle function the Insulin-Like Growth Factor Binding Protein 3 / Insulin-Like Growth Factor 1 (IGFBP-3/IGF-1) ratio was determined and shown to decrease only in the MFGM in combination with exercise group, while it increased in all other groups, including the MFGM-only group (H. Kim et al., 2015). The IGFBP-3/IGF-1 is an important ratio in aging populations as it is associated with muscle health and physical function, which are key factors in combating frailty (Theou et al., 2011).

Nakayama et al. (2025) studied the effects of MFGM-WPC and exercise on physical strength in young adults. No immune-related measures were evaluated, daily consumption of MFGM-WPC (0.2 g carbohydrate, 7.6 g protein, 1.7 g fat and 160 mg sphingomyelin, providing 46 kcal of energy per package) for 4 weeks, in combination with exercise (power training) significantly improved instantaneous physical strength, such as muscle strength and power, compared with placebo in healthy young adults. These results are consistent with outcomes observed in older (elderly) adult populations suggesting beneficial effects of MFGM as a part of a healthy lifestyle inclusive of exercise, together with confirming the acceptability and tolerance of MFGM through adulthood to old age.

2.5.3.3 Conclusion

Beneficial immune-related effects have been documented at both extremes of the lifespan, specifically during infancy and older adulthood. Accordingly, it is reasonable to propose that these immune-related effects operate along a continuum across the lifespan, rather than being strictly confined to discrete developmental windows. This expectation would include beneficial effects in young children. While some variation in magnitude and expression can be anticipated due to differences in diet, environment, lifestyle, and health challenges at various ages, the underlying mechanisms are unlikely to be entirely age specific.

2.5.4 Clinical trials in young children – MFGM and immune function

Three (3) clinical trials investigating outcomes related to immune function and regulation in cohorts that included young children were identified, with 1 study subject to subsequent sample analyses to evaluate potential mechanisms. Growth and development are a continuum, and potential nutritional benefits of substances can be expected to continue to perform across age and ongoing development.

An early study by Veereman-Wauters et al. (2012) investigated the effect of a daily MFGM intervention for 4-months on common infectious symptoms (fever, diarrhoea, cough) and gastrointestinal comfort (constipation) as primary outcome measures, together with evaluation of acceptability, safety, and behavioural regulation. Two hundred and fifty-three (n = 253) children were recruited from preschools in Flanders, Belgium to participate in the prospective, double blind, randomised placebo-controlled trial (1 November 2005 to 1 May 2006) (Veereman-Wauters et al., 2012). Participants (2.5 – 6 years) were randomly allocated to either the control group or intervention group and received either formulated chocolate UHT milk drink (200 mL/day) or a flavour and overall composition matched formulated chocolate UHT milk drink (200 mL/day) containing 500 mg of phospholipids from a dairy derived MFGM concentrate, respectively. Participants were required to consume the 200 mL allocated drink daily for 4 months (120 days). Overall, there were 58 spontaneous dropouts, a dislike of the product the main reason given. A further 13 participants were excluded for intake non-compliance (<80% of total trial days). Trial analysis was completed on a per protocol basis (n = 182), with the groups remaining evenly distributed. The control group, 76.4 % of original children allocated, had a mean age 4.3 ± 0.9 years, and the intervention group, 67.5 % of original children allocated, a mean age 4.3 ± 0.8 years. Primary outcome measures were incidence of fever (>38.5 °C), diarrhoea (more than three liquid stools per day), coughing (more than three bouts per day) and constipation (3 d without stools). Secondary outcomes included number of doctors' visits, number of school days missed because of illness, acceptability of the study products (amount left in the bottle (mL), medication intake and type of medication, safety of the intervention (any adverse event, need for hospitalisation (registered as a SAE)). The trial was also designed to enable evaluation of potential emotional and behavioural regulation that the MFGM intervention the product may have had. Daily outcome measures were recorded in a diary together with recording any visits outside of Belgium, and other remarks. At the end of the intervention period (after 120 d), a validated questionnaire to assess emotional and behavioural problems in 1.5- to 5-y-old children or the Achenbach System of Empirically Based Assessment (ASEBA) for older children were submitted to the parents and teachers of the children who completed the study in order to determine any effect of the intervention on behavioural regulation in healthy preschool children.

The study outcomes included confirmation that the addition of a MFGM concentrate to a formulated milk for young children is safe and well tolerated. Outcomes relevant to immune function and regulation showed a significant (p = 0.028) difference in the occurrence of days with fever (>38.5°C) observed between the control (2.60 ± 3.06 days [mean ± SD]) and intervention group (1.71 ± 2.47), with no significant differences in frequency of diarrhoea, constipation, coughing, doctors' visits, medication or days off school between the 2 groups. Further analyses where febrile episodes were categorised as short (<3 days) or long (> 3 days) duration showed that the statistically significant

difference between the MFGM intervention and control groups only remained significant for the short febrile events ($p < 0.032$). The potential immune-modulatory benefits observed, are plausibly contiguous with the 1-to-3-year age group. Veereman-Wauters et al. (2012) concluded, based on the reduction in febrile episodes, MFGM may have a clinically relevant regulatory effect on the immune system.

Participants in a randomised, double blind controlled study of Peruvian children consumed a complementary food for 6 months containing the recommended dietary allowance (RDA) of micronutrients and a protein source as either MFGM-WPC (Lacprodan®-MFGM-10, MFGM group) or skim milk powder (control group) (Zavaleta et al., 2011). The primary outcomes of the study were the efficacy of a 6-month intervention of a MFGM-fortified complementary food on the incidence of diarrhoea, anaemia and micronutrient status. The study site was Villa El Salvador, a peri-urban shanty town in Lima, Peru, and the study was run from 2004 through 2005. Diarrhoea was defined as greater than 3 loose stools or 1 bloody stool within 24-hours, and episodes separated by at least 2 diarrhoea-free days.

A total of n = 550 infants aged 6 to 11 months at enrolment entered the study, with n = 499 completing the 6-month intervention. Sample size was based on a power of 0.80 and an α–level of 0.05. Assuming an incidence (per child day) of diarrhoea of 0.0137 in the control group, 250 children per group followed for 6 months was required to allow detection of a 15% reduction in all diarrhoea types [redacted] Participants were randomly assigned to receive either the MFGM supplemented complementary food, or the control intervention.

Of the total n = 550 participants enrolled, 40% (n = 101) of those in the MFGM trial group (total n = 253) and 42% (n = 103) of the control group (total n = 246) were aged 9 – 11 months at time of enrolment (Zavaleta et al., 2011). For these older participants, the 6-month intervention period predominantly extended into the age relevant for young children. As dietary intakes and nutritional status change markedly during this period from infancy to young childhood, results were analysed for both the entire cohort, and the 6 – 8 and 9 – 11 months cohorts. Results based on age at enrolment were only reported where there was a significant difference ($p < 0.05$) between the cohorts. Where no significant difference was found results were presented for the entire cohort.

