The complexity of milk structure and why this makes dairy products healthy

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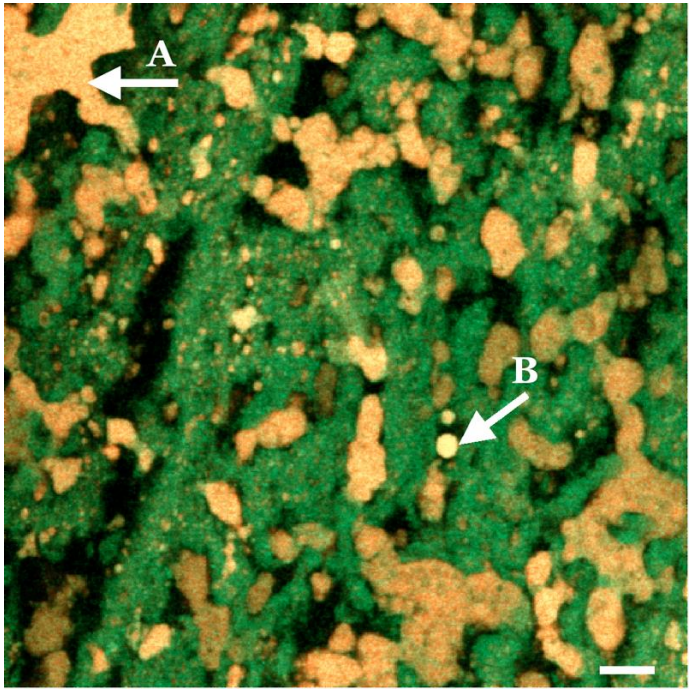
Complex structures



Pizza cheese
A: pool of free oil; B: emulsified fat globule

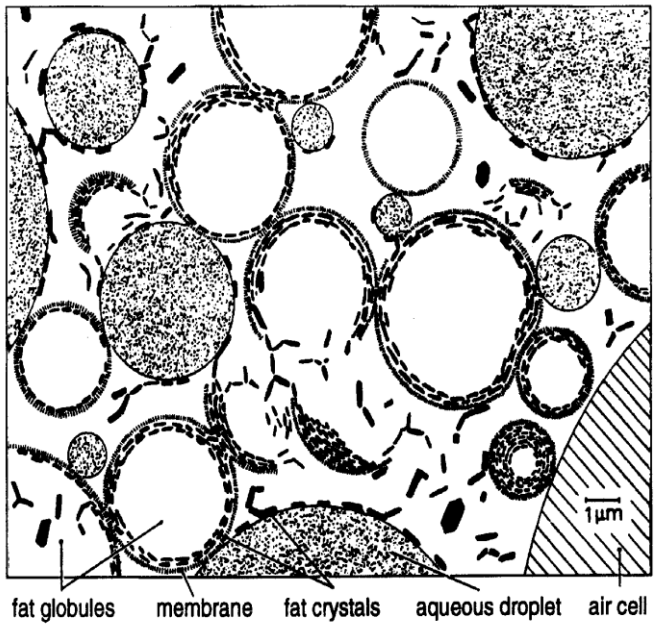
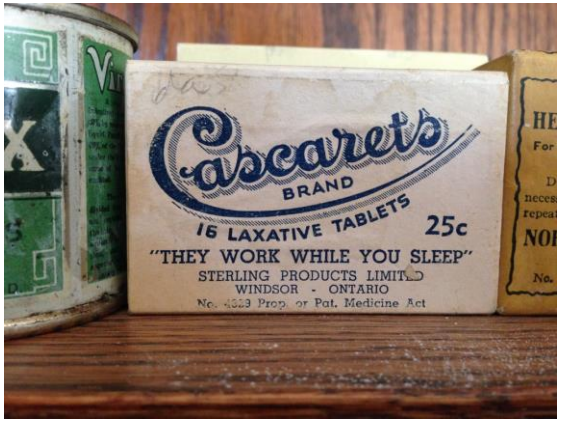


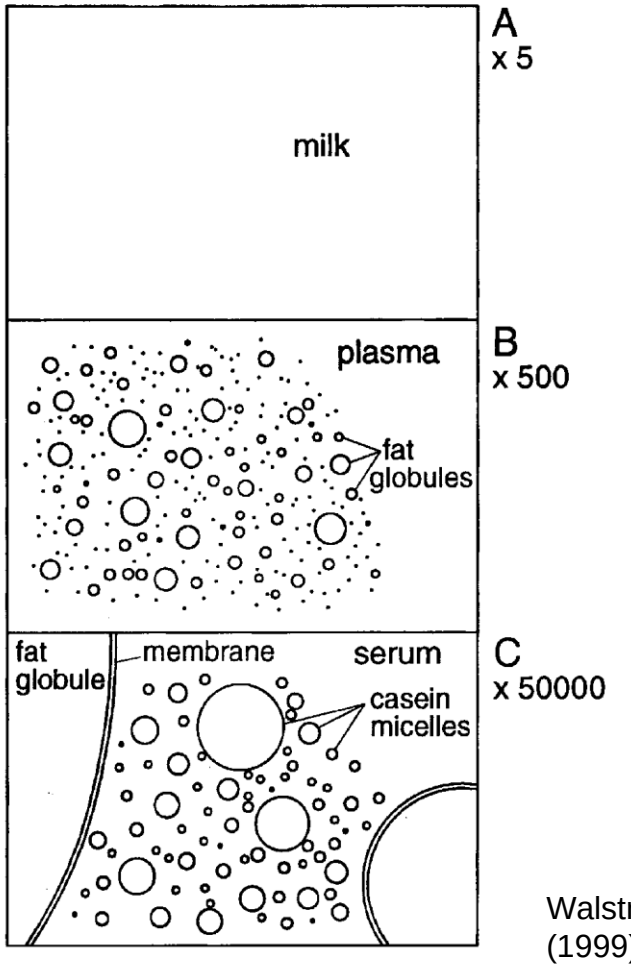
FIGURE 19.11 Butter microstructure at room temperature. Liquid fat is white. Membrane thickness is much (about 10 times) exaggerated. After H. Mulder and P. Walstra, *The Milk Fat Globule* (Wageningen: Pudoc, 1974).

Butter

A diet where structure is not as important



Interfaces in bovine milk



Water	87%
Lactose	4.5%
Fat	4.0%
Casein proteins	2.8%
Minerals (calcium phosphate)	0.8%
Whey proteins	0.7%

Fat contains β-carotene (yellow color)

4% C4 butyric acid in sn-3 position

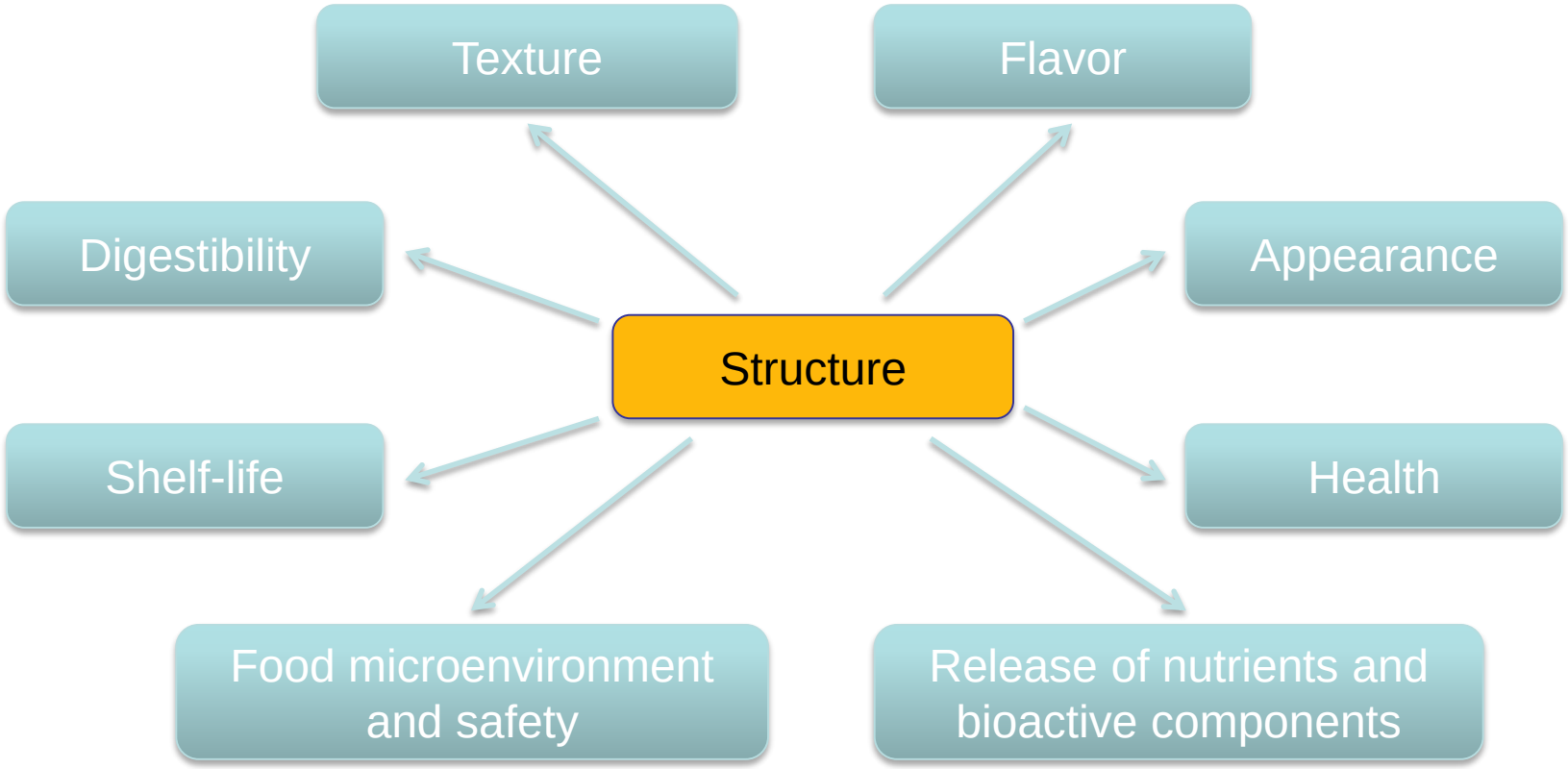
Exist as milk fat globules, 0.1 to 15 µm in size

75% smaller than 1 µm

15 billion globules per mL

Walstra, P., T. J. Geurts, A. Noomen, A. Jelema, and M. A. J. S. van Boekel (1999). Dairy Technology: Principles of Milk Properties and Processes. Marcel Dekker, Inc., New York.

