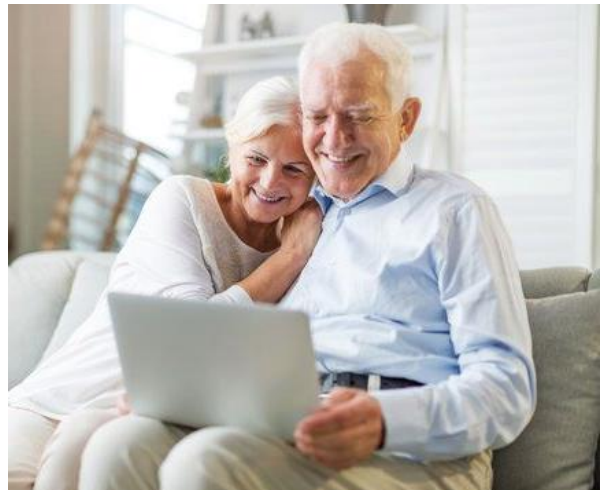
Bioactives from Dairy Streams

Dr. Daniela Barile

UCDAVIS
FOOD SCIENCE AND TECHNOLOGY



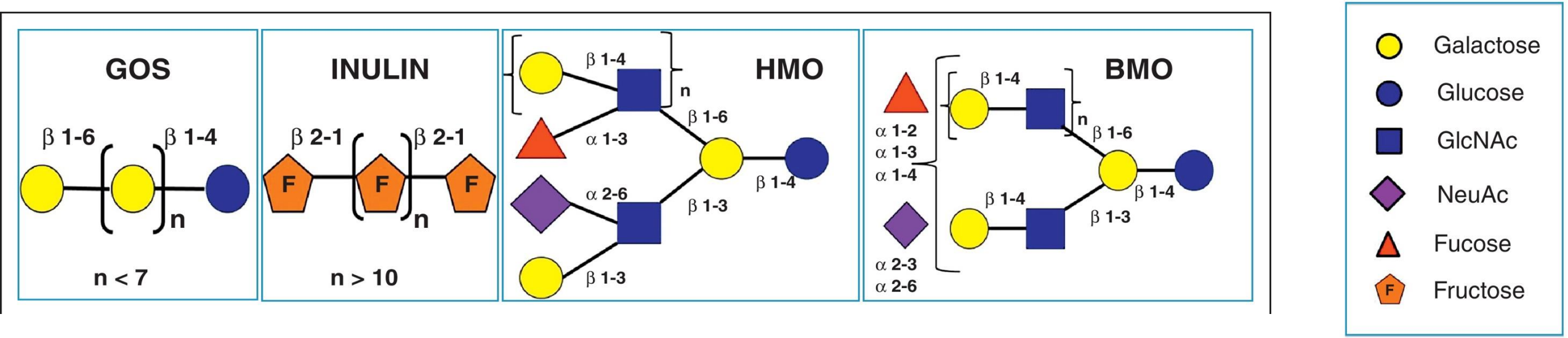
Yogurt is no longer just milk + starter cultures

Category	Examples	Why they are added
Extra protein	Milk protein concentrate, **whey protein concentrate**, casein, **ultrafiltered milk**	To make Greek-style, skyr-style, or **high-protein** yogurt
Prebiotics/fiber	Inulin, **chicory root fiber**, soluble corn fiber, pectin, beta-glucan, resistant starch	To support **gut-health** claims
Texture modifiers	**Pectin**, gelatin, starch**, tapioca starch**, locust bean gum, guar gum, carrageenan	To improve **viscosity**, reduce syneresis, and **stabilize** fruit-on-bottom or drinkable products

Current Opinion in Biotechnology
Volume 24, Issue 2, April 2013, Pages 214-219

Human milk and related oligosaccharides as prebiotics

Daniela Barile [1], Robert A Rastall [2]



What if one ingredient could could support texture, nutrition, and bioactive delivery ?

..and it came from dairy streams..

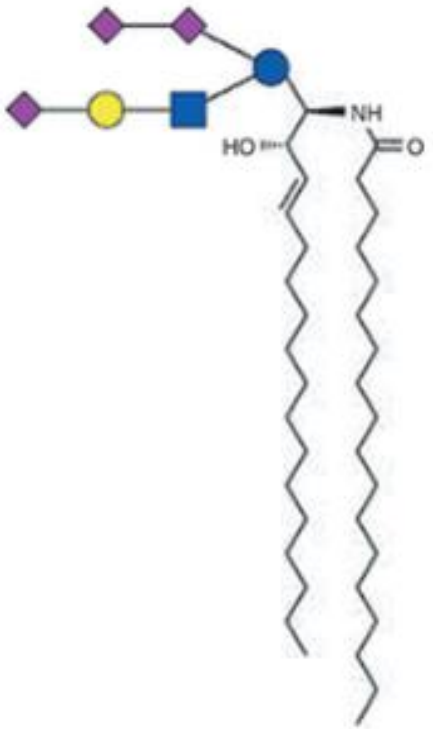
..without the need for expensive extraction..

...and could also provide benefits across the lifespan, supporting brain, immunity, cardiovascular and gut health?

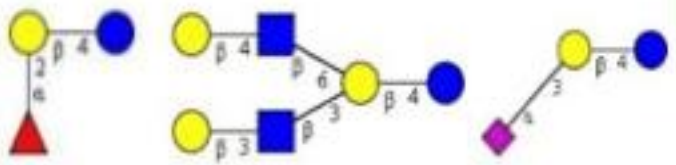
A recent science: Glycomics

Glycans are on of the surface of milk components.

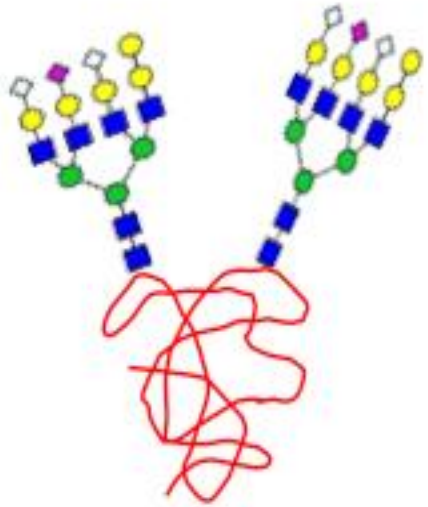
Glycolipids



glycosphingolipids

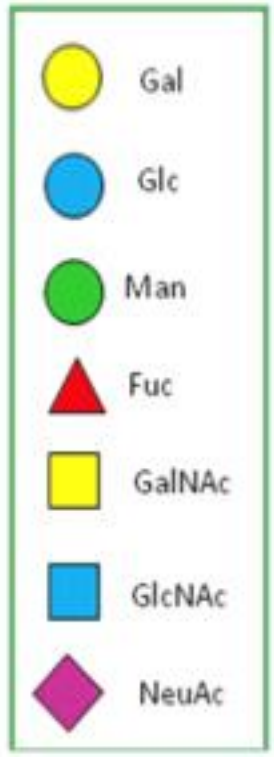


Free oligosaccharides



Protein-linked glycans

Symbolic nomenclature: monosaccharides denoted by colored geometric shapes



(Consortium for Functional Glycomics)

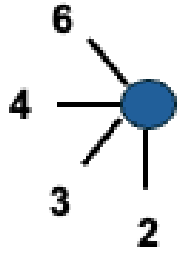
Characterizing glycans is not simple !

Many building blocks..