The protein content of the complementary food was met by the addition of MFGM-WPC, or for the control meal skim milk. For the MFGM supplemented meal (protein content 15.04 g/100 g) the addition rate of MFGM-WPC was 20.6 g/100 g. Based on the typical composition of MFGM-WPC each 20 g serve provided participant with 290 mg MFGM phospholipids, with 2 x 20 g serves per day providing a total of approximately 580 mg MFGM phospholipids.

A total of 1549 episodes of diarrhoea were recorded during the study, 750 in the MFGM group and 799 in the control group. Of the total cohort 45 participants (9%) did not have any diarrhoea episode (21 in MFGM group and 24 in the control group). Across the duration of the study a significant ($p < 0.05$) difference in the prevalence (mean (standard deviation) of diarrhoea (3.84 (4.38) % in the MFGM group versus 4.36 (5.33) % in the control group) was observed, i.e., the % of children in the MFGM group with any episode of diarrhoea was significantly less than that in the control group. There was no significant difference between groups in the duration of diarrhoeal episodes (MFGM 2.37 (1.51) days in the MFGM group and 2.36 (1.36) in the control group). The incidence of all episodes of diarrhoea per child per

year was 5.81 in the MFGM group and 6.38 in the control group; there was no significant difference between treatment groups for both the unadjusted and adjusted odds ratios (Zavaleta et al., 2011), or the incidence of severe diarrhoea (1.63 and 1.84 episodes per child per year in the MFGM group and the control group, respectively). In longitudinal multivariate regression modelling for diarrhoea outcomes, adjusting for initial anaemia (haemoglobin < 105 g/L) and potable water facilities, there was a 46% reduction in episodes of bloody diarrhoea in the MFGM group as compared to the control group, with an odds ratio (OR) of 0.54 (0.31–0. 93) ($p < 0.025$). In both groups a number of different serotypes of diarrhoeagenic *E. coli* (totalling 45% of all pathogens), and *Campylobacter* (25%) were isolated, with no significant difference between groups. The frequency of rotavirus diarrhoea (8.0% in the MFGM group; 9.55% in the control group) was not significantly different between groups, nor was the incidence of parasites (*Giardia lamblia* and *Blastocystis hominis*). Zavaleta et al. (2011) concluded that the significant reduction in the likelihood of bloody diarrhoea and prevalence of acute diarrhoea observed in the MFGM study cohort may result from a direct effect of the MFGM on gut microflora, to enhanced immune function, or by components functioning as a decoy for pathogens.

To explore the mechanism(s) behind these potential immunomodulatory outcomes, H. Lee, Zavaleta, et al. (2018) investigated the effects of the study treatments on the serum metabolome and cytokine levels, as markers for systemic immune responses, using serum samples collected at baseline and completion of the original study (Zavaleta et al., 2011), and when participants were not affected by diarrhoea. The study aimed to determine how the MFGM complementary food altered metabolic pathways, that together with changes in cytokine levels, micronutrient status and anthropometric measures may provide mechanistic information as how MFGM may enhance immune function and possible beneficial changes in gut microbiome.

Analysis of the serum metabolome showed that several metabolites were significantly (unadjusted $p < 0.05$) or approaching significance (unadjusted $0.05 < p < 0.10$) affected by MFGM supplementation; leucine, lysine, valine, alanine, arginine, proline, tyrosine, ornithine, urea, aspartate, choline, mannose, pyroglutamate, and taurine, which increased in MFGM-supplemented participants, as well as acetone, 3-OHB (3-hydroxybuty-rate), phenylalanine, and trimethylamine-N-oxide (TMAO), which decreased in the MFGM-supplemented group. Significance of diet effect was adjusted for sex.

The effect of the MFGM supplement appeared to be stronger in females for some metabolites (isoleucine, leucine, lysine, threonine, valine, alanine, arginine, proline, tyrosine, ornithine, urea, choline, pyroglutamate, and taurine, which were higher, and 3-OHB, acetone, and propylene glycol, which were lower), with moderate (0.28–0.43) to strong (>0.43) effect sizes. In male participants consuming the MFGM supplement, fewer significant or near significant changes were observed in metabolites, however overall, the trends were similar. Significantly higher levels of the bacterial choline metabolite TMAO were observed in the control group post intervention, relative to the MFGM group (unadjusted $p < 0.001$, effect size = 0.75).

Irrespective of the treatment group, the levels of all 10 cytokines analysed decreased from baseline to completion of the study (H. Lee, Zavaleta, et al., 2018). Because of the large inter-individual variation in cytokine levels, data was analysed looking at changes from baseline for everyone to determine fold change (FC) values where a FC of 1 means no change in cytokine level. On this basis interleukin (IL)-2 levels decreased more in the MFGM-supplemented group (median FC value: 0.457) compared with the control group (median value: 0.609) (unadjusted $p = 0.004$, Benjamini–Hochberg

false discovery rate (B-H FDR) adjusted p = 0.039; Mann–Whitney U-test). Decreases in IL-10 levels in the MFGM group were also observed but did not reach significance. To investigate the patterns in cytokine profiles, the dominance of T-helper 1 (Th1; cell-mediated) activity or T-helper 2 (Th2; humoral mediated) activity was estimated by the Th1/Th2 ratio defined as the ratio of Th1 (e.g., interferon-γ (IFN-γ) and IL-2) to Th2 cytokines (e.g., IL-4, IL-5, and IL-10). The Th1/Th2 ratio of the MFGM group decreased from 0.58 ± 0.05 to 0.46 ± 0.08, showing a possible shift toward Th2 dominance. In contrast, the Th1/Th2 ratio of the control group increased from 0.59 ± 0.06 to 0.66 ± 0.06, which was significantly different from the MFGM group at the final time point (unadjusted p = 0.007, B-H FDR adjusted p = 0.015; Mann–Whitney U-test)(H. Lee, Zavaleta, et al., 2018). Further analyses were completed to determine if significant correlations between the serum metabolome, cytokine profiles, and micronutrient status (including serum haemoglobin, folate, zinc, ferritin, and vitamin B_{12}) exist. No metabolites correlated with TMAO ($\rho < 0.30$), but vitamin B_{12} and IL-6 levels were negatively correlated ($\rho = -0.42$ and -0.41, respectively). Changes in bacterial metabolites may also reflect changes in the gut microbiota that may have been influenced by MFGM in the diet. Interestingly, IL-2 was inversely correlated with the concentrations of several amino acids and related metabolites. Positive correlations between vitamin B_{12} and IL-5 ($\rho = 0.40$) or IFN-γ ($\rho = 0.34$) were also observed. This positive correlation between IL-5 and vitamin B_{12} status agrees with previous findings in mice suggesting vitamin B_{12} deficiency caused a cytokine shift toward a Th2 response (Funada et al., 2001). Serum zinc had a weak negative correlation with IFN-γ ($\rho = -0.30$). The decrease in Th1 response observed in the MFGM group was mainly attributed to the significant decrease in IL-2 concentration, whereas decreased Th2 response in the control group was mainly due to a reduction in IL-5.