The importance of dairy food structure



Generation of new food structures

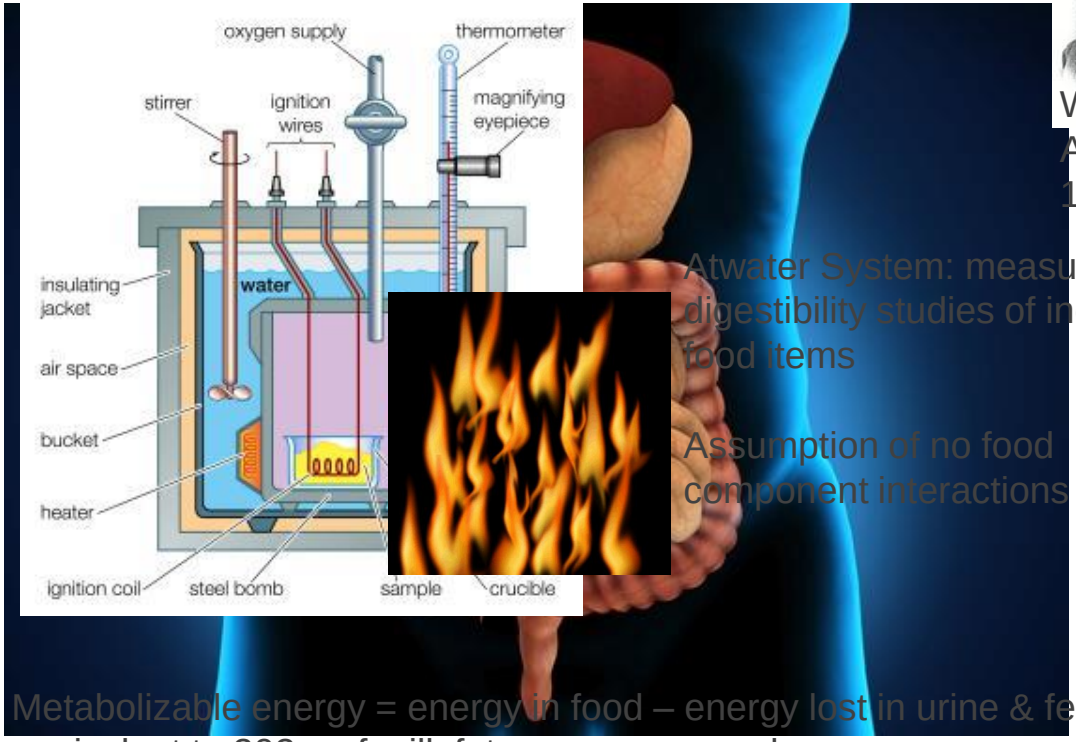
Texture and flavour reactions generated at surfaces…

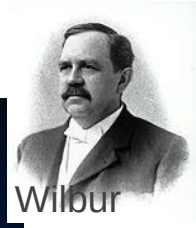
- Partitioning effects of flavour volatile precursors
- Impact of phase incompatibility
- Effect of extraction method
- Effect of milk processing conditions (heating, cooling, churning, homogenization, pulsed electric field processing
- Proximity of enzymes and substrate
- Matrix protection of enzymes
- Impact of oral processing conditions

It's much more than just calories!

Minimum energy requirements of 7500 kJ per day (FAO)

Bomb calorimeter

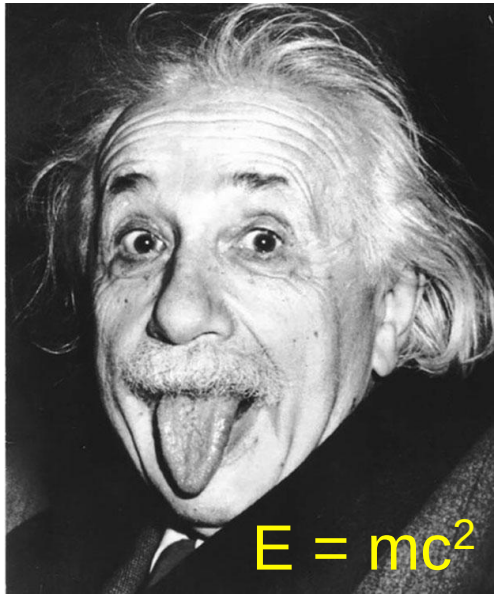




Wilbur Atwater
1844-1907

Atwater System: measured from digestibility studies of individual food items

Assumption of no food component interactions



Metabolizable energy = energy in food – energy lost in urine & feces; equivalent to 202 g of milk fat per person per day

Equivalent to 0.08 µg of food per person per day!

Human digestive conditions

www.dreamstime.com

Large intestine (cecum, colon, rectum, and anal canal): 2 m^2 area, absorbs water; intestinal microbiota

Stomach: 2nd phase of digestion; secretes proteases, gastric lipase; volume 1 L; pH 1-3, increases when food present; transit of 2 h; chyme enters the small intestine

Small intestine: duodenum (25 cm; neutralizes stomach acid; release of bicarbonate, trypsin, lipase and amylase by pancreas), jejunum (pH 7-9; 2.5 m; sugars, amino acids, and fatty acids absorbed), ileum (pH 6-7.5; 3 m; absorbs bile salt micelles)

Total transit time: 12-50 hours

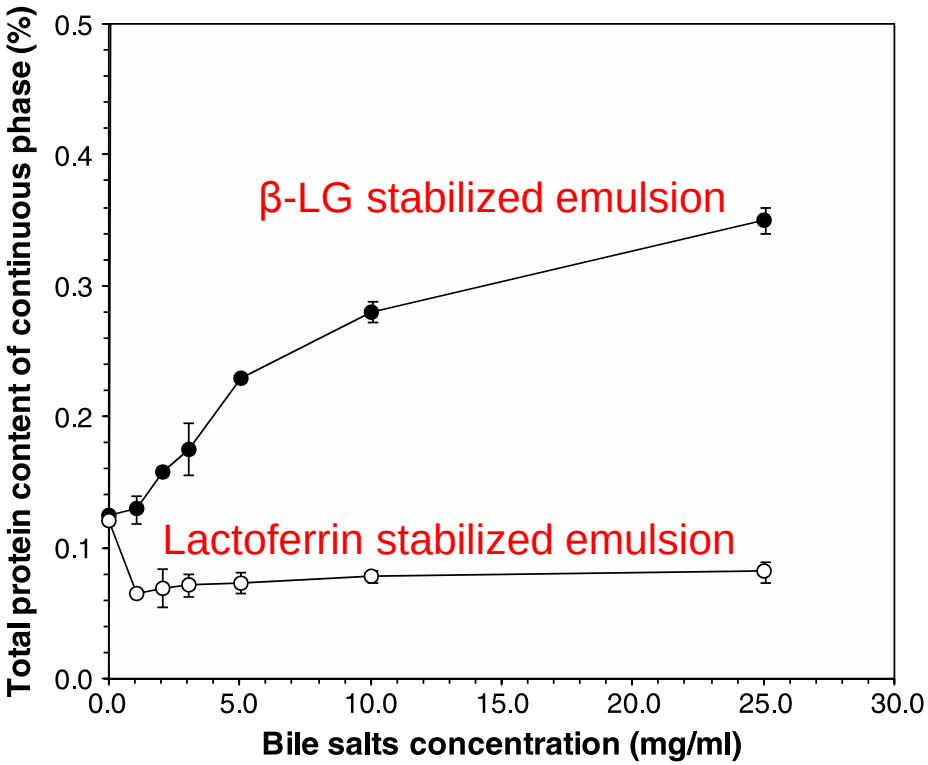
When stretched out, the GI tract is ~9 m long; folds increase surface area

Bile salts synthesized in liver from cholesterol; stored in the gall bladder; transported to duodenum as cholesterol, phospholipid and fatty acid mixed micelles

Protein digestion

- Thermal treatment and high pressure processing can partially unfold β-LG with increased digestibility
- Enzymatic cross-linking of proteins can reduce digestibility
- Adsorption of proteins to interfaces can increase digestibility through partial unfolding

Digestion of adsorbed proteins on emulsion surfaces



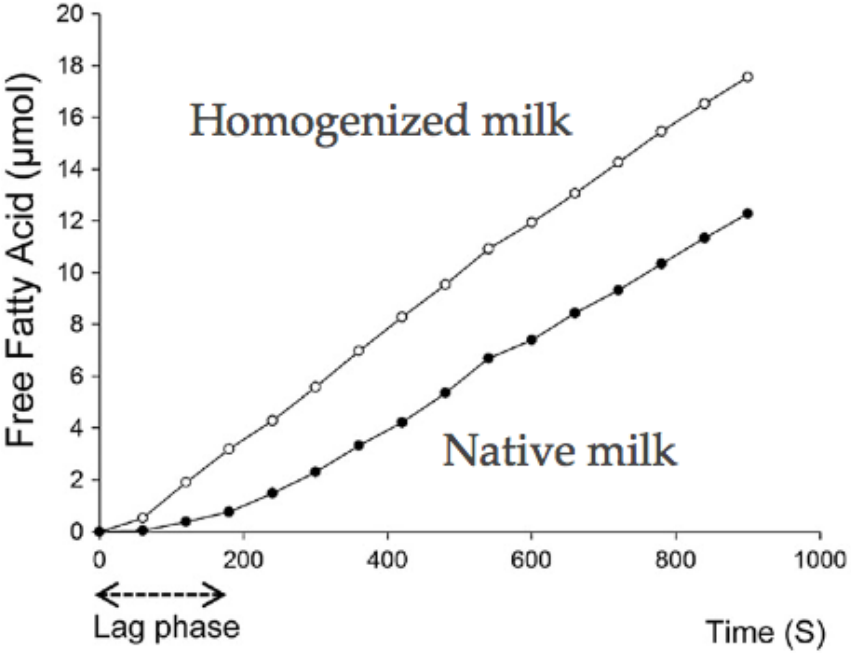
Adsorption of bile salts caused partial displacement of β-LG