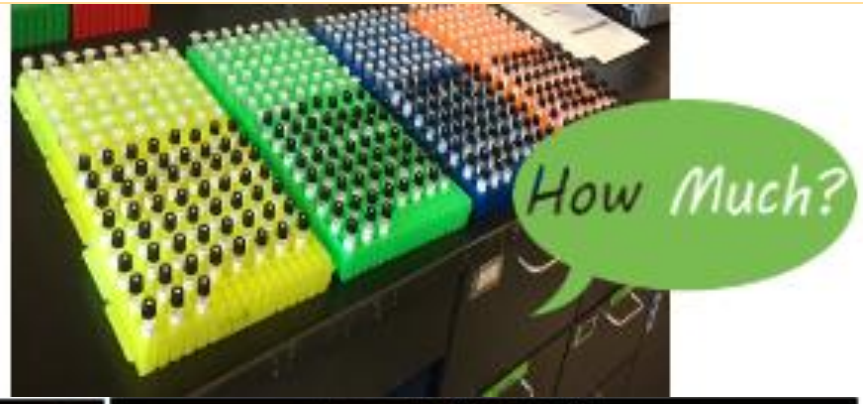
..limited software automation

Name	Abbreviation	Symbol	Structure	Name	Abbreviation	Symbol	Structure
D-glucose	Glc			D-xylose	Xyl		
D-galactose	Gal			L-arabinose	Ara		
D-mannose	Man			D-ribose	Rib		
D-fructose	Fru	Fru		D-apiose	Api		
L-fucose	Fuc			D-glucuronic acid	GlcA		
L-rhamnose	Rha			D-galacturonic acid	GalA		

- Lack a chromophore
- Diverse monosaccharides
- Complex branched structures
- Many isomers
- Manual annotation



Glycomics Analytical Platform



NANO-LC Q TOF

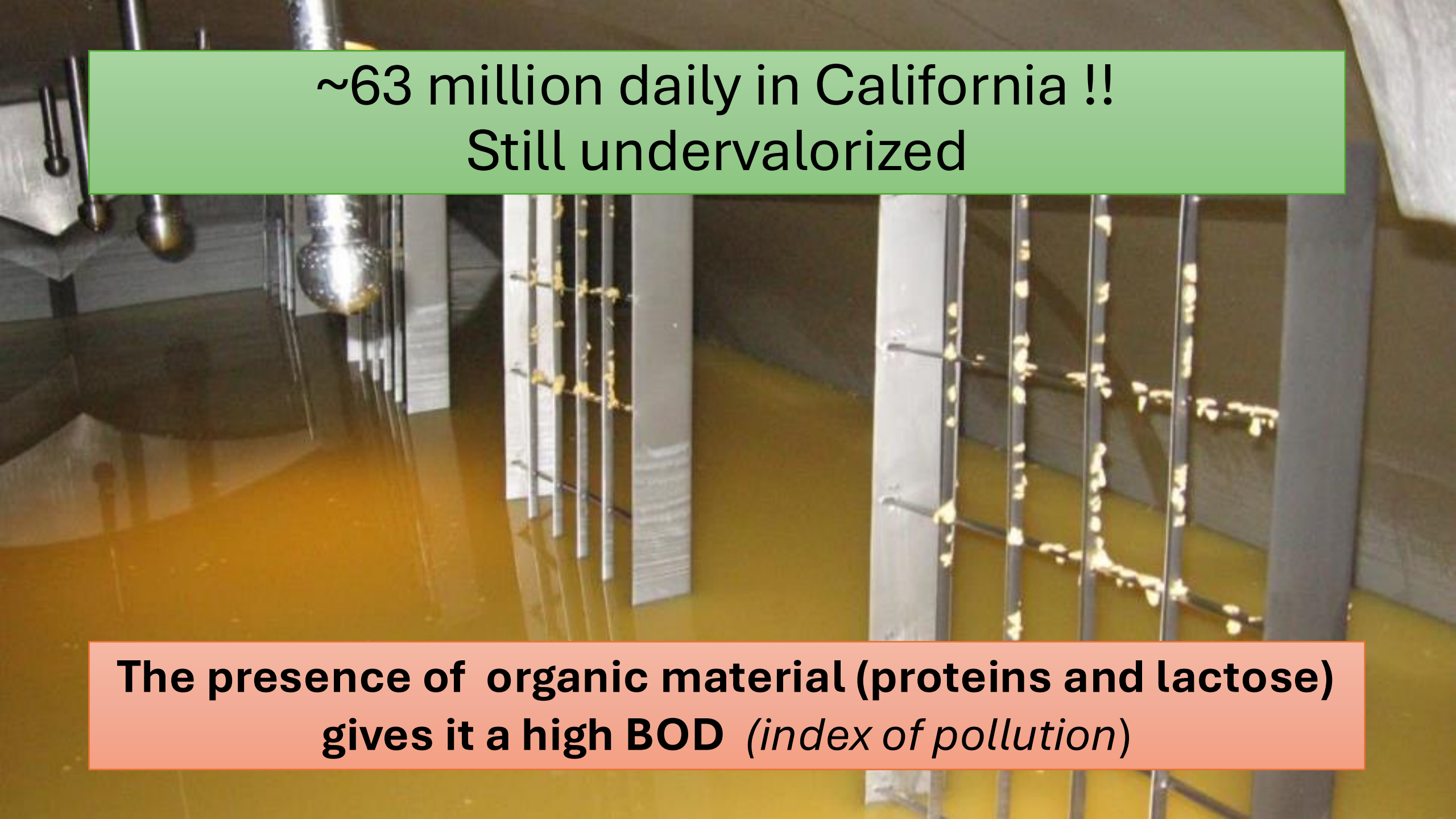
HPAE-PAD

MALDI-TOF

6470A Triple Quad LC-MS

- **Guide processing and ensure the purity of ingredients**
- Utilize the appropriate amount of ingredients for testing

Barile Lab



~63 million daily in California !!
Still undervalorized

The presence of organic material (proteins and lactose) gives it a high BOD *(index of pollution)*

Can dairy streams become functional ingredients for yogurt?

What if we skip extraction – can we still get prebiotics?

Milk

Cheese

Whey

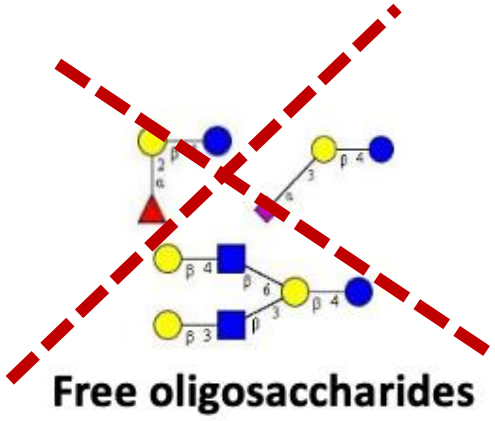
Whey Proteins

Whey Permeate

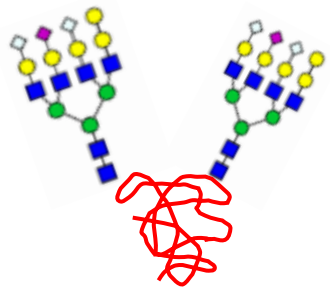
Whey Protein phospholipid concentrate

Lactose

Delactosed permeate



Free oligosaccharides



Protein-linked glycans

Looking at available whey streams for protein-linked glycans

The “other side” of whey protein isolates

Identify & recover bioactives:

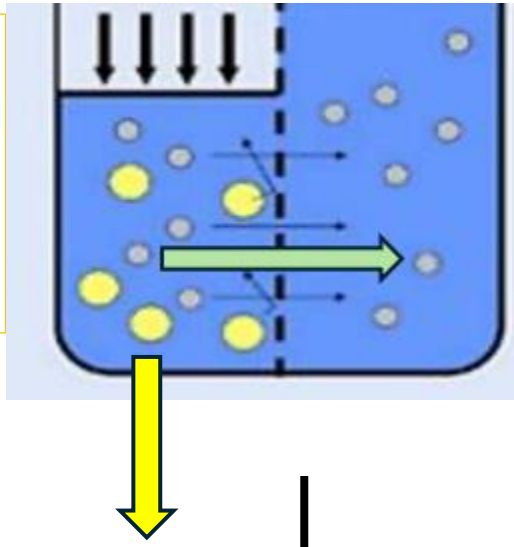
- Add value
- Demonstrate dairy health benefits
- Increases the economic viability

LC-MS/MS

Low value

Poorly characterized

Microfiltration



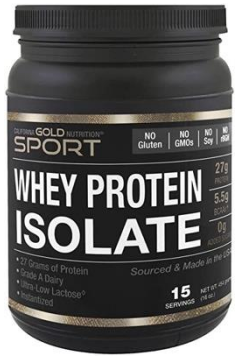
Microfiltration of WPC

WPPC

Whey Protein Phospholipid Concentrate

Retentate

WPI



Whey Protein Isolate (fat free)

Permeate

Successful Market **$$$**

Why is it called Whey Protein Phospholipid Concentrate?