Based on these outcomes, H. Lee, Zavaleta, et al. (2018) concluded there are two possible pathways through which MFGM functions: (1) providing bioactive compounds to support intestinal maturation that improves mucosal absorptive capacity and immune systems; and/or (2) modulating gut microbial structure and/or function to impact systemic immunological and metabolic pathways. Both support the relationship between MFGM consumption and immune function in this age group through to 17 months of age.

Poppitt et al. (2014) investigated the potential of a MFGM derived complex milk lipid (CML) to prevent rotavirus infections and diarrhoea in young Indian children. Acute gastroenteritis is a leading cause of post-natal under-five mortality in India, with rotavirus the most common cause. Between 2011-2013 in India, rotavirus caused annually an estimated 78,500 deaths, 872,000 hospitalizations, and over 3.2 million outpatient visits in children <5 years of age. This translates to that by 5 years of age, 1 in every 334 – 356 Indian children died from rotavirus diarrhoea, 1 in every 22 – 45 children will be hospitalized, and 1 in every 6 – 12 children will have visited an outpatient clinic for rotavirus diarrhoea (John et al., 2014). In a randomised, double-blind, controlled trial, 450 young children (aged 8 to 24 months at recruitment) were enrolled at 3 study centres in Northern India. Based on an assumption each child would experience at least 1 incidence of diarrhoea (all cause (ACD)) over the 12-week study period, the power calculation identified that a total of 300 infants (150 in each group) were required to achieve a power of 90% and a significance level of 5% for comparing the two groups (CML and control). A difference of 38% of the within-group standard deviation (SD) was considered meaningful. Allowing for lower-than-predicted diarrhoea incidence, noncompliance, or dropout, the sample size was increased to 450 infants. The trial ran from December 2009 to September 2010. The peak hospitalisation period for severe RVD is October to December (Poppitt et al., 2014). Compared

to the predicted rate of ACD (1 episode per child over the 12 week trial i.e. 450) only 110 episodes occurred (62 in the CML group and 48 in the control group) of which 10 were RVD (CML, n = 4; control, n = 6) (no significant difference)), with the duration 9 days in the CML group versus 23 in the control group. Of those that did have RVD the mean duration was significantly less in the CML group (2.3 ± 0.5 days) compared to the control group (3.8 ± 1.3 days, p = 0.03). At the end of the trial only 3 of the 450 stool samples analysed were identified as RV+ (<1%; CML, n = 2; control, n = 1). With only 80/450 children (18%) experiencing ≥ 1 episode of diarrhoea during the trial, this unexpectedly low rate of diarrhoea and RVD limited the ability of the researchers to fully and reliably assess the efficacy of CML in preventing RVD (Poppitt et al., 2014).

2.5.4.1 Conclusion

Taken together these studies provide evidence for the potential benefits of MFGM-containing foods on immune-relevant function in young children aged 1 to 3 years. These studies which cover and are contiguous to the 1-to-3-year age range demonstrate reduction in the prevalence and severity infection-related incidences. Furthermore, they are further supported with mechanistic data providing biological plausibility for potential for immune modulation around 12 – 18 months. Research across the lifespan further reinforces the immune-related benefits of MFGM, and its long history of safe use and tolerability in vulnerable populations—including infants and the elderly—supports the reasonable conclusion that MFGM will be safe and well tolerated in young children. Taken together, these findings provide a strong rationale for the inclusion of MFGM-WPC in FSFYC to deliver additional immune-related benefits in the 1 to 3 year old age group.

Table 2-30 Intervention studies with MFGM-ingredients in young children

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of outcomes	Study limitations
Veereman-Wauters et al. (2012)	To evaluate the acceptability, safety, effect on intestinal comfort (constipation), common infectious symptoms (fever, diarrhoea, cough), and emotional & behavioural regulation of a 4-months daily intake of 200-mL formula with or without enrichment of MFGM	Prospective double-blinded randomised controlled trial	Belgium	Healthy children 2.5-6 years of age. n = 253 children were included. n = 58 dropped out (authors speculate mainly due to not liking the milk product). n = 13 excluded due to low intake of the formulated milk. PP analysis was conducted on 182 children (97 girls, 85 boys). Mean age of MFGM group 4.3 ± 0.8 years of age; control 4.3 ± 0.9 years, at enrolment.	Subjects received a daily amount of a 200-mL formulated chocolate milk (Society Lactel, Laval, France) without MFGM (placebo group) or a 200-mL chocolate formula milk enriched with 500 mg of total phospholipids (PL) with the addition of 2.5% of INPULSE (MFGM enriched dairy ingredient) (intervention group). Control group chocolate milk contained 30 mg total PL per 200mL serve. Duration: Daily, for 4 months	Significant difference ($p < 0.028$) in number of days with fever: control (2.60 ± 3.06), intervention group (1.71 ± 2.47). When analysed on duration of the fever (short < 3 days & long > 3 days) the significant difference ($p < 0.032$) was only observed for short febrile episodes. Analysis of prevalence also showed a significant ($p < 0.002$) effect on fever after 6 weeks of the intervention. No significant difference between groups for diarrhoea, constipation, coughs, doctors' visits and days absent from school. No adverse events reported that lead to study discontinuation by participants.	Exploratory study to determine the ability to observe potential emotional and behavioural effects of MFGM. No emotional & behavioural evaluation completed prior to the intervention.
Zavaleta et al. (2011) [redacted]	To evaluate the efficacy of a milkfat globule membrane (MFGM) – enriched protein fraction in a complementary food, on diarrhoea, anaemia, and micronutrient status	Randomized, double-blind controlled study.	Peru	Healthy term infants aged 6-11 months with a birth weight ≥2500 g, primarily BF. Total enrolled n = 550 (290 males and 260 females). MFGM group: n=277 control group: n= 273 Cohort classified by age at recruitment: 6 – 8 months (MFGM n= 152; control n= 143), and 9 – 11 months (MFGM n = 101; control n = 103). At the end of the study total sample sizes remaining (91%) n=	Complementary food (40 g/d) divided into 2 x 20 g servings Control group: complementary food with skim milk (n=246) MFGM group: complementary food enriched with MFGM-WPC (Lacprodan®MFGM-10) (n=253) Per 20 g serve: • 4.1 g MFGM-WPC • 290 mg phospholipids from MFGM-WPC • 0.33 g MFGM proteins from MFGM-WP Total daily intake of PL from MFGM intervention ~580 mg per day	The primary outcome was diarrhoea morbidity. No difference was observed between the groups in the incidence of diarrhoea or as a function of age at recruitment, but global prevalence of diarrhoea was significantly lower in the MFGM group (3.84%) compared with the control group (4.37%) ($p<0.05$). The prevalence was significantly lower in participants enrolled at 9 – 11 months and were not anaemic at entry. Overall, the mean number of diarrheal episodes (2.96 MFGM group; 3.25 control) was not significantly different. Not difference between age grouping at entry was observed. In girls the mean number of episodes was significantly lower ($p = 0.049$) in the MFGM group (2.78) versus the control	Age of infants recruited (between 6 and 11 months of age), not all met the age requirement for "young children" (≥1 year) during the study.