Binds to positively charged lactoferrin without displacement

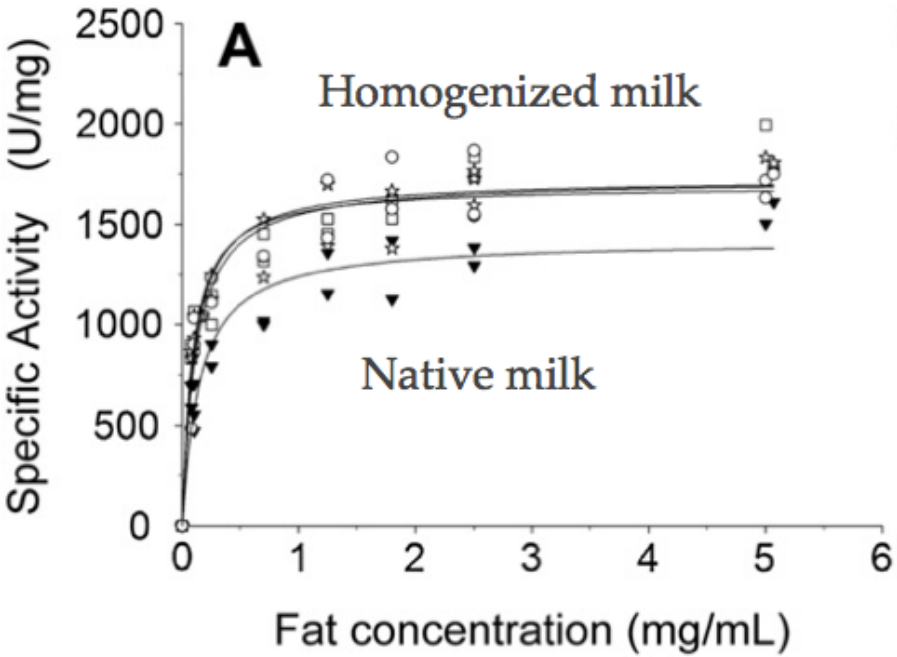
Singh & Sardar (2001). *Advances in Colloid and Interface Science 165*, 47–57

Digestion of homogenized globules

Lipolysis



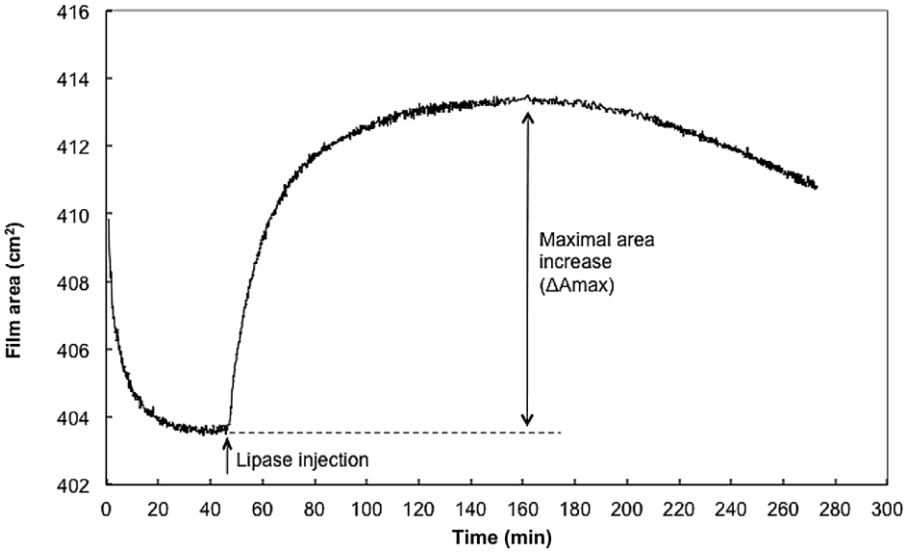
Pancreatic lipase activity

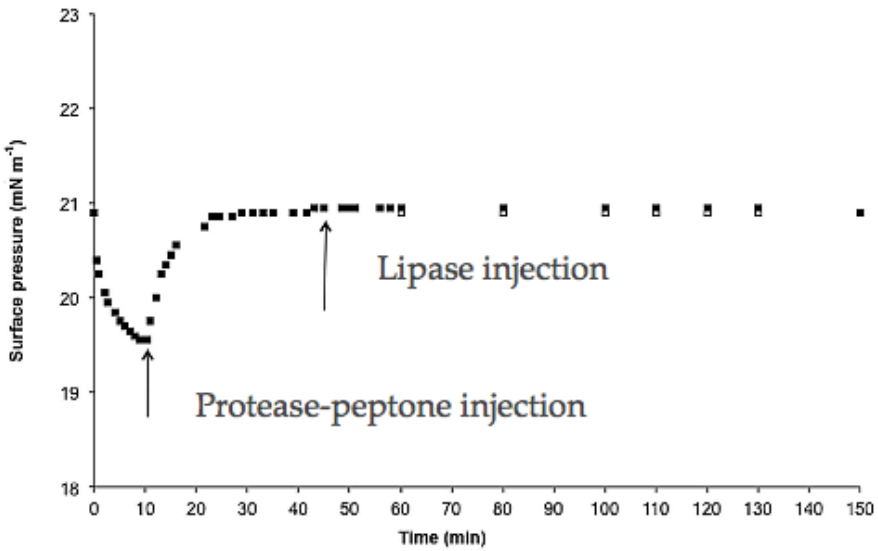


Berton, Rouvellac, Robert, Rousseau, Lopez, & Crenon (2012). *Food Hydrocolloids 29*, 123-134.

Homogenized globule surfaces in milk are less favorable to pancreatic lipase activity, but activity increases due to smaller size and greater total surface area.

Interfacial lipid digestion

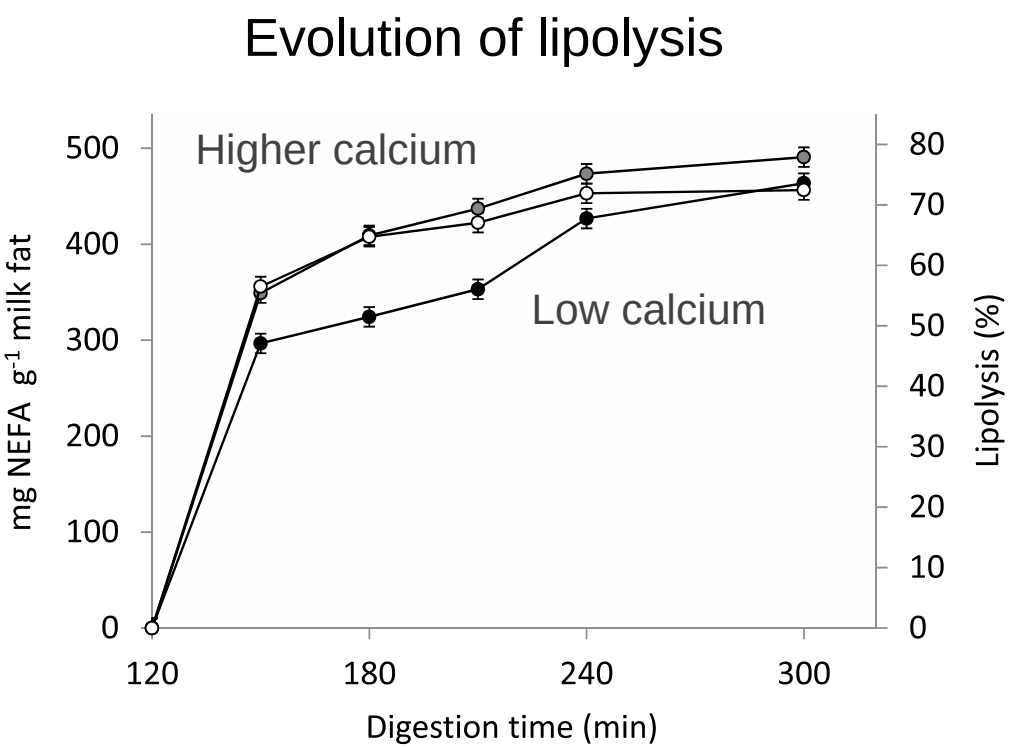




Danthine & Blecker (2014). *International Dairy Journal 35*, 81-87

- Lipases can penetrate native MFGM, increasing film area (and therefore interfacial tension at constant surface area)
- Lipolysis occurs more readily in homogenized globules
- Protease-peptone inhibits lipolysis by increasing globule interfacial tension

Digestion of cheese



Ca precipitates free fatty acid under near neutral intestinal conditions, allowing lipases access to neutral lipid interior.

Greater extent of fat globule aggregation at higher Ca.

Ayala-Bribiesca, E., Lussier, M., Chabot, D., Sylvie L. Turgeon, S.L., Britten, M. (2016). *International Dairy Journal 53*, 1-9.

Postprandial FA lymphatic absorption

- Dairy products containing 30 g of lipids with similar fatty acid profiles:
  - Butter < cream and cream cheese
  - Butter, mozzarella, and milk similar, but peak TAG delayed for butter (Type 2 diabetes subjects)
  - Differences attributed to the dispersive state of milk fat.

Fruekilde & Hoy (2004). *Journal of Nutrition 134,* 1110–3.