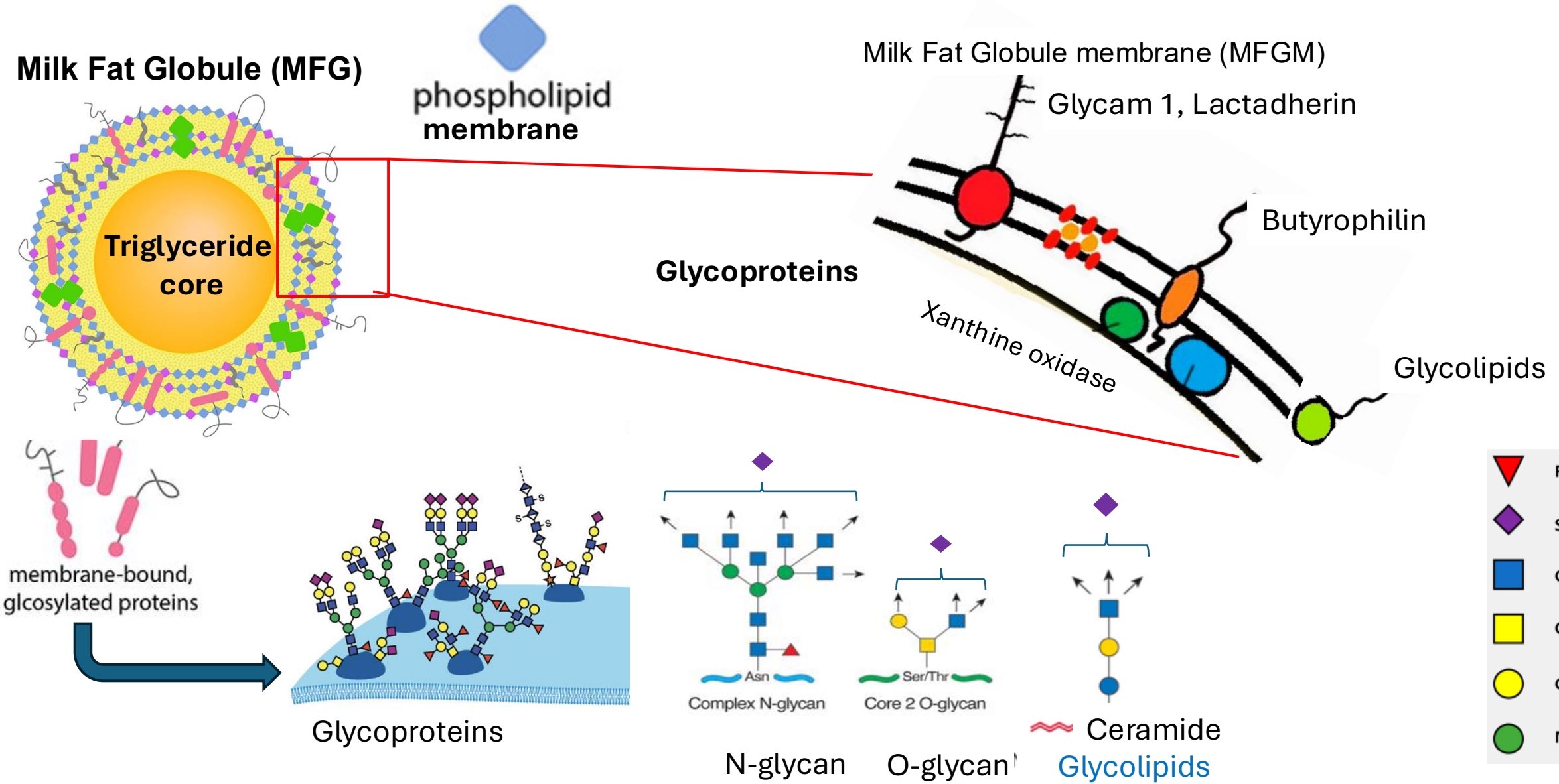


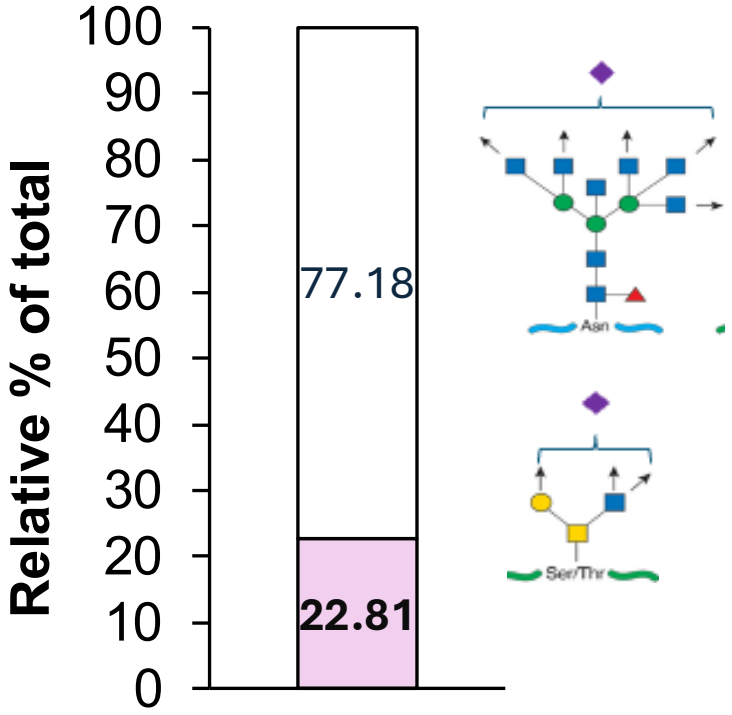
Image source: https://news.ku.edu/2023/08/31/study-demonstrates-adding-complex-component-milk-infant-formula-confers-long-term, Essentials of Glycobiology [Internet]. 4th edition

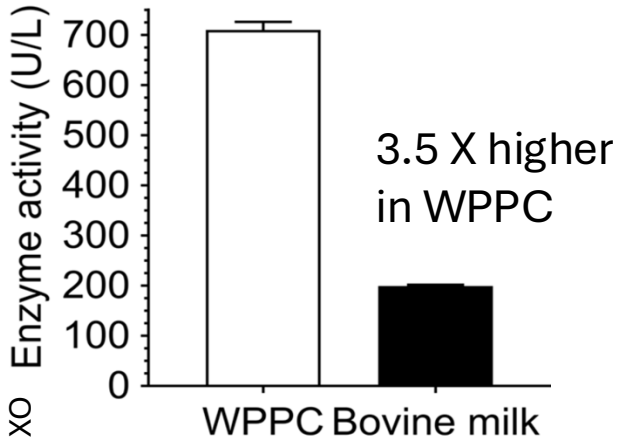
Proteomics of WPPC: 1300 proteins

>1300 proteins

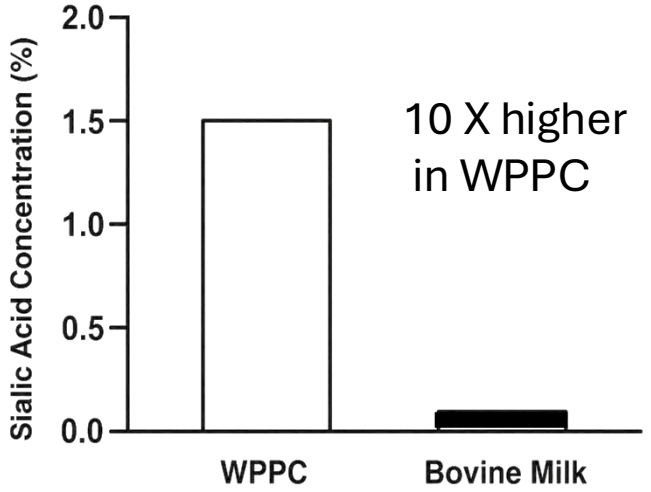
Description	Accession	# Peptides	MW [kDa]	Area
beta-lactoglobulin	P02754	24	19.9	4.73E+10
Serum albumin	P02769	66	69.2	1.72E+10
Glycosylation-dependent cell adhesion molecule 1	P80195	13	17.1	1.10E+10
Alpha-fetoprotein	Q3SZ57	2	68.5	8.29E+09
Butyrophilin subfamily 1 member A1	P18892	28	59.2	8.03E+09
Isoform Short of Lactadherin	Q95114-2	17	41.5	6.99E+09
Lactadherin	Q95114-1	25	47.4	6.99E+09
Lactotransferrin	P24627	60	78	5.97E+09
Polymeric immunoglobulin receptor	P81265	28	82.4	3.76E+09
Isoform Short of Polymeric immunoglobulin receptor	P81265-2	16	58.6	3.71E+09
Xanthine dehydrogenase/oxidase	P80457	58	146.7	3.53E+09
alpha-lactalbumin	P00711	11	16.2	3.25E+09
Fatty acid-binding protein, heart	P10790	14	14.8	2.75E+09
lactoperoxidase	P80025	29	80.6	2.08E+09
Alpha-S1-casein	P02662	10	24.5	1.92E+09