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of outcomes	Study limitations
				499: MFGM group: n=253 Control group: n=246 Reasons for dropout included: refused to continue, moved, dislike product, mothers work and final evaluation not completed.	Age groups 6 - 8 months and 9 - 11 months analysed separately as dietary intake and nutritional status change during this period. Where no significant differences between age groups, results for total cohort were reported. Duration: 6 months (2 serves per day)	group (3.43) but this was not observed in boys, or haemoglobin level at entry. Consumption of the MFGM test product reduced episodes of bloody diarrhoea when adjusting for anaemia and potable water facilities as covariates (odds ratio 0.54; 95% confidence interval 0.31–0.93, p = 0.025). There was no significant difference between groups in pathogens detected during diarrhoea episodes (*Salmonella, Shigella,* diarrheagenic *E. coli,* Enterotoxigenic (ETEC), Enteropathogenic (EPEC), Shiga toxin-producing (STEC), Enteroinvasive (EIEC), Enteroaggregative (EAEC), and Diffusely adherent *E. coli* (DAEC), *Vibrio cholerae, Campylobacter*, rotavirus and parasites (*Giardia, Ascaris*). There were no overall differences between groups in anaemia, serum ferritin, zinc, or folate.	
H. Lee, Zavaleta, et al. (2018)	To investigate potential of alterations in metabolic pathways to enhance immunity and reduce infection-related diarrhoea in 6–17-month-old Peruvian children.				Analysed the serum metabolome and 10 cytokine levels (as markers of systemic immune response) from blood samples collected prior to and following the study, at times when the children were no affected by diarrhoea.	MFGM significantly affected a range of metabolites and pathways over the 6-month intervention period. Increased levels of essential and non-essential amino acids were observed in the MFGM group, particularly in females. Urea cycle intermediates tended to increase in the MFGM group. Ketone body metabolites, decreased in the MFGM group. Decreased levels of TMAO (trimethylamine-N-oxide) in the MFGM group suggest functional changes in the intestinal microbiota. A significant decrease in IL-2 levels I MFGM group FC 0.457 vs. 0.609 (control) Unadjusted p = 0.004; Benjamini–Hochberg false discovery rate (B-H FDR) adjusted p = 0.039 (Mann–Whitney U-test) was observed.	

Reference	Objective (s)	Study design	Country	Study population, age at baseline and number	Study groups and intervention	Summary of outcomes	Study limitations
						Th1/Th2 ratio decreased in MFGM group 0.58 ± 0.05 to 0.46 ± 0.08 (indicated shift toTh2) versus increase in Control group 0.59 ± 0.06 to 0.66 ± 0.06 unadjusted p = 0.007; B-H FDR adjusted p = 0.015 (Mann–Whitney U-test). IL-10 showed greater decrease in MFGM group (median FC: 0.433) compared to the control group (median FC: 0.608) (Unadjusted p = 0.108; B-H FDR adjusted p = 0.540 (not significant). A significant shift toward Th2 dominance which may reduce aggressive immune responses, improve intestinal health, lower systemic stress and potentially reduce infection.	
Poppitt et al. (2014)	To evaluate the acceptability, safety, and efficacy of a MFGM derived high-ganglioside complex milk lipid (CML) in preventing rotavirus (RV)infection and diarrhoea in children aged 8 to 24 months in northern India.	Prospective double-blind, randomised controlled trial	Northern India (3 study sites)	Young children aged 8 to 24 months at recruitment; N = 450 (n = 284 boys; n = 166 girls) Average age (± SD) CML group 15.4 ± 4.6 months; control 15.6 ± 4.5 months. Each trial group: n = 225; (CML (135 boys, 90 girls) and control (149 boys, 76 girls)). Each study site enrolled and randomised 150 children. No (0%) participants had rotavirus vaccination (exclusion criteria). Both groups were analysed on an ITT basis (n = 225), however int eh CML group 2 were lost to follow-up and 11 discontinued the intervention, and in the control group 1 lost to follow up and 19 discontinued.	CML group: 2 g CML + 3 g whole-milk powder in 1 glass of fresh milk Control group: 5 g whole-milk powder in 1 glass of fresh milk Treatments given once daily. Parental diaries to record episodes and severity of participant diarrhoea, AE, compliance, health etc. daily. Stool samples to test for RV at each episode. Study duration: 12 weeks. Study ran between December 2009 and September 2010.	Trial confirmed the acceptability and safety of long-term CML in 8- to 27-month-old children. Similar rates of AE reported in CML (41% and control (46%) groups with majority mild and not related to intervention. No SAE in CML group. Growth of both groups similar. Very low prevalence of diarrhoea throughout trial • Total episodes of all-cause diarrhoea (ACD): 62 in the CML group vs. 48 in the control group. • Total episodes of RVD: 4 in the CML group vs. 6 in the control group. • Total days of RVD: 9 days in the CML group vs. 23 days in the control group. The mean duration of RVD was significantly lower in the CML group (2.3 ± 0.5 days) compared to the control group (3.8 ± 1.3 days, p = 0.03). At baseline, 20 stool samples tested positive for rotavirus (RV+), but only 2 infants had symptomatic diarrhoea. At 12-weeks only 3 (of 450) stool samples tested positive for rotavirus (RV+), indicating a >80% decrease in prevalence.	The overall low incidence of RV compromised ability to assess efficacy of CML on RV infection rate and severity.

2.6 Information related to the potential impact on consumer understanding and behaviour

2.6.1 Information to demonstrate the level of consumer awareness and understanding of MFGM in FSFYC products

Whilst manufacturers and brand owners of FSFYC products are aware of MFGM and its benefits as it has been used for some years in export products, this is a new ingredient for consumers in Australia and New Zealand. It has not previously been used as a dietary supplement and therefore expected awareness is anticipated to be currently low. If permitted for use, consumers (as in caregivers of young children consuming FSFYC) may seek information on the benefits of adding MFGM (from MFGM-WPC) to FSFYC. In countries, such as the USA, where MFGM enriched ingredients may be added to FSFYC-type products, brand owner websites, together with product labelling often provide top level information providing consumers with a simple understanding of the ingredient benefits. A sample mock-up of a front of pack and information flyer that may be representative of a hypothetical FSFYC brand are shown below (Figure 2-32 and Figure 2-33).