Clemente, Mancini, Nazzaro, Lasorella, Rivieccio, Palumbo, et al. (2003). *Nutrition, Metabolism and Cardiovascular Diseases. 13,* 377–83.

Novel ingredients from milk

Case study: the milk fat globule membrane (MFGM)

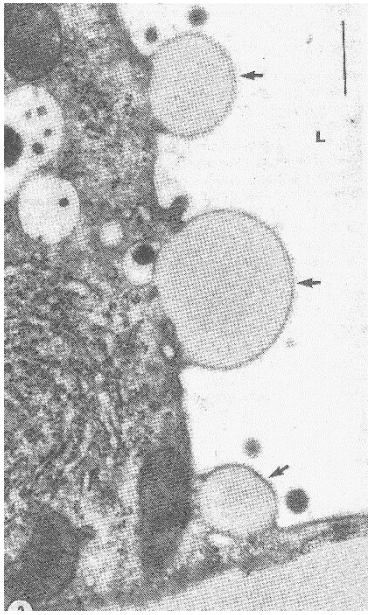
Originates from lipid droplet extrusion from the mammary epithelial cells

Protects milk fat globule from lipolysis and coalescence

Contains components with bioactive functionality

Major component of buttermilk

Emulsification, carriers of flavor compounds



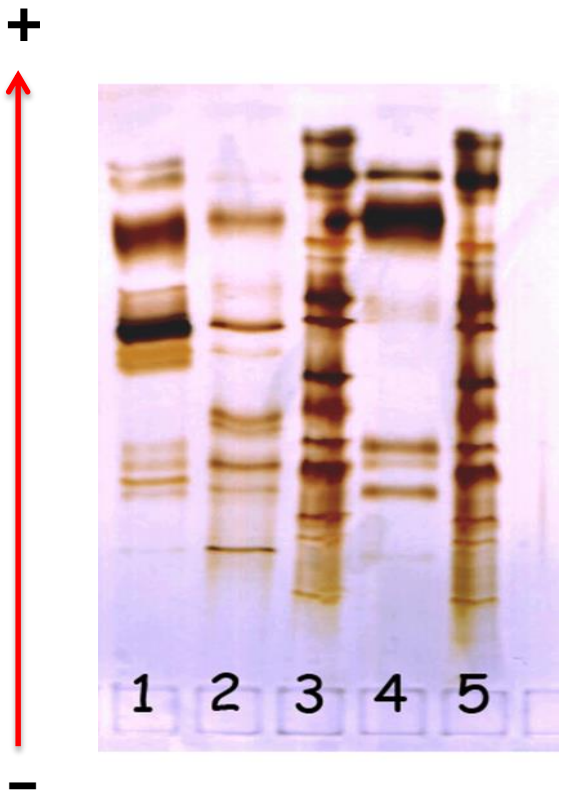
Keenan, Mather & Dylewski, Physical Equilibria Lipid Phase, In N.P. Wong, *Fundamentals of Dairy Chemistry*, 3rd ed. (1988)

Composition of the MFGM

Component	mg/100g fat	mg/100mg MFGM
Proteins	1800	70
Phospholipids	650	25
Cerebrosides	80	3
Cholesterol	40	2
Monoacylglycerides	Present	Unknown
Water	Present	0
Carotenoids	0.04	0
Total	>2570	100

Composition depends upon the method of extraction

MFGM proteins



1: Low-heat skim milk powder
2: MFGM extract
3. Wide-range MW markers
4: Xanthine oxidase extract
5: Wide-range MW markers

Estimated MW/kDa	Protein identification
(150)	mucin 1
130 (150)	xanthine oxido-reductase
84 (95-100)	PAS III
72 (76-78)	CD 36
65 (67)	butyrophilin
59 (48-54)	Periodic acid/Schiff 6/7
56 (52)	adipophilin
35	unknown
31	unknown
23	α_s andβcaseins
14	α-lactalbumin
11 (13)	fatty acid binding protein

XO accounts for around 20% of membrane proteins

Polar lipids in MFGM

Polar lipid class	% of total polar lipids
Phosphatidylcholine (PC)	36
Phosphatidylethanolamine (PE)	27
Sphingomeylin (SM)	22
Phosphatidylinositol (PI)	11
Phosphatidylserine (PS)	4
Lysophosphatidylcholine (LysoPC)	2

Composition depends upon the method of extraction

Factors that affect composition

- Age of cow
- Breed of cow
- Stage of lactation
- Bacteriological state of milk
- Seasonality
- Diet of cow
- Milking frequency
- Processing effects: homogenization, storage temperature

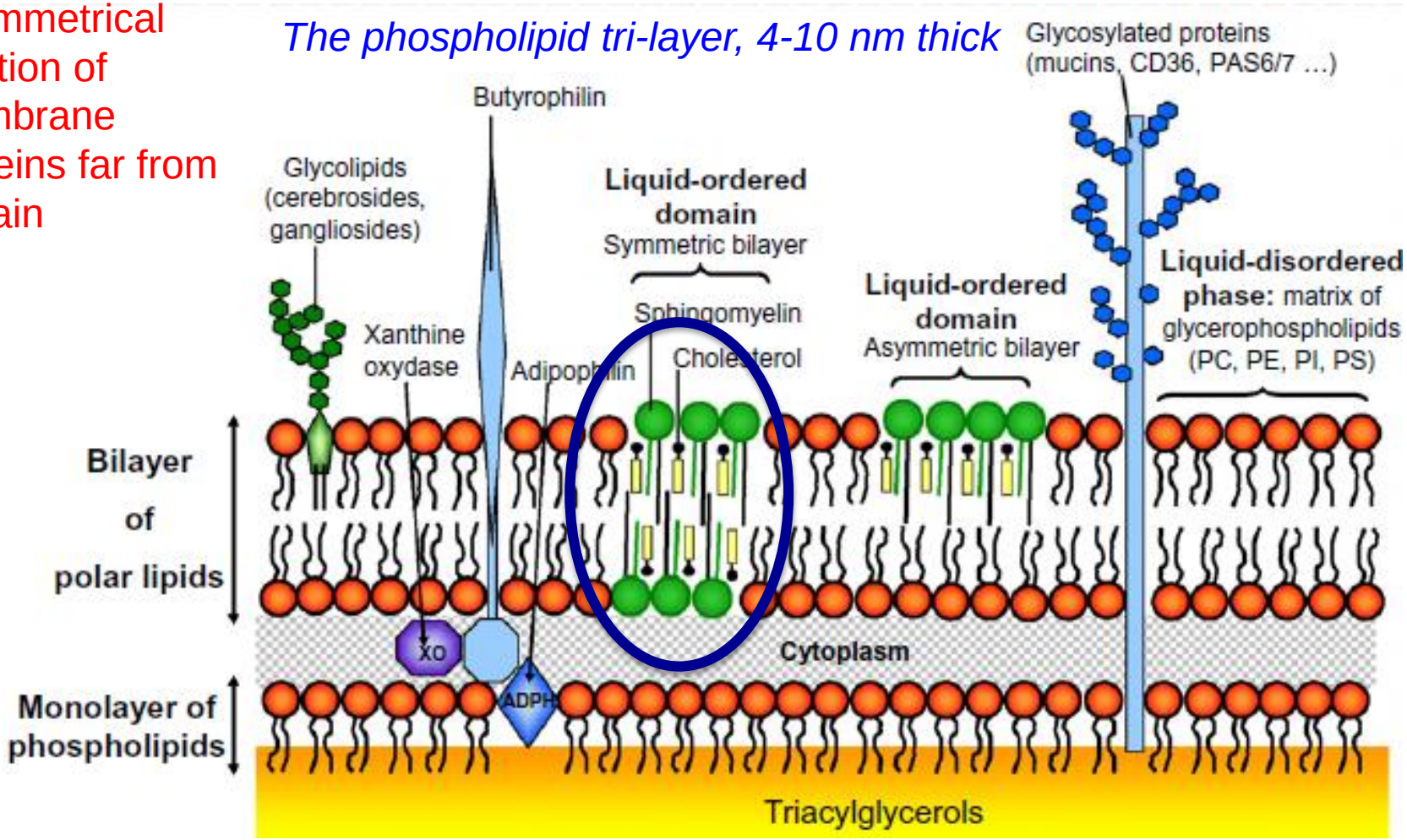
Factors impacting upon lipid digestibility

- Gastric lipase must adsorb to globule surface — displacement, depending upon the surface tension
- Smaller fat droplets result in slower gastric emptying, and greater lipolysis due to increased total surface area.
- Native human milk fat globules have faster gastric lipolysis than smaller, homogenized droplets in infant formula.
- Rate of lipid hydrolysis: casein < phospholipids
- Human gastric lipase more efficient when globules coated with PC, PI or PS; lowest for SM (PC dominant component of outer surface)
- Lipolysis by pancreatic lipase greater when globules coated with whey proteins or caseins (homogenized vs. unhomogenized) — possible size effect.
- Increased viscosity delays gastric emptying