WPPC contains very small globules





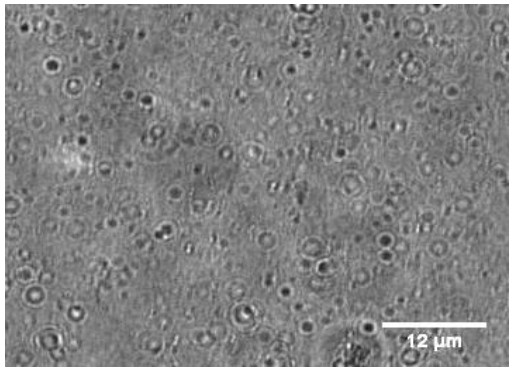
Xanthine Oxidase



Sialic acid

MFGM-proteins accounted for 22% of protein abundance

A single producer in CA accumulates **2 million kg/year** WPPC

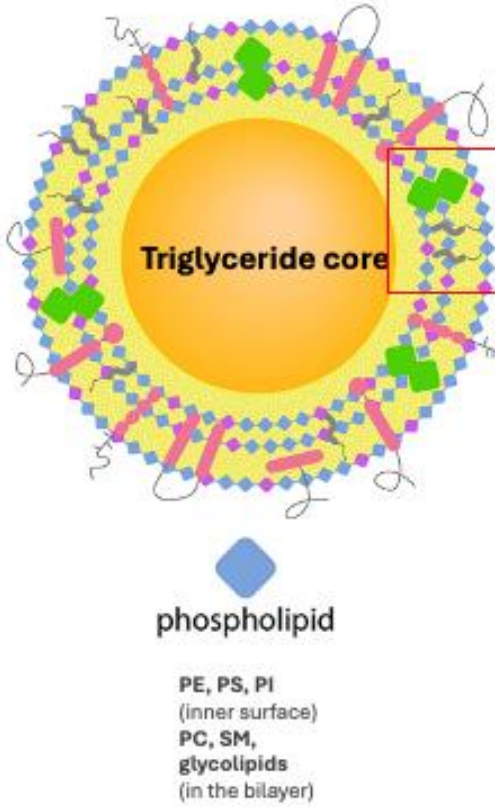


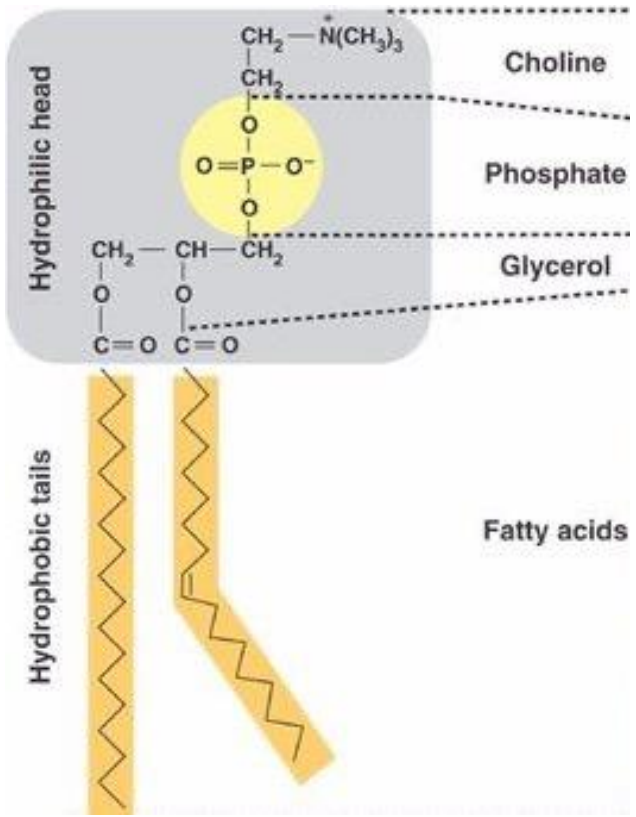
Microscopy of WPPC

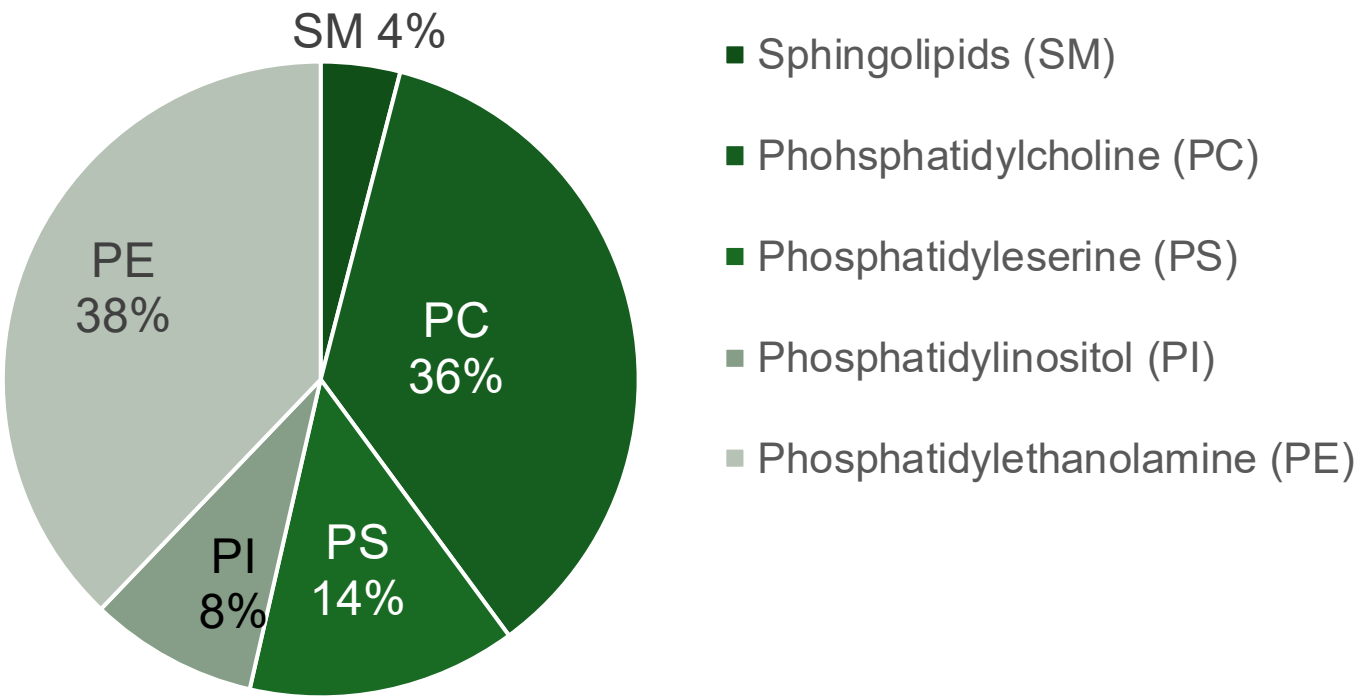
Smaller globules contain more membrane proteins and less fat!

Phospholipids distribution in WPPC

Prof. Ameer Taha
(UC Davis)