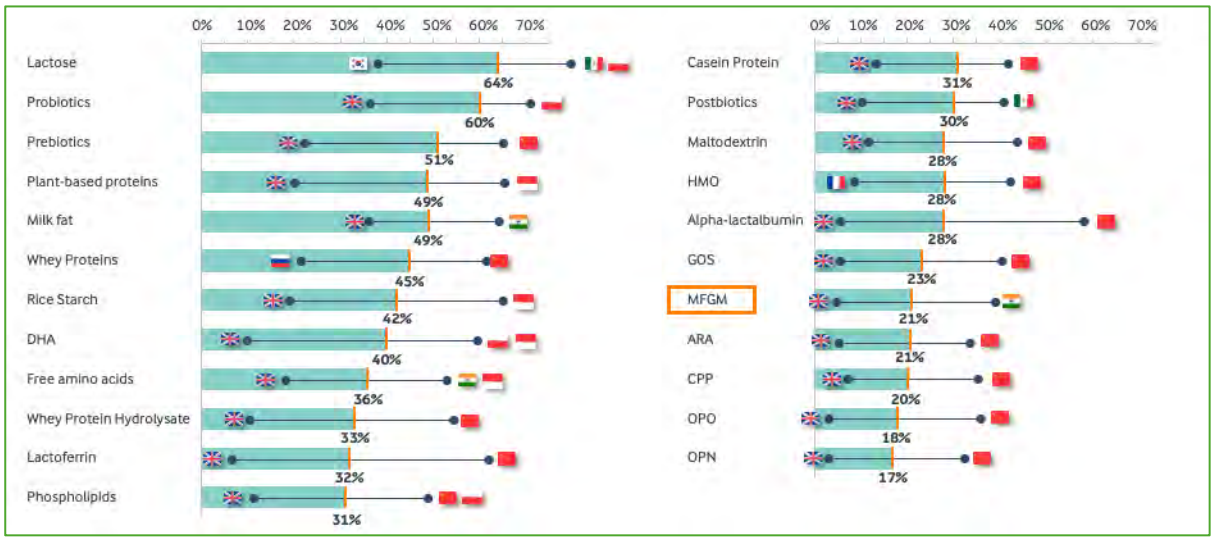
Figure 2-32 Mock up pack shot of hypothetical FSFYC containing MFGM-WPC

Figure 2-33 Mock up information flyer of hypothetical FSFYC containing MFGM-WPC

No specific consumer awareness research on the use of MFGM-WPC or MFGM in FSFYC is available in Australia and New Zealand.

Market research completed in 2021 “the Mum Survey” on behalf of AFI was conducted across 11 countries to gain insights into the perception and purchase behaviour of formulas for infants and young children. The college educated mothers surveyed (n = 7600) were 18-45 years of age, ad had children aged 0 to 4 years old, or were pregnant, at the time of the survey. The survey did not include ANZ. In terms of awareness and knowledge of MFGM as an ingredient 23% of respondents were aware of it, ranking between arachidonic acid (ARA) and casein phosphopeptide (CPP) (Figure 2-34). Of note was that in general all ingredients were significantly more well known in China compared to the rest of the countries in the study (UK, France, South Korea, Germany, USA, Indonesia, Poland, Russia, Mexico, India).

Figure 2-34 Overall awareness of MFGM in formula for infants and young children



Of those who were aware of or had knowledge of MFGM 35% of respondents indicated they would prefer a formula that contained added MFGM and 44% indicated it would not affect their product choice (Figure 2-35).

Figure 2-35 Purchase preference based on presence of ingredient

Knowledge (Figure 2-36) and preference for MFGM as an ingredient (Figure 2-37) was further explored by country, with changes in responses since an earlier similar survey completed in 2018 noted.

Considerable country differences in knowledge and preference for MFGM as an ingredient in formula are apparent.

Figure 2-36 Knowledge of MFGM by country

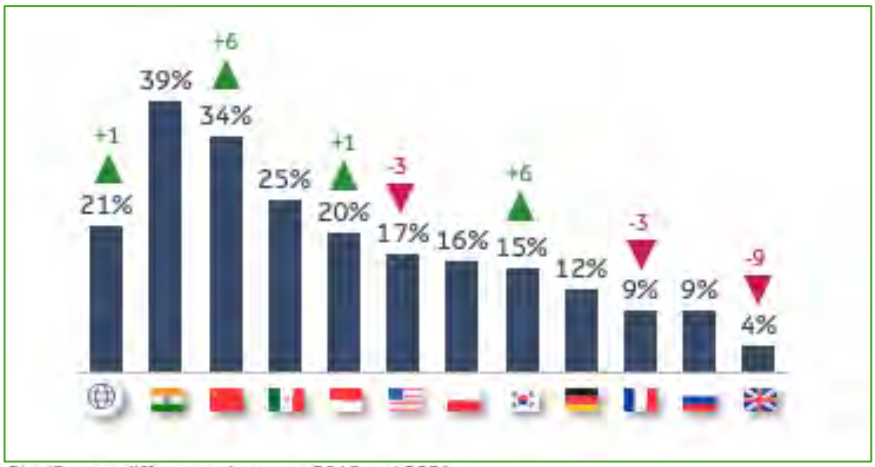
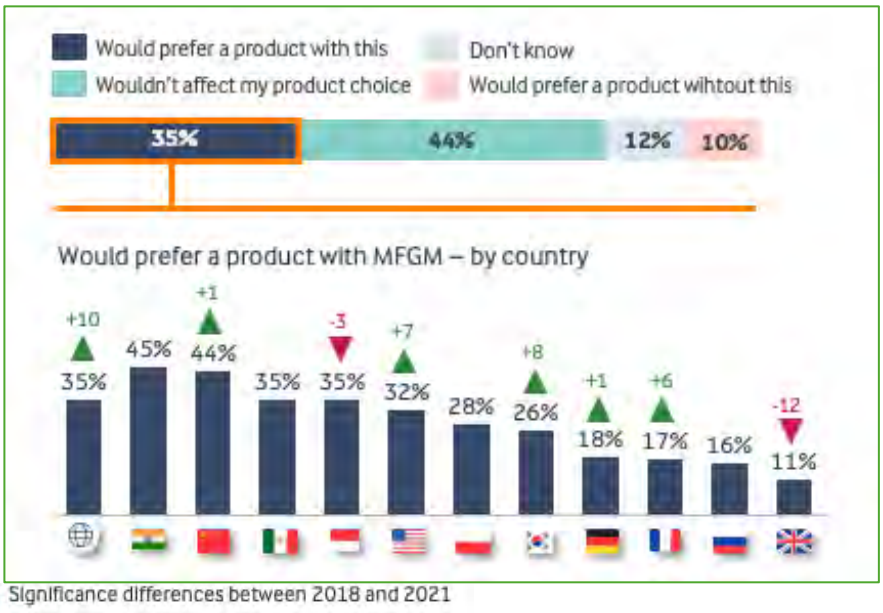


Figure 2-37 Preference for MFGM in formula by country



[redacted]

[redacted]

In the USA recent product releases have drawn attention to MFGM in general foods for young children (Figure 2-39), reflecting a growing consumer awareness of MFGM. In the product range developed under the Brainiac brand, buttermilk has been added to provide the MFGM.