MFGM topological model

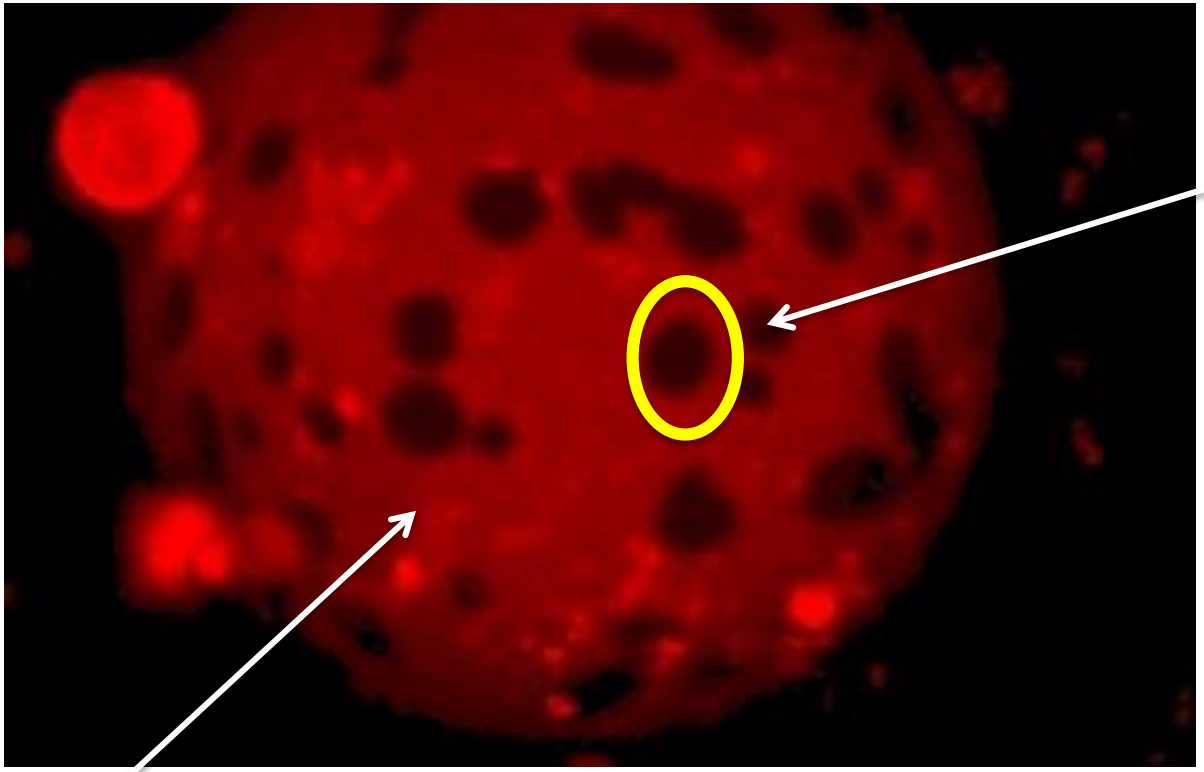
Asymmetrical location of membrane proteins far from certain

The phospholipid tri-layer, 4-10 nm thick



Lopez C. et al., Food Chemistry 125: 355-368 (2011)

Colloid surface reactions – milk fat globule



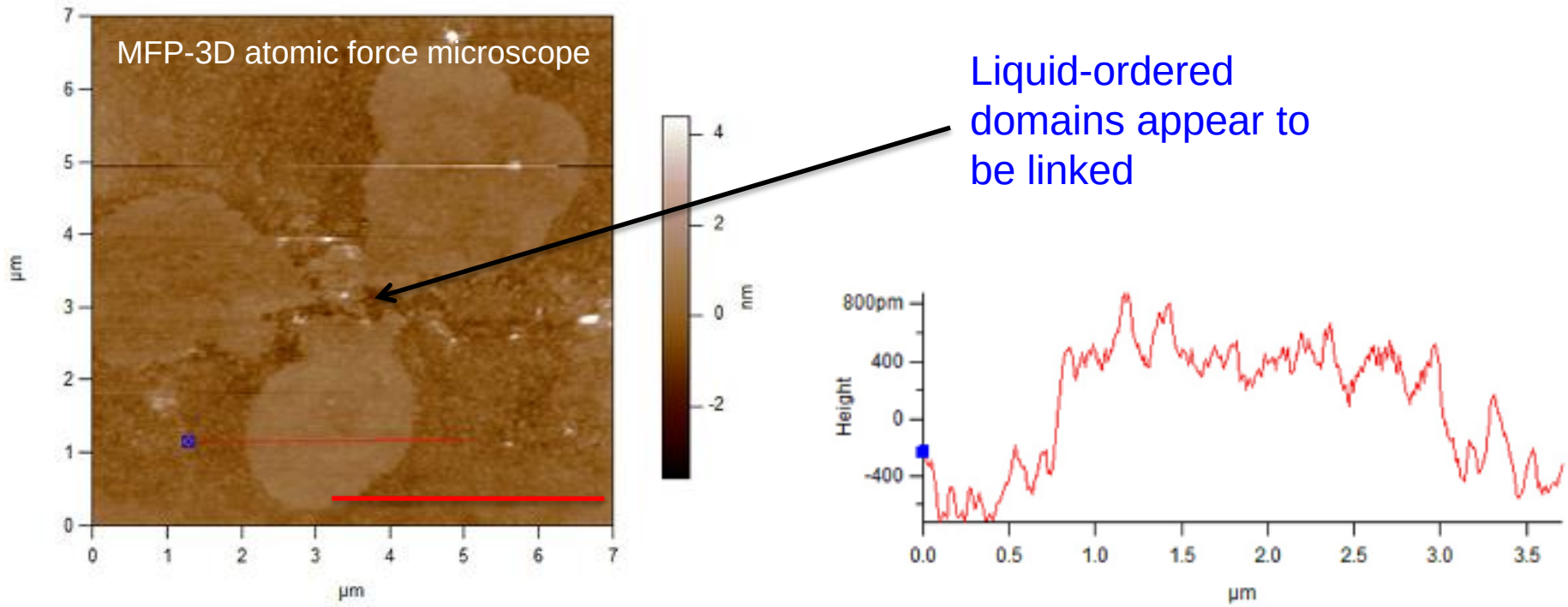
Liquid ordered L_0 regions rich in sphingomyelin (highly saturated, longer chain) and cholesterol

Two-dimensional reactions on emulsion and other colloidal surfaces to generate texture and flavour reactions

Liquid disordered region

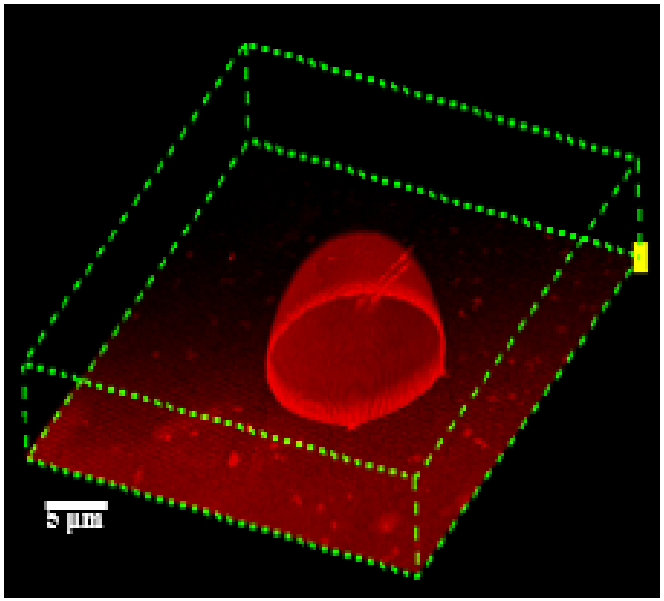
Gallier, S., Gragson, D., Jiménez-Flores, R., Everett, D.W., *J. Agric. Food Chem.* 58: 4250–4257 (2010)

Atomic force microscopy (AFM)

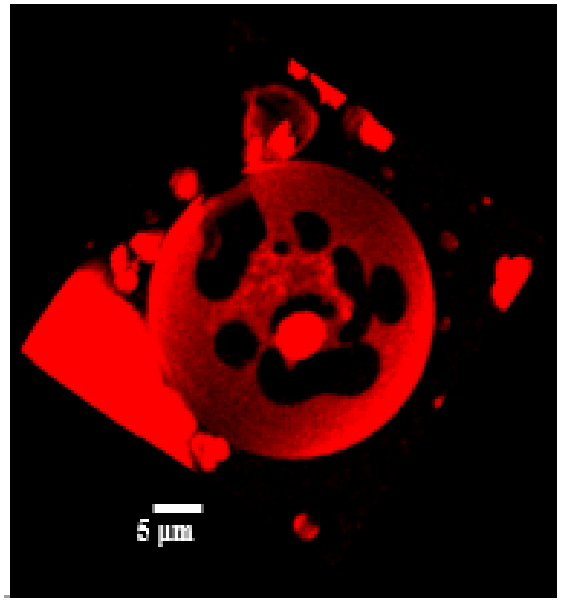


Liquid-ordered domains are thicker by approximately 1 nm compared to liquid disordered domains

Giant unilamellar vesicle model systems



GUV generated from electroformation.



Non-fluorescent lipid domains formed with DPPC/DOPE 3/7 mol/mol in a GUV system.

Model milk fat globule vesicles to examine surface structures

Zheng, H., Jiménez-Flores, R., Gragson, D. & Everett, D.W. *J. Agric. Food Chem.*, *62*, 3236-3243 (2014)

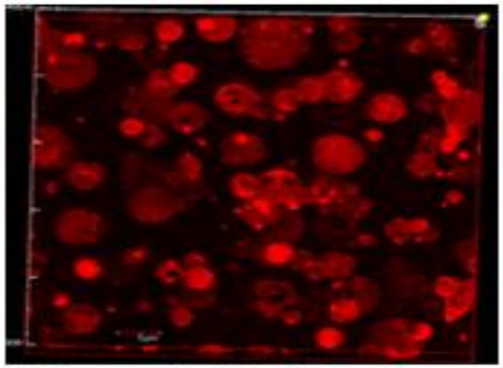
Compartmentalisation of enzymes

Redox enzymes
Xanthine oxidase
Cytochrome C reductase

Hydrolases
Acetylcholine esterase
Alkaline phosphatase
Acid phosphatase
5'-Nucleotidase
Glucose-6-phosphatase
Phosphodiesterase
Adenosine triphosphatase

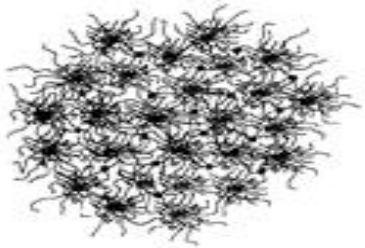
Lyase
Aldolase

Transferases
γ-Glutamyl transferase
Galactosyl transferase



Gallier, et al., J. Agric. Food Chem. 58: 4250–4257 (2010)