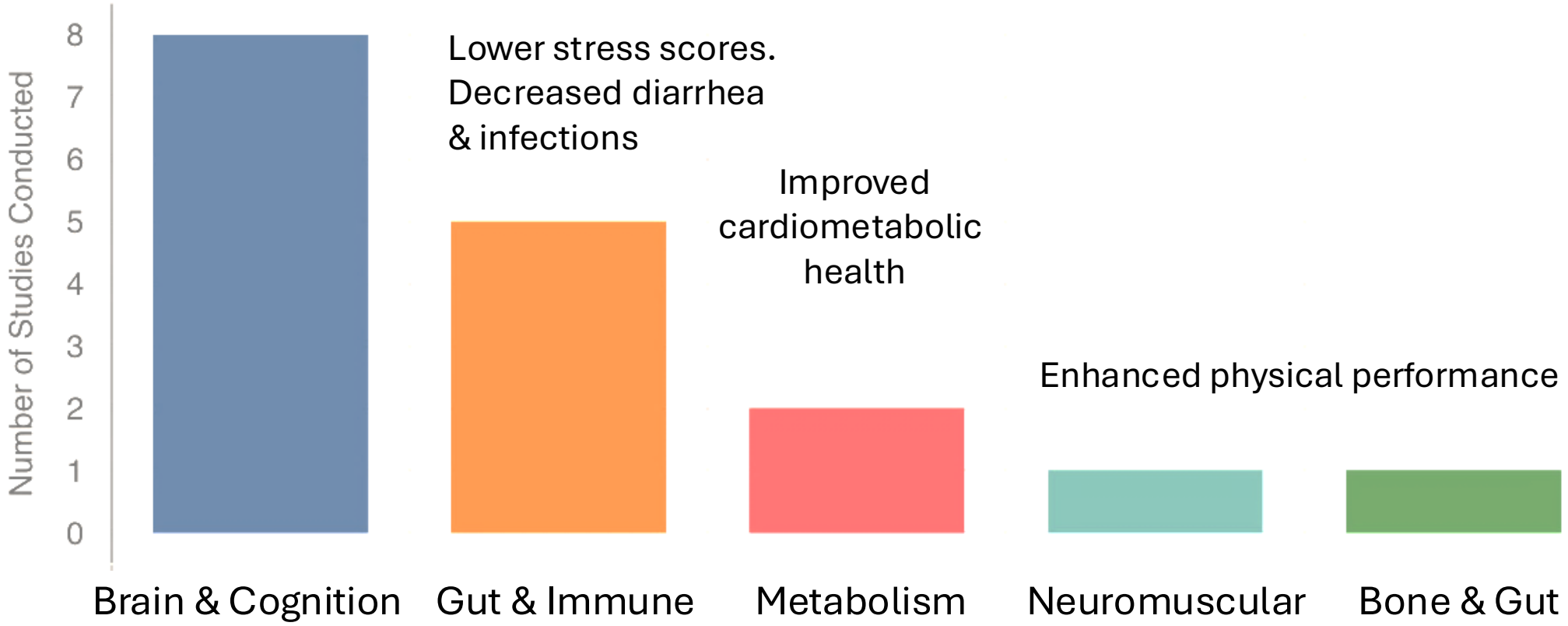
Phospholipids accounted for 20% of the total lipid pool in WPPC.

Ozturk et al, 2022, Dairy 3(2), 277-302

Clinical Studies with MFGM in the last 10 years

18 studies, 50% were done on human subjects.

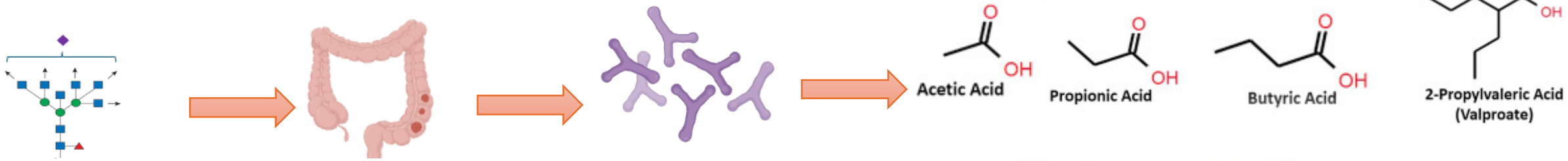
Animal studies included piglets, rats and mice



Novel opportunities for yogurt enhancement

- Prebiotic activity:

Bovine milk glycoconjugates resemble human milk glycoconjugates and reach the colon intact

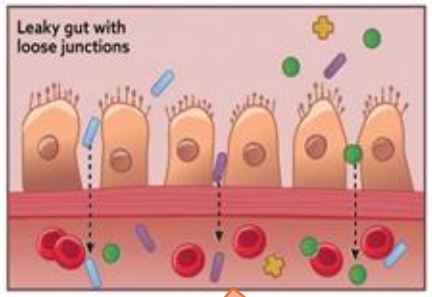


Environmental Microbiology

Oligosaccharides Released from Milk Glycoproteins Are Selective Growth Substrates for Infant-Associated Bifidobacteria

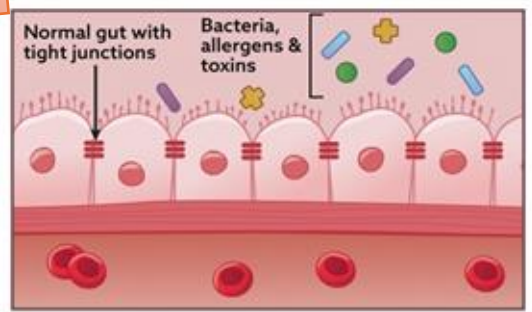
Sercan Karav, Annabelle Le Parc, Juliana Maria Leite Nobrega de Moura Bell, Steven A. Frese, Nina Kirmiz, David E. Block, Daniela Barile, David A. Mills

C. M. Dozois, Editor



- Direct gut-health mechanisms:

milk glycoconjugates can directly modulate epithelial barrier integrity and act as decoy receptors



doi.org/10.3390/foods6110093 doi.org/10.3389/fmicb.2018.00947

Biomedicine & Pharmacotherapy 196 (2026) 119009

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

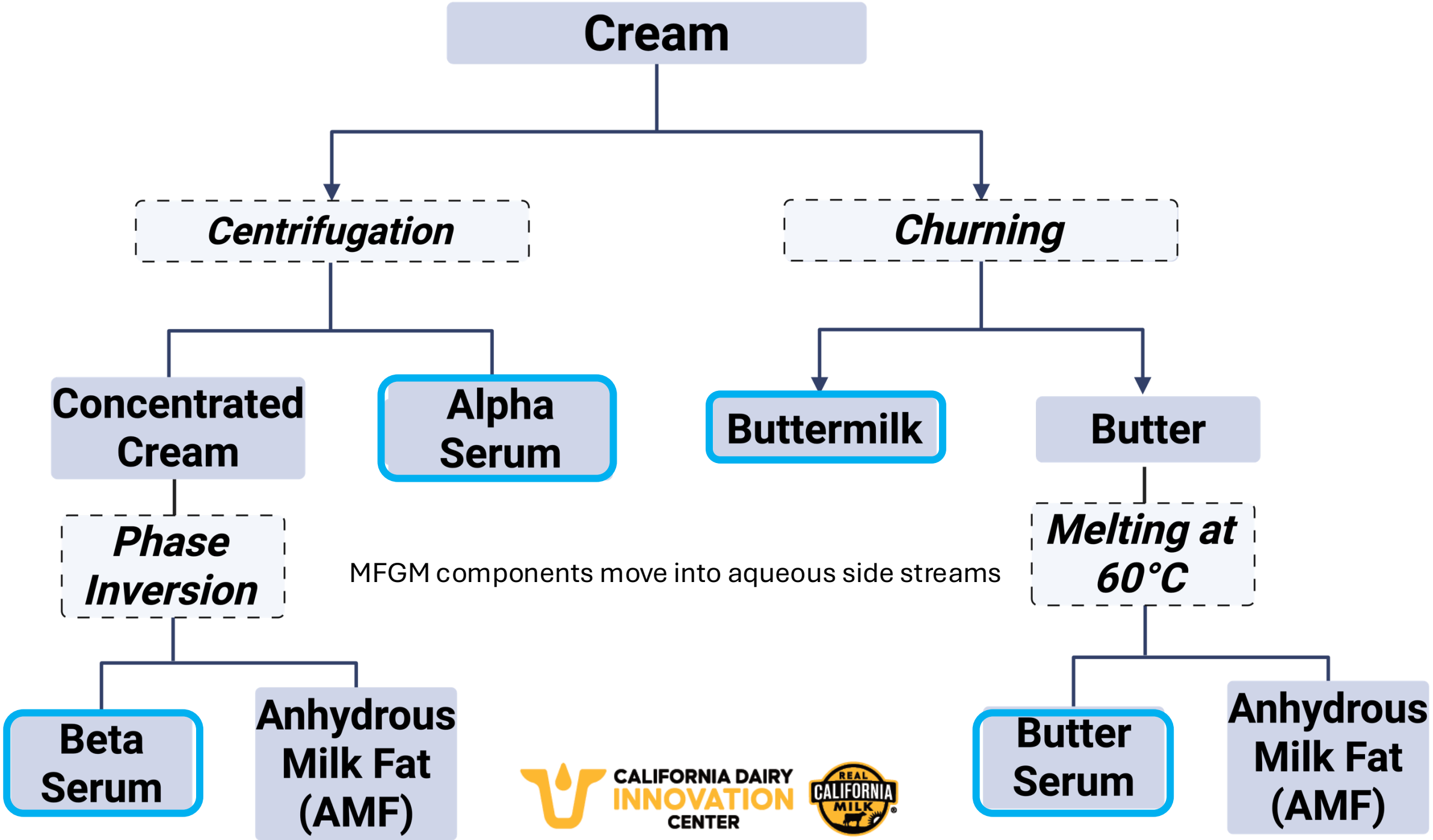
Milk fat-globules derived from whey protein phospholipid concentrate prevent high-fat diet induced cognitive impairment in wistar rats in a manner associated with increased brain neuronal connectivity and sphingolipid clearance

Duncan A. Sylvestre [a,b], Nuanyi Liang [a,c], James Guinto Galan [a], Amber Safar [a], Felipe de Costa Souza [a], Mitchell C. Bancks [a], Vedanth Sundaram [a], Brian W. Scott [d], Kasey Schalich [a], Michael Goodson [e], Jennifer M. Rutkowsky [e], Kristopher Galang [e], Gulustan Ozturk [f], David A. Mills [a], Daniela Barile [a], Ameer Y. Taha [a,g,h,*]

Upgrading butter side streams:

Can butter side streams become sources of MFGM-rich ingredients?

low-value stream → MFGM recovery → premium ingredient ?