Figure 2-39 Foods for young children new product releases (USA)

https://brainiacfoods.com/products/neuro-blueberry-carrot-blend
accessed 6th February 2025

Innovation in the product categories is now active in the EU with AFI raising industry awareness of the benefits of milk fat globule membrane (MFGM) beyond the infant formula category. The initiative follows confirmation that MFGM is not classified as a novel food. The decision, made by the Danish Veterinary and Food Administration, applies across the whole of the EU and allows MFGM to be declared clearly on products for age groups across the lifespan.

New concepts demonstrating its potential in functional nutrition products for adults and children include a squeezeable cheese, a UHT drink for children and a high-protein drinking yoghurt (adults) together with three easy-to-implement recipes demonstrate how MFGM can help create nutrient-rich "mini-meals" for toddlers: a drinkable fruit yoghurt with a straw, a squeezable smoothie, and an instant porridge mix [9] (Figure 2-40).

Figure 2-40 New MFGM containing product concepts

2.6.2 Information on the actual or potential behaviour of consumers in response to the proposed use of MFGM-WPC in FSFYC

ANZ products that contain added MFGM-WPC will have the addition listed as an ingredient in the ingredient list (Section 0). As a nutritive substance, the nutrition information panel may include the typical level of MFGM-WPC added and may also include sphingomyelin as the quantitative marker of the presence of MFGM-WPC. Parents who have chosen to feed FSFYC to their toddlers and are aware of MFGM may choose a formula containing MFGM and thereby replace a similar formula not containing MFGM. The choice of FSFYC containing MFGM-WPC will most likely be a substitution option, replacing a similar FSFYC that does not contain added MFGM-WPC. No nutritional concerns are known with the use of this product. All FSFYC sold in Australia and New Zealand must meet the regulatory standards defined for them.

The presence of MFGM-WPC in FSFYC may be noted on pack (Standard 1.2.7-13) and may be of interest to caregivers who are interested in product composition and nutritional components when making a choice in FSFYC selection.

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9 *https://www.arlafoodsingredients.com/about/press-releases/not-just-for-infants-arla-foods-ingredients-highlights-benefits-of-mfgm-throughout-life/*

Arla Foods Ingredients P/S does not anticipate that addition of MFGM-WPC to FSFYC will change overall consumption of FSFYC, rather, it will provide consumers with more choice. Parents opting to switch to or choose the FSFYC containing MFGM-WPC do so as a consumer choice option.

[redacted]

[redacted]

[redacted]

2.6.3 Information to demonstrate the consumption of foods containing MFGM-WPC will not adversely affect any population groups.

There is no evidence to support the addition of MFGM-WPC will adversely affect young children consuming FSFYC. The protein composition of FSFYC is regulated to ensure a minimum protein content (min 2.5 g protein per serve[10]) and whilst there is no maximum protein content set, elevated protein levels are unlikely to be found in these products due to a combination of formulation constraints to meet energy levels, cost to manufacture limitations, and general competitor comparison. FSFYC are a supplement to a normal diet, with feeding guidelines typically 1 – 2 servings per day. This means for those young children consuming FSFYC the exposure is typically limited. The addition of MFGM-WPC to FSFYC will not change the gross composition or energy of FSFYC products as it will replace other sources of protein and fat currently in the formulation. Information on the safety of MFGM-WPC is detailed in Section 2.3. In summary, MFGM-derived components have a long history of safe use and tolerability in vulnerable populations, including infants and the elderly, supporting the reasonable conclusion that they will also be safe and well tolerated in young children.

[10] *Standard 2.9.3-7 (1) (a)*

3 Special purpose foods – Other foods (3.6.2)

3.1 Information related to the composition

3.1.1 Identity and need of target population

The target population for the addition of MFGM-WPC to FSFYC is young children within the general population aged 1 to 3 years.

FSFYC are intended to supplement the normal diet of young children to address situations where intakes of energy and nutrients may not be adequate to meet an individual's requirements.

In New Zealand, and potentially Australia, FSFYC have a role in supporting the intakes of essential nutrients such as iron and folate (Netting et al., 2022; Szymlek-Gay et al., 2024), and optional components such as those of the MFGM to further support normal growth and development.

3.1.2 Purpose of the compositional change

The purpose of the compositional change is to permit levels of MFGM-WPC in FSFYC to support nutritional outcomes. The nutritional purpose is to support overall developmental outcomes and is specifically based on the weight of evidence supporting immune relevant outcomes in young children receiving MFGM-WPC fortified formula and foods compared to those not fortified with MFGM components. The addition of MFGM-WPC to formulated foods replenishes and enriches milk-based products with MFGM-derived lipid and protein components.

In summary, the primary purpose of adding MFGM-WPC to FSFYC products is, based on the available evidence, to provide continued nutritional support for the normal growth and development of young children, including supporting immune-relevant outcomes, such as reduction in severity and rates of common childhood infections.

3.1.3 Safety of proposed compositional change

A limited number of clinical trials in young children have addressed the use of MFGM-WPC added to FSFYC. Standard whey-based ingredients, including WPC is a common ingredient used in FSFYC. There are different WPC ingredient options for use in nutritional products such as FSFYC which typically vary in protein content. Commonly used and widely available are WPC 34/35 (approximately 35% protein) and WPC 80 (approximately 80% protein). There are no restrictions on the use of WPC in FSFYC that are manufactured as a standard WPC. MFGM-WPC does not contain any additional components added that are not normally present in dairy-based FSFYC made with WPC, however the level of MFGM components will be higher than products without added MFGM-WPC. The safety and tolerance of MFGM-WPC have primarily been assessed in infant products, and on that basis the extrapolation of the safety outcomes into products for young children can be substantiated (Billeaud et al., 2014; Hari et al., 2015; Jaramillo-Ospina et al., 2022; B. Jiang et al., 2022).