Casein micelle
Sulfhydryl oxidase
Lactoperoxidase
Superoxide dismutase
Ribonuclease

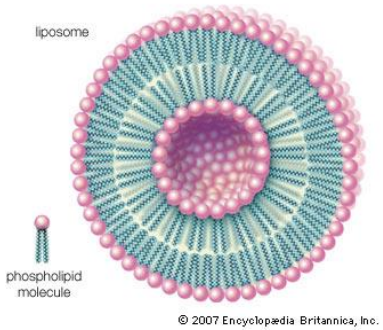


Holt, C., Advances in Protein Chemistry, vol. 43: 63-151 (1992)

Serum phase
Lipoprotein lipase
Plasmin (lower pH)

MFGM functionality

- Biologically relevant membrane with multitude of components
- Functional flavor and texture properties
  - Emulsification
  - Liposomes as carriers and flavor masking agents
  - Impact of isolation procedure
- Impact on cheese (added as buttermilk)
  - Increases moisture content and yield
  - Decreases free oil in pasta filata varieties
  - Improved flavor (bacteria congregate near fat globule interface, and MFGM components may provide a carbon source)



MFGM extraction from buttermilk

Commercial cream separation

Buttermilk powder available on a large scale, is inexpensive, but has functional problems

Buttermilk powder isolation

Washing of cream

Wash with water, simulated milk ultrafiltrate, dissociating agents, or surfactants

Churn cream into butter

Butter

Cool temperatures ~15°C

Buttermilk

Removal of caseins by dissociation with citrate, rennet to remove casein aggregates, ultracentrifugation, dialysis, aggregation of MFGM fragments at low pH, microfiltration, super-critical fluid extraction

Lyophilization or heat drying

Cold-drying retains functionality of labile components

Buttermilk powders

(Bakery, dairy and ice cream applications)

Valio sweet butter milk powder

Historically considered a low-value product…but huge potential!

Current global market (2-2-2016) price $1513 ($1480-1700 on 2-12-2016) per ton
Global prices dropped 43% over last 12 months

Compare with SMP $1792 ($1350-2300); WMP $1952 ($2000-3040); rennet casein $4362 per ton

Fonterra butter milk powder

Products usually promoted for their good solubility, clean flavor, and emulsifying efficacy.

High heat butter milk powder

MFGM health claims

Functional health properties* of MFGM phospholipids

- Sphingolipids, including sphingomyelin and metabolites (ceramide, sphingosine, sphingosine-1-phosphate, cermaide-1-phosphate)
  - colon anti-carcinogenic properties
  - cholesterol and LDL adsorption lowering effects by lowering liposome membrane fluidity, raises HDL levels
  - trans-membrane signal transduction and regulation of immune cell development
  - cell growth and apoptosis
  - lipoprotein formation
  - mucosal growth in the gut
  - associated with age-related diseases, such as Alzheimer's
  - ameliorate inflammatory processes in atherosclerosis
  - treatment for insulin resistance, dyslipidemia, cardiovascular diseases
  - protection against bacterial and virus infections
- Fatty acid binding protein
  - anti-carcinogenic properties (colon, breast)

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* Scientific literature reports - evidence in some cases is weak, and in some cases insufficient

MFGM health claims

Functional health properties* of MFGM phospholipids

- Phosphatidylserine
  - positive effects on Alzheimer's patients
  - restoration of memory
  - alleviate muscular soreness
- Phosphatidylcholine
  - support liver recovery
  - protect human gastrointestinal mucosa against toxic attack
  - reduced life-threatening necrotizing enterocolitis
- Lactadherin
  - protection against gut viral infection
- Butyrophilin
  - suppression of multiple sclerosis
- Lyso-phosphatidylcholine and xanthine oxidase
  - bacteriocidal and bacteriostatic properties (*S. aureus, E. coli, Sal. enteritidis*)

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* Scientific literature reports - evidence in some cases is weak, and in some cases insufficient

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Neo Kid-Plus

Sphingolipids
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Nucleotides
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Phospholipid concentrates: sphingomyelin
Cell growth and regulation

Gangliosides: mono-sialo ganglioside 3 (GM3), di-sialo ganglioside (GD3) and phosphatidylserine
Infant learning and development, maintain gut health and balancing the immune system

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frieslandcampina.com

Nutritional milk powders for children
5x Docosahexaenoic acic
Sialic acid

Dutch Lady Growing Up Milk 123 for ages 1+
Dutch Lady Growing Up Milk 456 for ages 3+
Dutch Lady Growing Up Milk 6+ for ages 6+

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Lacprodan® PL-20
Phosphatidylserine and sphingomyelin
Performance boost, contributes to healthy ageing, promotes cognitive development in infancy

Lacprodan® MFGM-10 for infant nutrition.
Lactoferrin, IgG, sialic acid, phospholipids and gangliosides
Neonatal gut maturation and myelination of the central nervous system
Gangliosides for beneficial gut microflora, and intestinal maturation and cognitive development
MFGM components for anti-pathogenic effects
Lactoferrin protects against microbial infections

Meiji – Global Brands Marketing

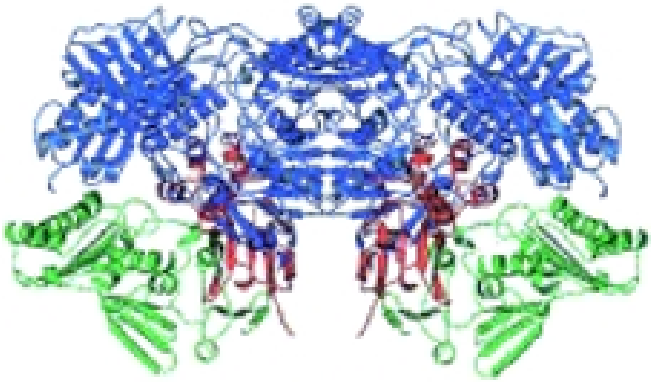
Meiji FM-T
Meiji mamilac
Meiji Fu

Fortified with docosahexaenoic acid
Cerebral and retinal development

Xanthine oxidase (XO)

In bovine milk, xanthine oxido-reductase is in the XO form

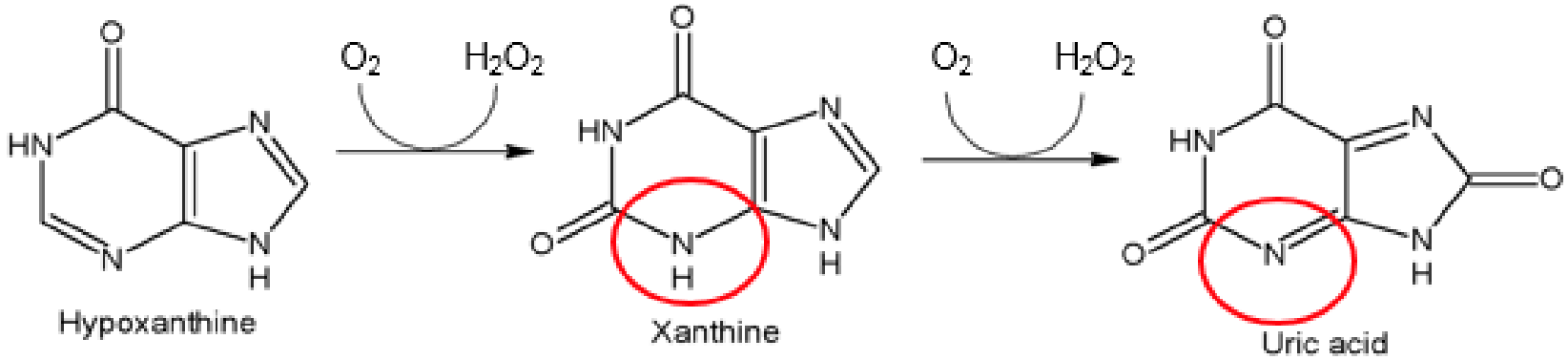
Capable of oxidising a wide range of aldehydes



XO has both bacteriocidal and bacteriostatic properties brought about by—

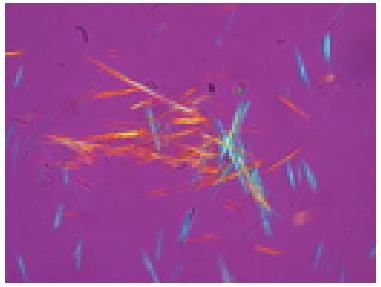
1. Production of reactive superoxide and hydrogen peroxide in the gut
2. Reduction of nitrite to nitric oxide, and to peroxynitrite
3. Stimulating lactoperoxidase system in milk (reductant + H_2O_2 → oxidant + H_2O)

Oxidation reactions of XO



Uric acid elevated in blood; crystals implicated in gout*.

XO is the target of the widely used anti-gout drug, Allopurinol, an isomer of hypoxanthine and a xanthine oxidase inhibitor.