CALIFORNIA DAIRY INNOVATION CENTER

REAL CALIFORNIA MILK

Annual Production Volumes?

No official production data available

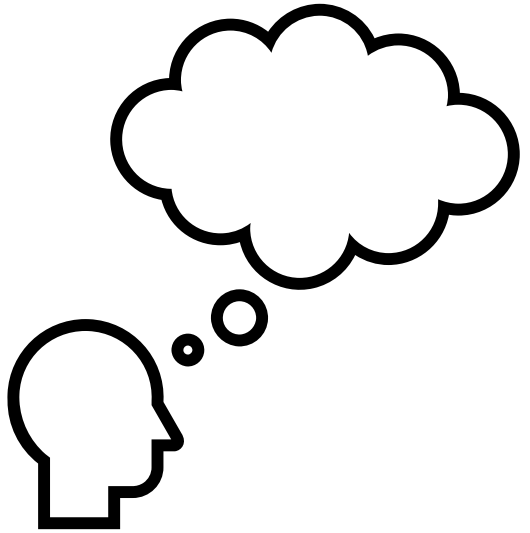
Single Producer (interviews)

Product	Annual Production (mil kg/yr)
WPPC	2.05
Buttermilk	1.5
Beta Serum	0.502
Alpha Serum	2.07

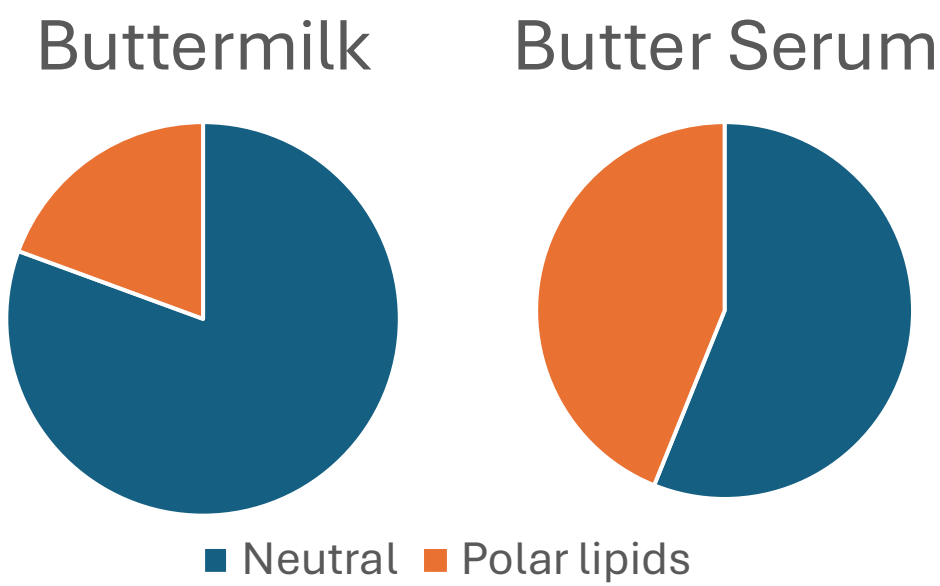
=4.072 million kg/year

**Displayed as dry weight for powdered products*

Will they have the same bioactive activity?



Knowledge and Gaps for Butter Streams



Presence of **phospholipids** in Buttermilk and especially in **Butter Serum**

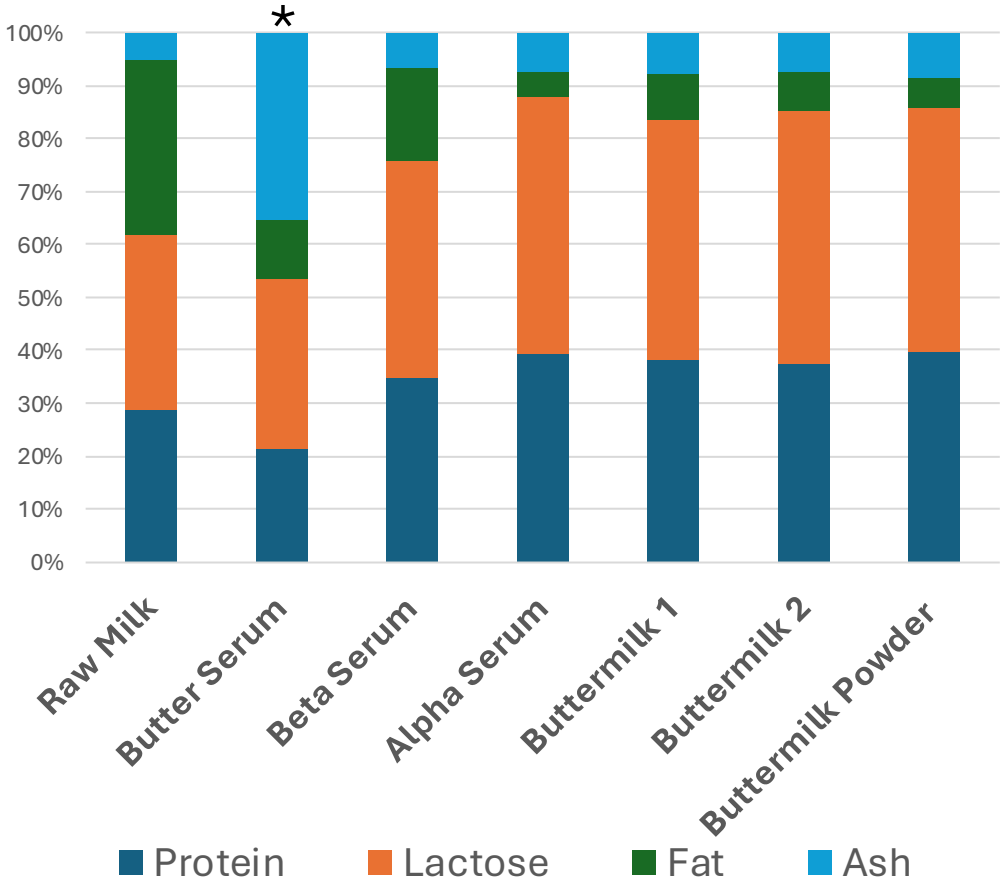
- Only **one research study** investigated siome basic composition of **Alpha Serum**
- **Confusion** between **butter serum and beta serum** in the literature
- **Lack of standards of identity** for all these by-products

(Bourlieu et al., 2018)(Señoráns et al., 2023)