Only one study in young children was identified that included specific measures of safety and tolerance of a MFGM-ingredients (Figure 2-2). A complementary food (UHT beverage) was consumed for a 4 month period by healthy preschool children aged 2.5 to 6 years enrolled in a clinical study investigating the potential protetective effects of MFGM against gastrointestinal and other

infrections, intestinal discomfort and behavioural changes (Veereman-Wauters et al., 2012). No reports of adverse effects, safety or intolerance were reported.

In the first published study on the addition of MFGM added to infant food, marginally nourished Peruvian term infants (enrolled at 6-11 months old) received on a daily basis for 6 months (through 12-17 months of age) a complementary food containing MFGM-WPC as the protein source, with an average daily MFGM-WPC intake of 5.9 g, or a control complementary food with skim milk as the protein source (Zavaleta et al., 2011). The two groups did not differ in growth measurements (weight/height, height/age, or weight/age ratios). Furthermore, anaemia and micronutrient status were not different between the two groups (Zavaleta et al., 2011). Further exploration of serum metabolome and immune markers was undertaken by H. Lee, Zavaleta, et al. (2018) using serum samples collected at the start and completion of the study for a subset (n = 100) of the cohort. This study found supplementation with MFGM tended to improve micronutrient status, energy metabolism, and growth reflected as increased levels of circulating amino acids and weight gain, particularly in female infants compared to those in the control group. No adverse events or negative effects associated with the consumption of the MFGM-enriched complementary food were reported.

No adverse events or negative effects of the MFGM have been reported associated with the original intervention study or in the follow up studies of the original Peruvian cohort through to 14 years of age (Lazarte et al., 2022; Lazarte et al., 2021; Lazarte et al., 2023).

In the COGNIS study (Nieto-Ruiz et al., 2019) a MFGM ingredient was included In the experimental formula fed through to 18 months. There were no reports of safety or tolerance concerns related to this trial. Follow-up studies through to 6 years have not reported any safety concerns related to the the trial product (Diéguez et al., 2023; Nieto-Ruiz et al., 2022).

Together these studies support the safe use of MFGM-WPC in foods for young children and that there were no long-term safety concerns arising later in life (to 14 years) in children who had consumed the MFGM-WPC as young children. Furthermore, a substantial body of research across the lifespan further reinforces the immune-related benefits of MFGM, and its long history of safe use and tolerability in vulnerable populations—including infants and the elderly—supports the reasonable conclusion that MFGM will be safe and well tolerated in young children.

3.2 Information related to the dietary intake or dietary exposure

3.2.1 Dietary exposure data

As discussed in Section 2.4.3 estimated mean intakes of FSFYC are approximately 2 x 200 mL serves per day. Previously FSANZ have estimated the 90th percentile (high) intake to be twice that of the mean intake (Food Standards Australia New Zealand (FSANZ), 2009), equating to 4 serves per day.

The proposed maximum level of MFGM-WPC is 2.3 g per serve (Section2.4.2). At this highest addition rate, the 90th percentile for MFGM-WPC is 9.2 g/day for young children.

Dietary exposure data for quantifiable components of MFGM-WPC (whey and SM) have been determined using a simulated formulation of a FSFYC that is typical of products found in market. For this model a larger serve size of 224 mL ensures a conservative approach. It is also a typical serve size for commercially available FSFYC.

Simulation models:

Protein content:	13.5%
Fat content:	15.5%
Reconstitution rate:	14 % w/w (28 g FSFYC powder g in total volume 200 mL)
Serve size:	200 mL
Mean serves per day:	2
90th percentile serves per day:	4

Simulated FSFYC milk formula models are:

i. Whole milk only to achieve protein content (no MFGM-WPC)
i. Skim milk only to achieve protein content (no MFGM-WPC)
ii. Whole milk (50% of total protein) + skim milk (50% of total protein) (no MFGM-WPC)
iii. Skim milk (50% of total protein) + WPC35 (50% of total protein)
iv. MFGM-WPC added at 1.4 g per serve + skim milk as balance of protein content.
v. MFGM-WPC added at 2.3 g per serve + skim milk as balance of protein content.
vi. MFGM-WPC added at 3.4 g per serve + skim milk as balance of protein content.

These are compared to normalised values for liquid whole milk and liquid skim milk (Table 3-1and Table 3-2). Compared to FSFYC whole milk contains significantly more fat (3.8 % w/v) and protein (3.3% w/v), meaning the level of MFGM consumed is high as it is associated with the milkfat. Total phospholipids are approximately 0.8% of the milkfat, and the documented SM range is 22-26% of the total PL (see section 2.2.1.1). For comparative purposes a typical SM of liquid whole milk is 27 mg / 100 ml, or 54 mg SM per 200 ml serve size.

The model simulation shows that to match the MFGM, based on SM, content of consuming a single 200 mL serve of whole milk, a FSFYC formula made from SMP and MFGM-WPC using the model composition as above would require the addition of 3.4 g of MFGM-WPC per 200 mL serve (17.0 g/L). It is reasonable to conclude this level of MFGM consumption is common amongst young children who regularly consume whole milk.

It is possible to make a FSFYC milk-based formula using only WMP as the protein source. The formula model for this option predicts a SM content of 29 mg /200 mL serve. However FSFYC milk formulas are more typically made using SMP, or SMP and WPC to provide the protein, meaning the level of

MFGM (represented by SM) is significantly less than with whole milk or WMP-based FSFYC formula (Table 3-2).

Accordingly, this Application proposes to permit the addition of MFGM-WPC up to a maximum addition rate of 2.3 g/serve (11.5 g/ mL) to restore the MFGM content to an equivalent level as if the formula were made using whole milk. A constraint of a maximum sphingomyelin content of 40 mg SM per serve means the total MFGM material (represented by SM) will always be less than that of 200 mL of whole milk consumed, and any possible high rate of addition of MFGM-WPC to a whole milk based FSFYC formula is restricted by the total SM maximum limit.

MFGM-WPC is foremostly a whey powder, differing only in that it is enriched in MFGM material. Whey powder is commonly used in FSFYC formula as a protein source. As whey protein is the main component (> 70 %) of MFGM-WPC dietary exposure data for whey protein has been determined and presented together with what would be consumed through milk consumption at the same intake level (Table 3-1). This data demonstrates the addition of MFGM-WPC to FSFYC does not result in an elevated intake of whey protein that may otherwise be achieved through an equivalent volume consumption of FSFYC that is made with a standard SMP and commonly used WPC.