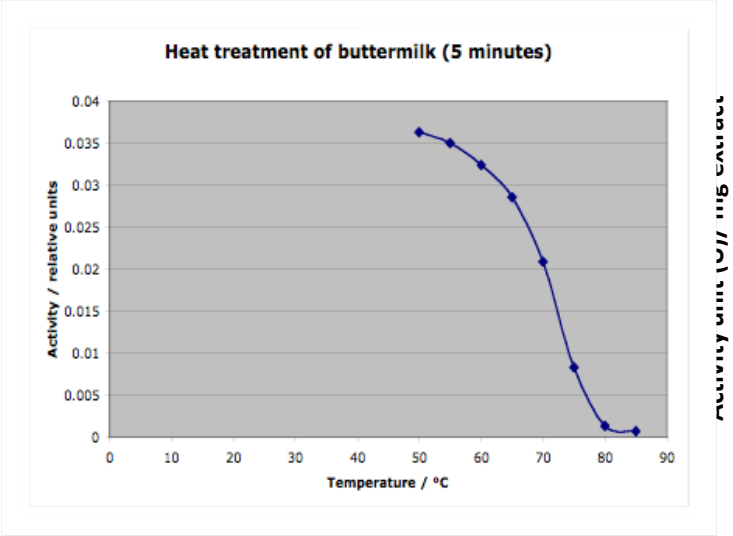
Xanthine oxidase	$XH + H_2O + O_2 \longrightarrow$	$X{=}O + H_2O_2$
Xanthine dehydrogenase	$XH + H_2O + NAD^+ \longrightarrow$	$X{=}O + NADH$
Aldehyde oxidase*	$RCHO + H_2O + O_2 \longrightarrow$	$RCOOH + H_2O_2$

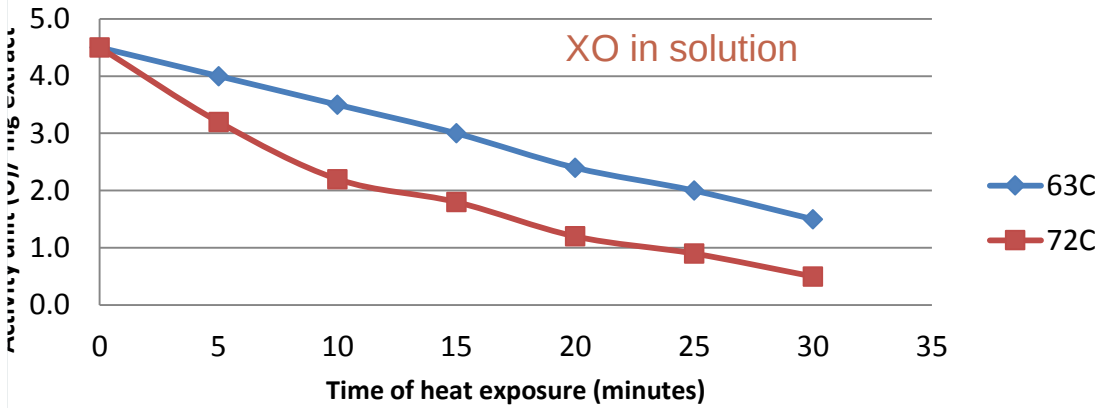
* Found mainly in liver

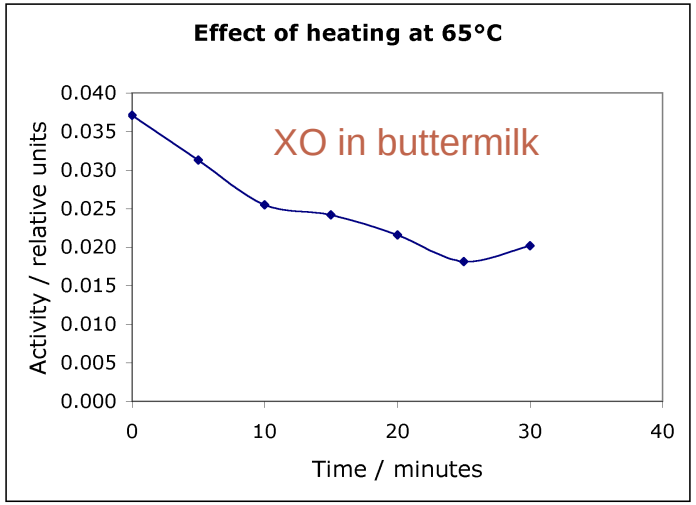
*Genetic, diet, and lifestyle causes
Consumption of alcohol, fructose-sweetened drinks, meat, seafood
Known as "rich man's disease, or "the disease of kings"

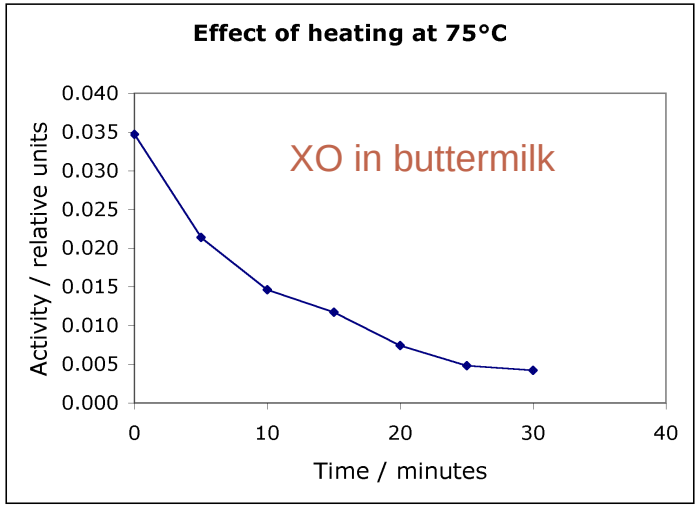
Matrix effect on XO activity

XO in solution and in buttermilk









CAL POLY
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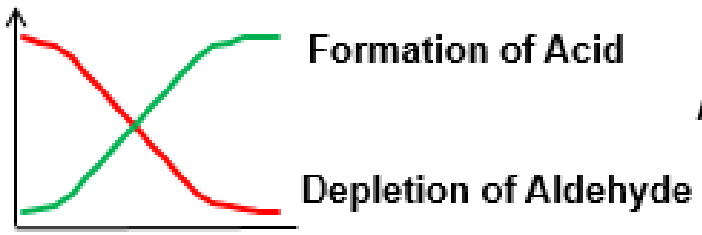
Volatiles produced by XO action



$$\text{Aldehyde} \xrightarrow{\text{XO}} \text{Acid}$$

Aldehydes acids pairs:

- acetaldehyde → acetic acid
- 2-methylpropanal → 2-methylpropanoic acid
- 3-methylbutanal → 3-methylbutanoic acid
- 2-methylbutanal → 2-methylbutanoic acid

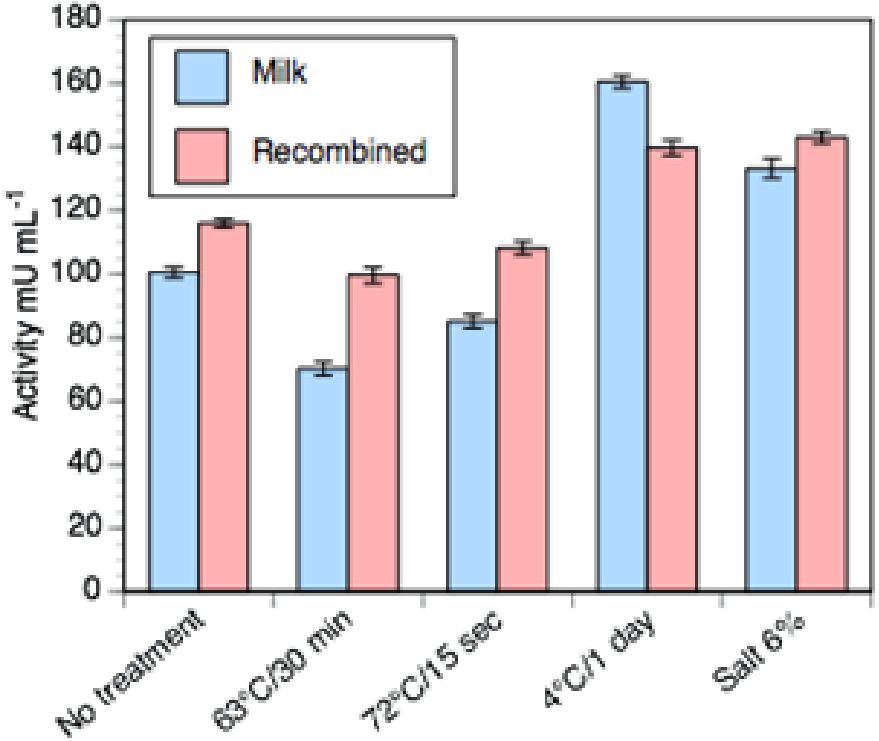
Short chain aldehydes are volatiles and can be analyzed by **headspace GC**

Corresponding acids are not sufficiently volatile at this concentrations to analyse by headspace GC, requiring derivatization to an ester

Xanthine oxidase activity in processed milk

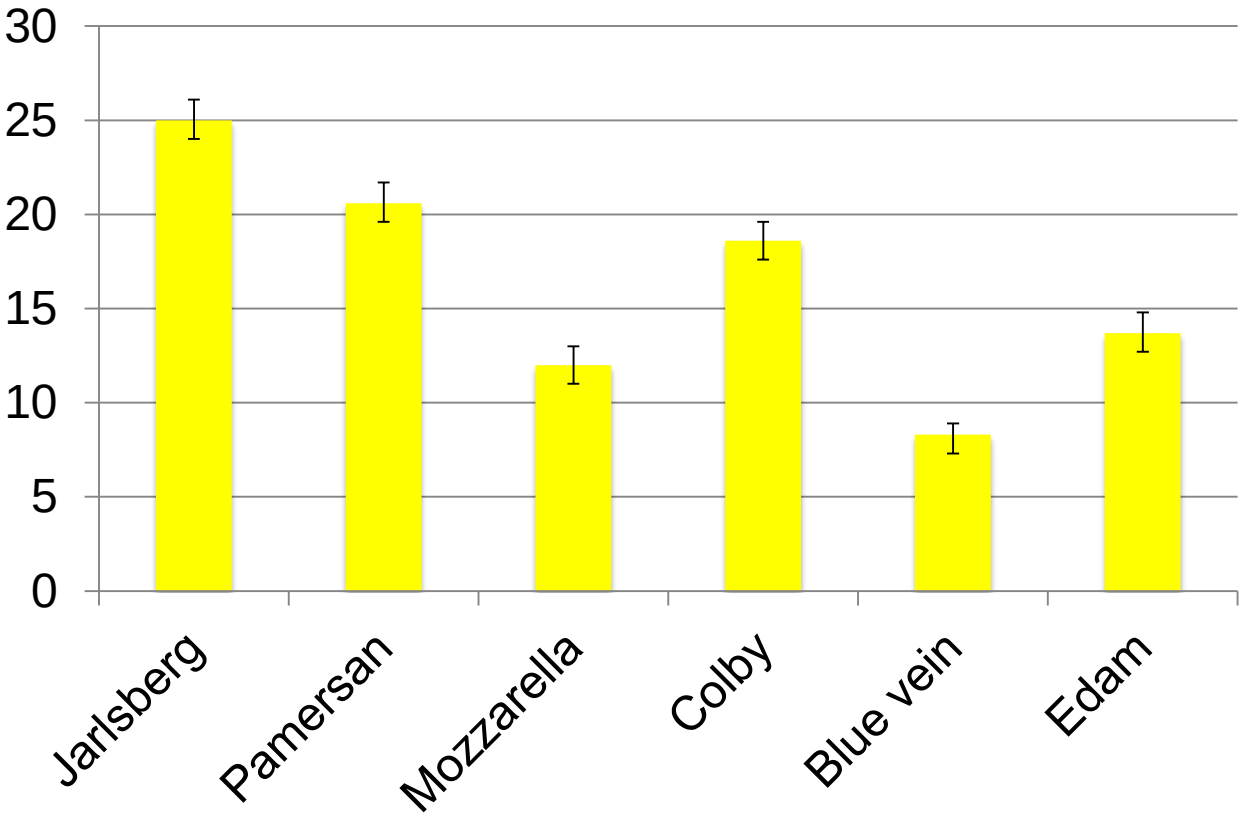
$$XH + H_2O + O_2 \longrightarrow X{=}O + H_2O_2$$