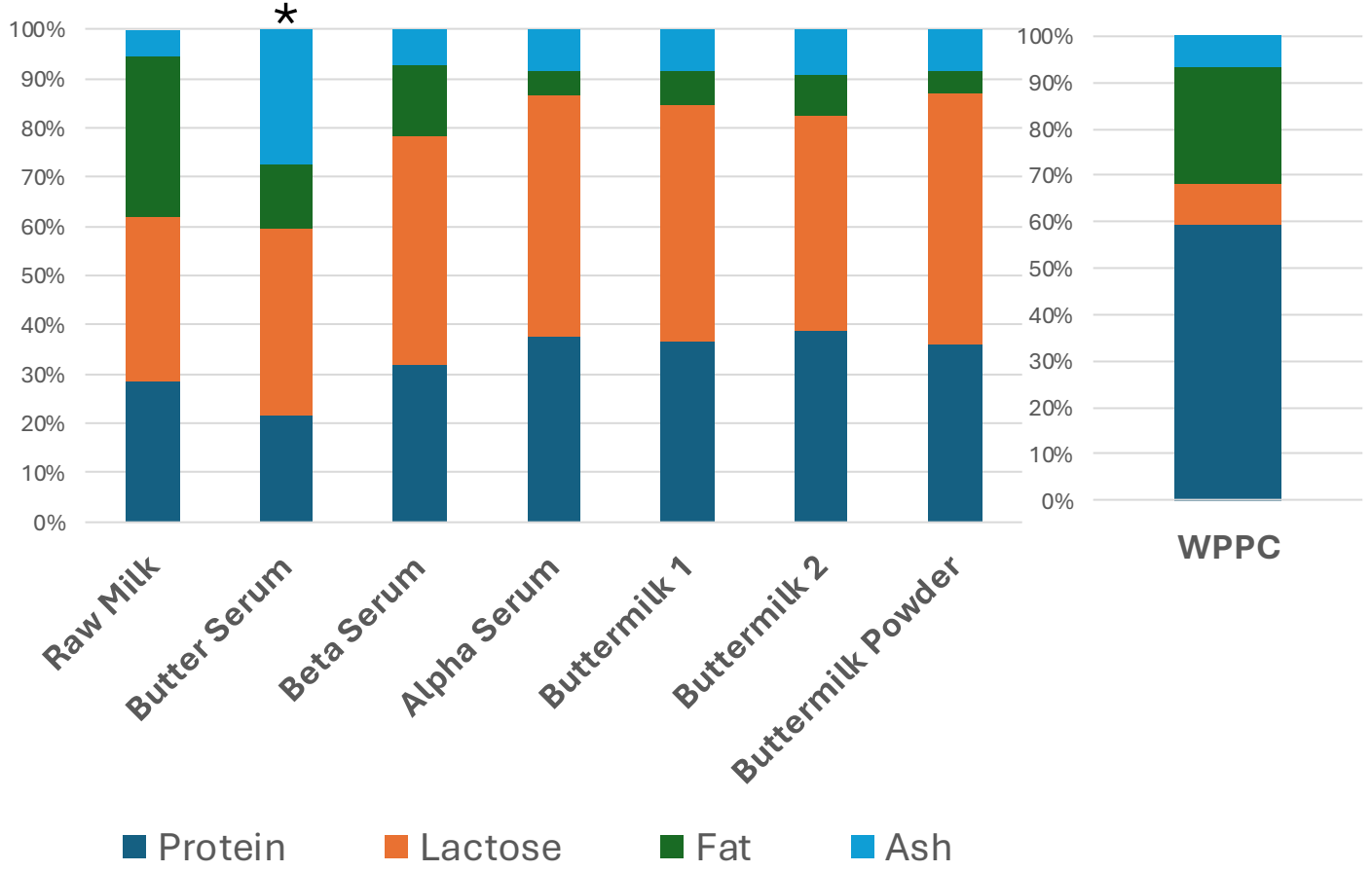
Measuring basic composition

Component	Assay
Protein	Dumas-Nitrogen Method
Lactose	LC-QQQ
Fat	Mojonnier Method
Ash	Dry Ashing

Batch 1



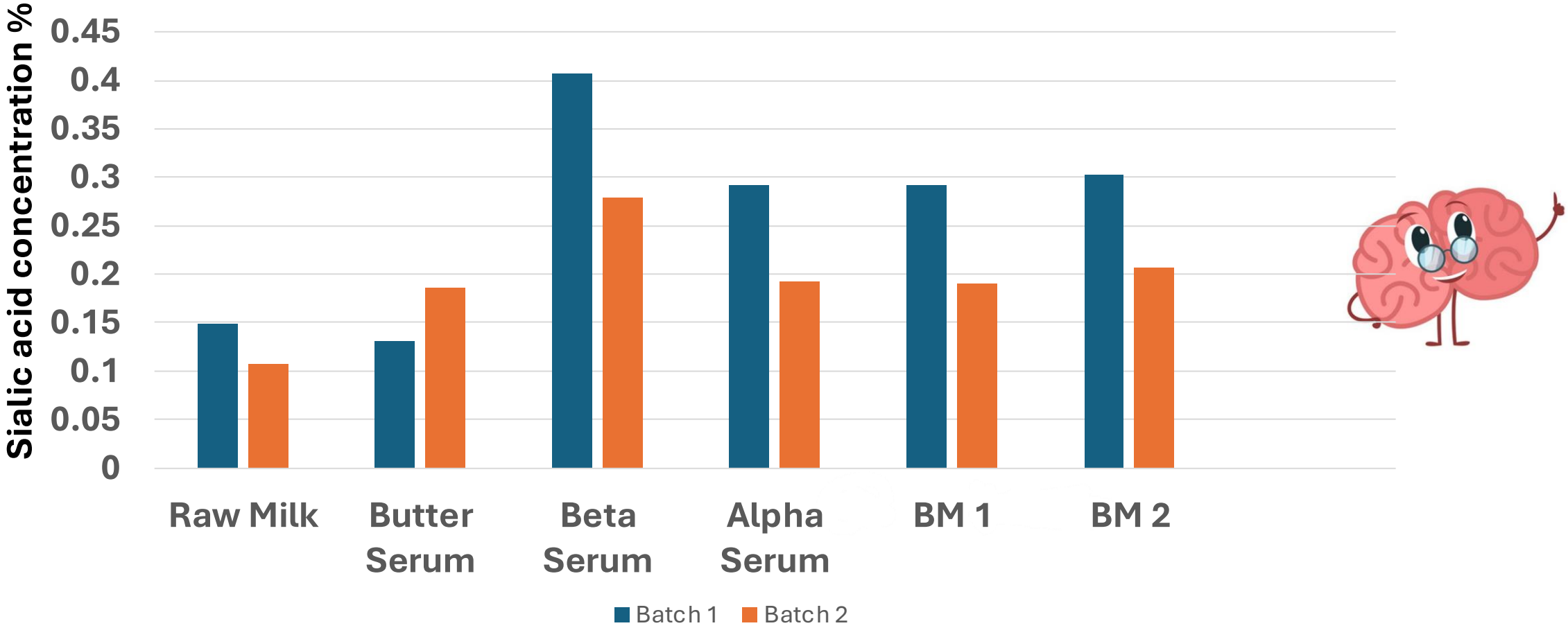
Batch 2



**Significant batch-to-batch variability was observed in butter serum, necessitating multiple sample requests and experimental replicates*

Sialic Acid Quantification

LC-QQQ MS (% dry matter)



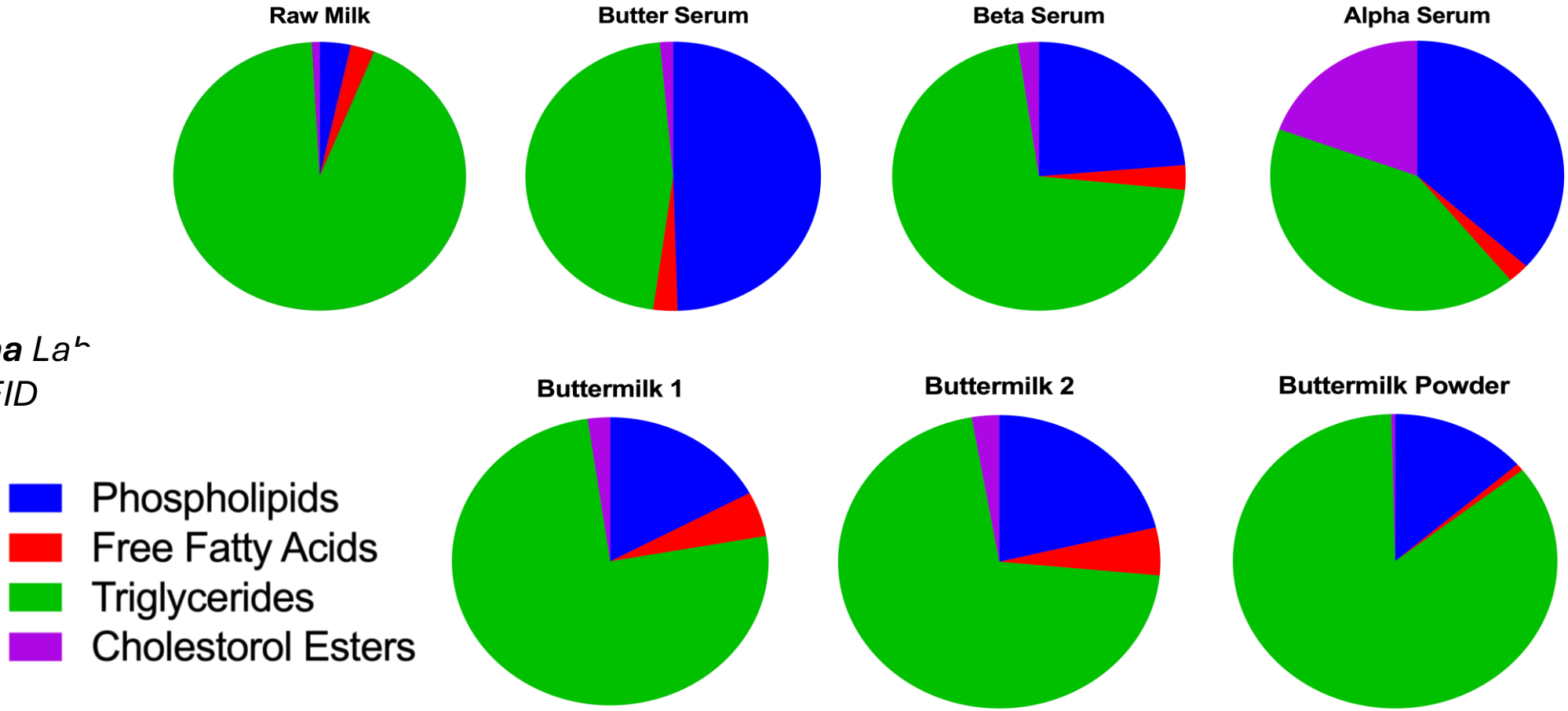
Higher than raw milk, but still lower than WPPC..