Table 3-1 Simulated FSFYC formulation models of whey protein intake of milk or FSFYC as consumed

Formulation / milk	**Whey protein (g/100 mL)**	**Whey protein (g/200mL serve)**	**90th Percentile whey protein intake (g/day)**
Skim milk *	**0.68**	**1.4**	**5.6**
Whole milk *	**0.66**	**1.3**	**5.2**
FSFYC (WMP only base)	**0.38**	**0.76**	**3.0**
FSFYC (WMP 50% protein; SMP 50% protein base)	**0.38**	**0.76**	**3.0**
FSFYC (SMP only base)	**0.38**	**0.76**	**3.0**
FSFYC (WPC35 50% protein; SMP 50% protein base)	**1.13**	**2.3**	**9.0**
FSFYC (MFGM-WPC + SMP) to match addition rate permitted in IFP FSFYC with MFGM-WPC at **max** 1.4 g/serve (SMP base) (equivalent to 7 g /L) (5% MFWPC powder basis)	**0.78**	**1.6**	**6.4**
FSFYC (MFGM-WPC + SMP) to match SM of WMP-based FSFYC FSFYC with MFGM-WPC at **max** 2.3 g/serve (SMP base) (equivalent to 11.5 g /L) (8.1% MFWPC powder basis)	**1.0**	**2.0**	**8.2**
FSFYC (MFGM-WPC + SMP) to match SM of liquid whole milk FSFYC with MFGM-WPC at **max** 3.4 g/serve (SMP base) (equivalent to 17.0 g /L) (15.4% MFWPC powder basis)	**1.6**	**3.2**	**12.8**

* *Typical milk protein composition: 20% whey protein and 80% casein protein (Barth & Behnke, 1997).*

Using the same simulated formulations, together with data on the typical SM content of dairy products and ingredients (WM (liq.), 0.125 g SM/100 g milk fat (Contarini & Povolo, 2013); SMP, 0.04 g /100 g (Moloney et al., 2018); WPC35, 0.176 g/100 g (Moloney et al., 2018)), the dietary exposure to SM has been determined (Table 3-2). This identifies the intended effect of the adding MFGM-WPC to FSFYC to increase the dietary intake of components of the MFGM including phospholipids such as SM. As wholemilk contains high levels of milk-fat–associated phospholipids including sphingomyelin, reproducing its SM contribution in a FSFYC requires the deliberate addition of MFGM-rich material. To match the SM content in liquid wholemilk a FSFYC requires the addition of 3.4 g / serve of MFGM-WPC (equivalent to 22g /L), while matching the SM level of a FSFYC where the protein

content is provided solely from the use of wholemilk or WMP would require the addition of 2.3 g/ serve of MFGM-WPC,equivalent to 11.3 g/L.

Table 3-2 Simulated FSFYC formulation models of SM intake of milk or FSFYC as consumed

Formulation / milk	**SM (mg/100 mL)**	**SM (mg/200 mL serve)**	**90th Percentile intake (g/day)**
Skim milk	**0.085**	**0.17**	**0.68**
Whole milk	**27**	**54**	**216**
FSFYC (WMP only base)	**14.5**	**29.0**	**116**
FSFYC (WMP 50% protein; SMP 50% protein base)	**7.6**	**15.2**	**60.8**
FSFYC (SMP only base)	**0.67**	**1.4**	**5.6**
FSFYC (WPC35 50% protein; SMP 50% protein base)	**0.93**	**1.9**	**7.4**
FSFYC (MFGM-WPC + SMP) to match addition rate permitted in IFP FSFYC with MFGM-WPC at **max** 1.4 g/serve (SMP base) (equivalent to 7 g /L) (5% MFWPC powder basis)	**9.3**	**18.6**	**74.5**
FSFYC (MFGM-WPC + SMP) to match SM of WMP-based FSFYC FSFYC with MFGM-WPC at **max** 2.3 g/serve (SMP base) (equivalent to 11.5 g /L) (8.1% MFWPC powder basis)	**14.5**	**29.0**	**116**
FSFYC (MFGM-WPC + SMP) to match SM of liquid whole milk FSFYC with MFGM-WPC at **max** 3.4 g/serve (SMP base) (equivalent to 17.0 g /L) (15.4% MFWPC powder basis)	**27**	**54**	**216**

3.2.2 Level of consumption

When included in the diet of young children, typically 1 – 2 serves per day of FSFYC are consumed. FSFYC are a supplement to the diet. As a part of the diet of young children, the consumption of FSFYC is *in lieu* of milk, rather than in addition to (Food Standards Australia New Zealand (FSANZ), 2009).

At 1 – 2 serves per day as recommended, the consumption of MFGM-WPC would, at maximum addition rate, be 2.3 – 4.6 g /day.

The addition of MFGM-WPC to FSFYC will not change the level or frequency of FSFYC consumption, nor will it change the current potential consumption of whey protein by young children. The addition of MFGM-WPC will provide increased levels of MFGM, but not necessarily whey or other minor components, to the diets of young children compared to those consuming equivalent volumes of milk or standard formulations of FSFYC.

3.3 Information related to the labelling requirements under Part 2.9 of the Code

3.3.1 Safety or nutritional impact of the labelling change

The proposed changes to the label are discussed in Section 2.2.9. Options for how the MFGM-WPC may be labelled in both the ingredient list and NIP are proposed. There are no safety related issues relating to the labelling change.

For FSFYC it is possible, in addition to the ingredient list and NIP, to indicate on pack the presence of a nutritional substance. The presence of MFGM-WPC (or MFGM from MFGM-WPC) is permissible for FSFYC as a nutrition content claim (Standard 1.2.7 -13). Hence the nutritional impact of the labelling change will be the awareness of the presence of MFGM-WPC in the product.

A mock-up of a hypothetical FSFYC front-of-pack label is shown in Figure 2-32.

3.3.2 Demonstrated consumer understanding of the labelling change

No specific consumer data regarding consumer understanding of the labelling change is available within ANZ. However, given the simplicity of the changes – inclusion in ingredient list and NIP, and possible nutrient content claim, consumers should understand the presence of MFGM-WPC in the FSFYC based on clarity of the label. The possible labelling changes relate only to providing information stating the product contains added MFGM-WPC, including possible nutrition content claims, the level of addition, and potentially on-pack visual representations (Figure 2-32 by way of example).

3.4 Internationally recognised codes of practise and guidelines on labelling

CODEX has labelling requirement and guidelines for FSFYC-type products that fall within the scope of Section B of the Standard for follow-up formula for older infants and young children (CXS 156-1987 (Rev 2023)). Furthermore, the requirements of the General Standard for the Labelling of Pre-packaged Foods (CXS 1-1985), the Guidelines on Nutrition Labelling (CXG 2-1985) and the Guidelines for Use of Nutrition and Health Claims (CXG 23-1997) apply to this product category. These requirements include a prohibition on the use of nutrition and health claims for foods for young children except where specifically provided for in relevant Codex standards or national legislation

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