Lipid Classes Percent Composition

Duncan Sylvestre

Dr. Ameer Taha *Lab*

TLC and GC –FID

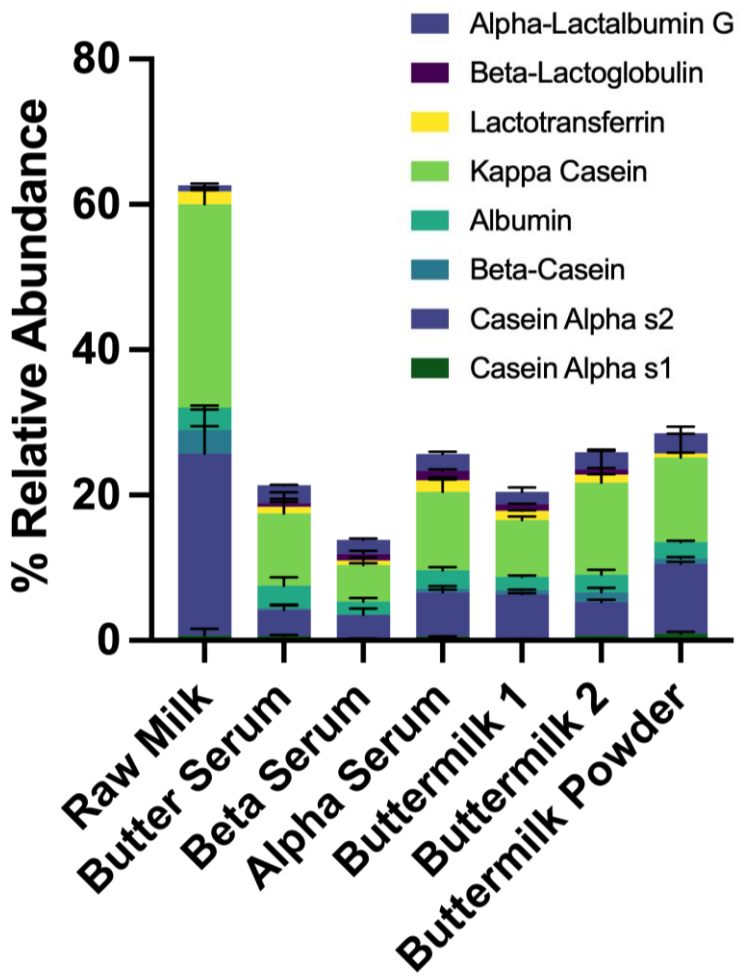


Increased abundance of phospholipids in the streams compared with raw milk

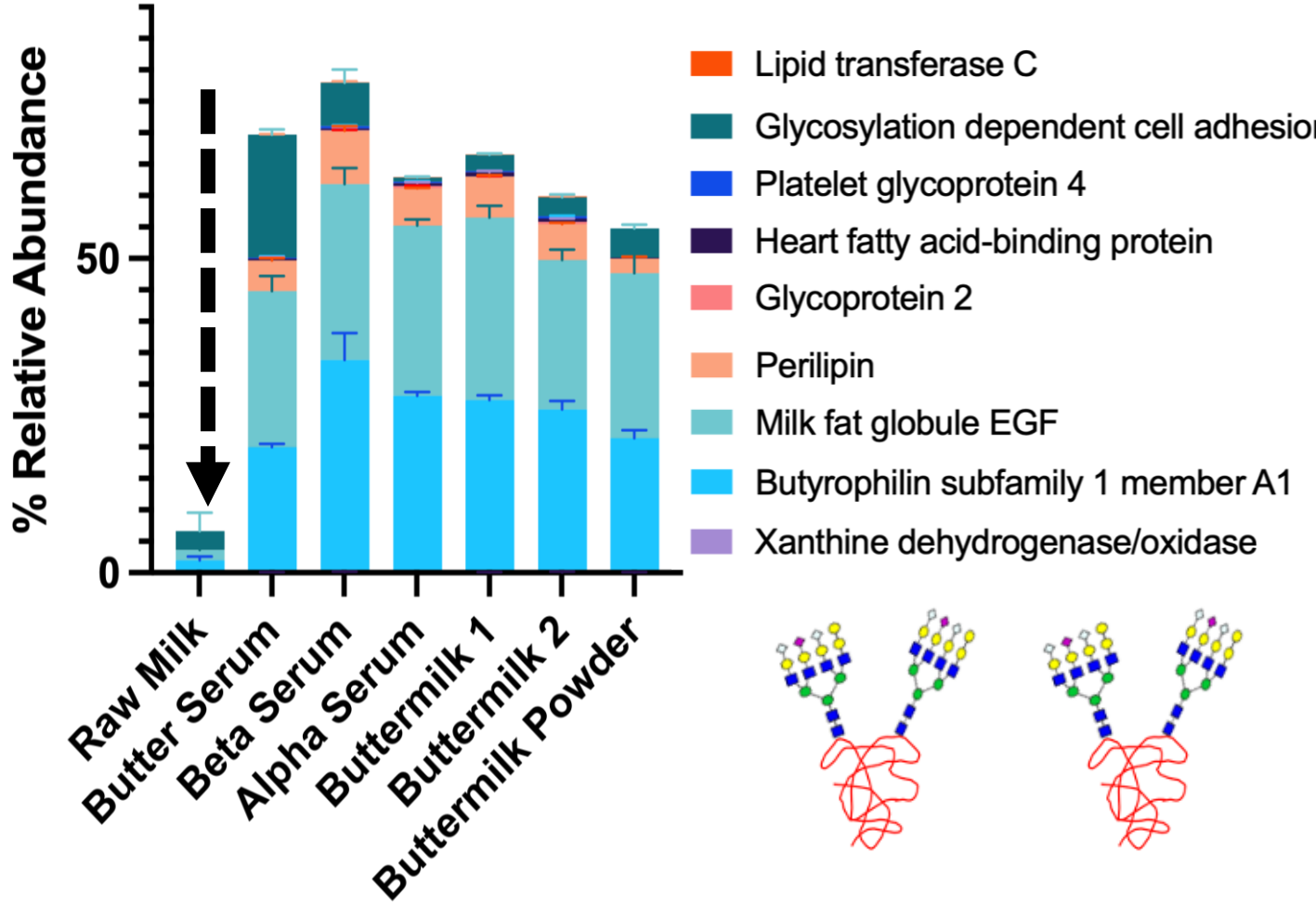
Proteomics Results

Identified over 1200 different proteins

Conventional Milk Proteins



MFGM Proteins



MFGM-enriched yogurt from butter streams: proof of concept already exists

Quality of Set Yogurts Made from Raw Milk and Processed Milk Supplemented with Enriched Milk Fat Globule Membrane in a Two-Stage Homogenization Process

by Joshua Oladapo Ibitoye [1], Binh Ly-Nguyen [1], Duy Nghia Le [1], Koen Dewettinck [2], Antoine P. Trzcinski [3] and Thi Thanh Que Phan [1,*]

Adding beta-serum MFGM improved set yogurt **texture and quality**.

Homogenization helped give a **denser, more compact yogurt gel** via MFGM interaction with milk proteins

https://doi.org/10.3390/foods10071534

Conclusions

- **First** comprehensive compositional characterization for **WPPC** and **all butter/AMF streams.**
- Glycomics and proteomics enabled molecular-level characterization
- All samples showed presence of MFGM materials, especially an **abundance of glycoproteins and phospholipids (compared to milk).**
- **Membrane filtration** is the next step to **reduce lactose** and **enrich MFGM-rich fractions** for functional testing.. and yogurt too!

Thank you!

California Dairy Innovation Center