Located in the cytoplasmic region of the MFGM
Oxidation of aldehydes to acids
Increase in n-fatty acids leading to methyl ketones, γ- and δ-lactones.



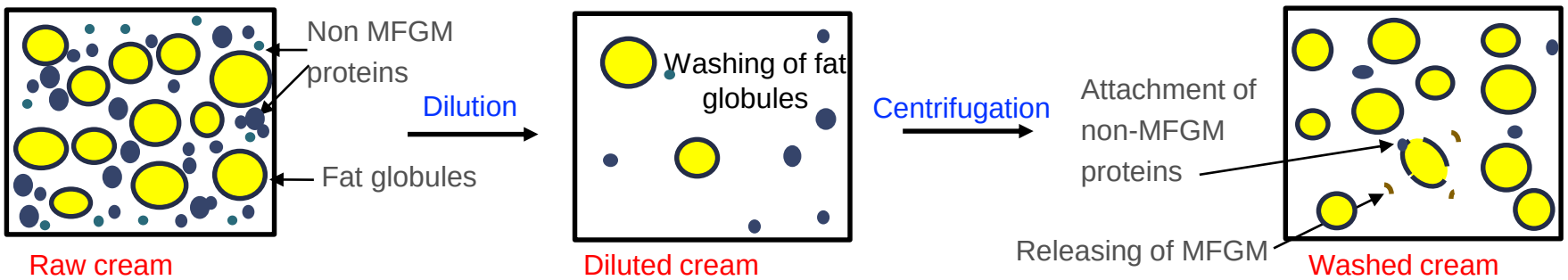
Kathriarachchi, K., PhD thesis, University of Otago (2015)

Xanthine oxidase activity in New Zealand cheeses



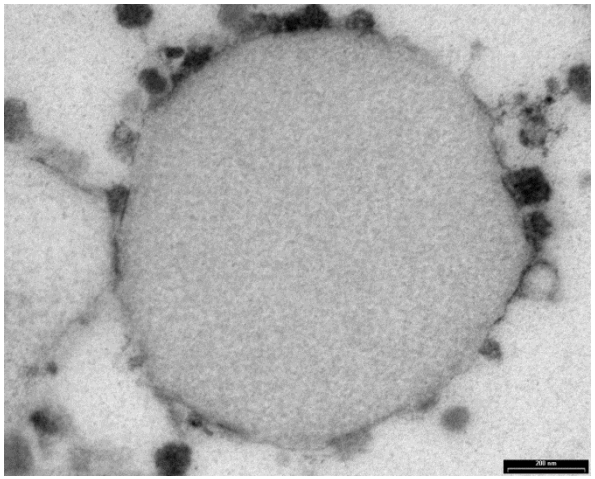
Ali Rashidinejad, PhD thesis, University of Otago (2015)

Washing cream with either simulated milk ultrafiltrate (SMUF) or water

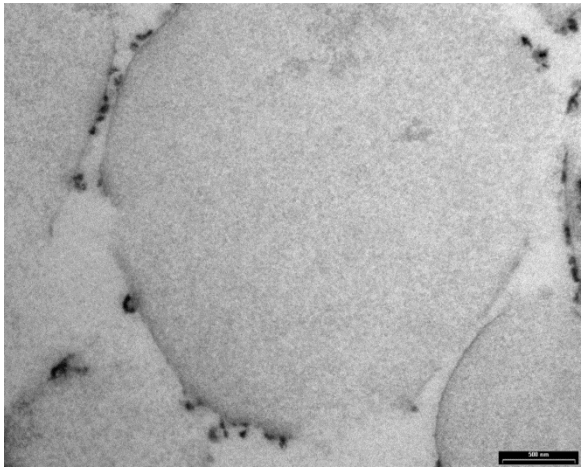


Washing fat globules

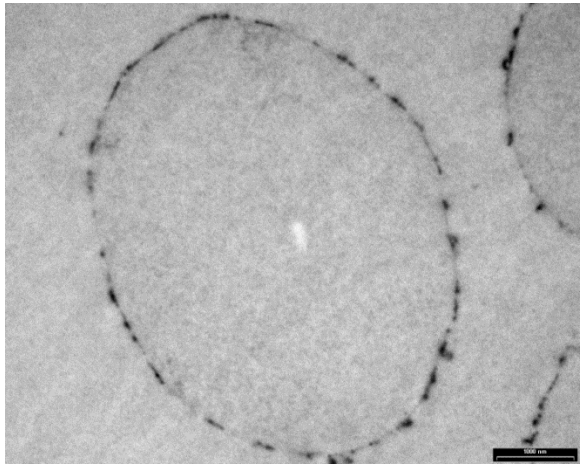
Scale bars: 5 µm



Native fat globule



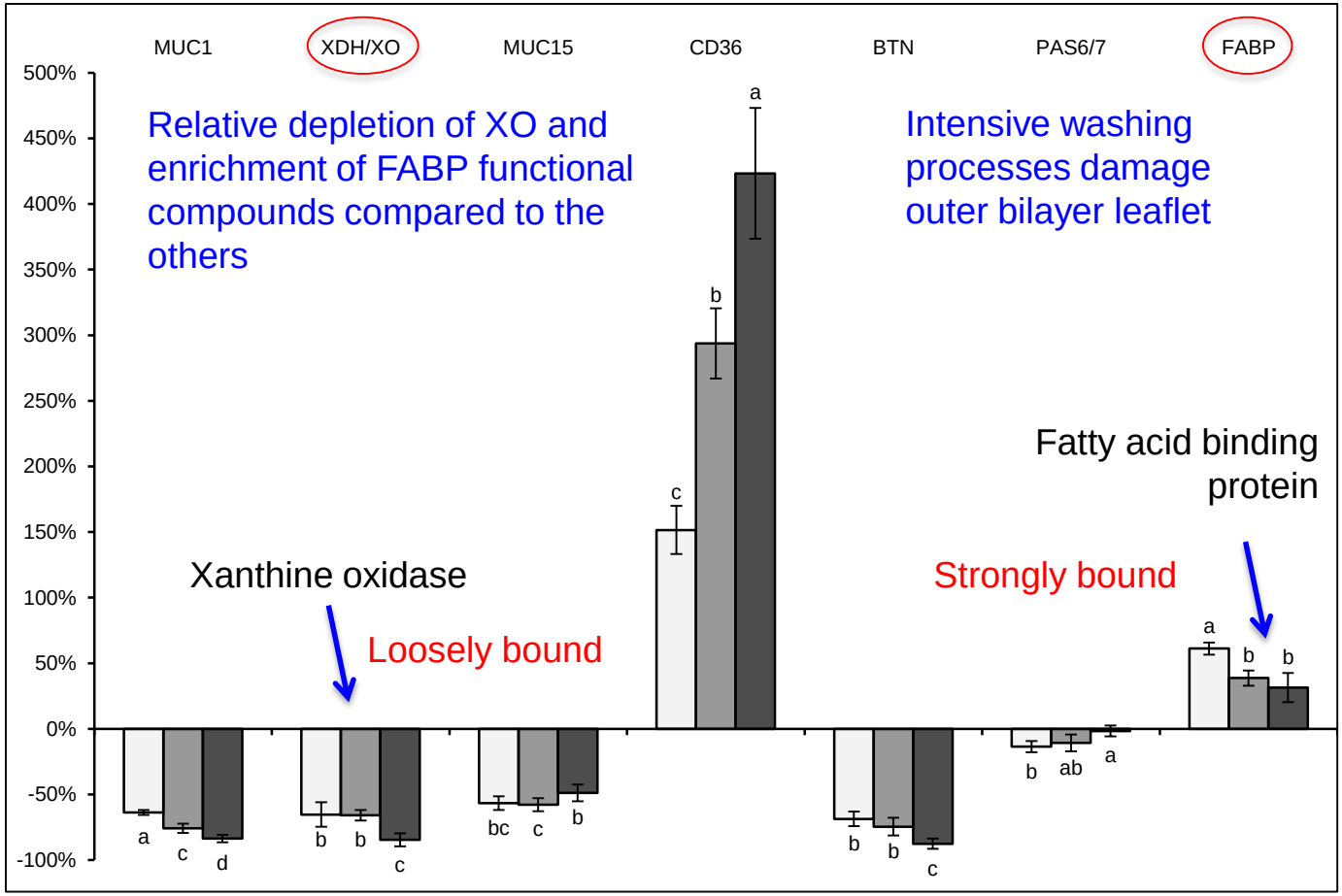
Fat globule washed with simulated milk ultrafiltrate (SMUF)



Fat globule washed with water

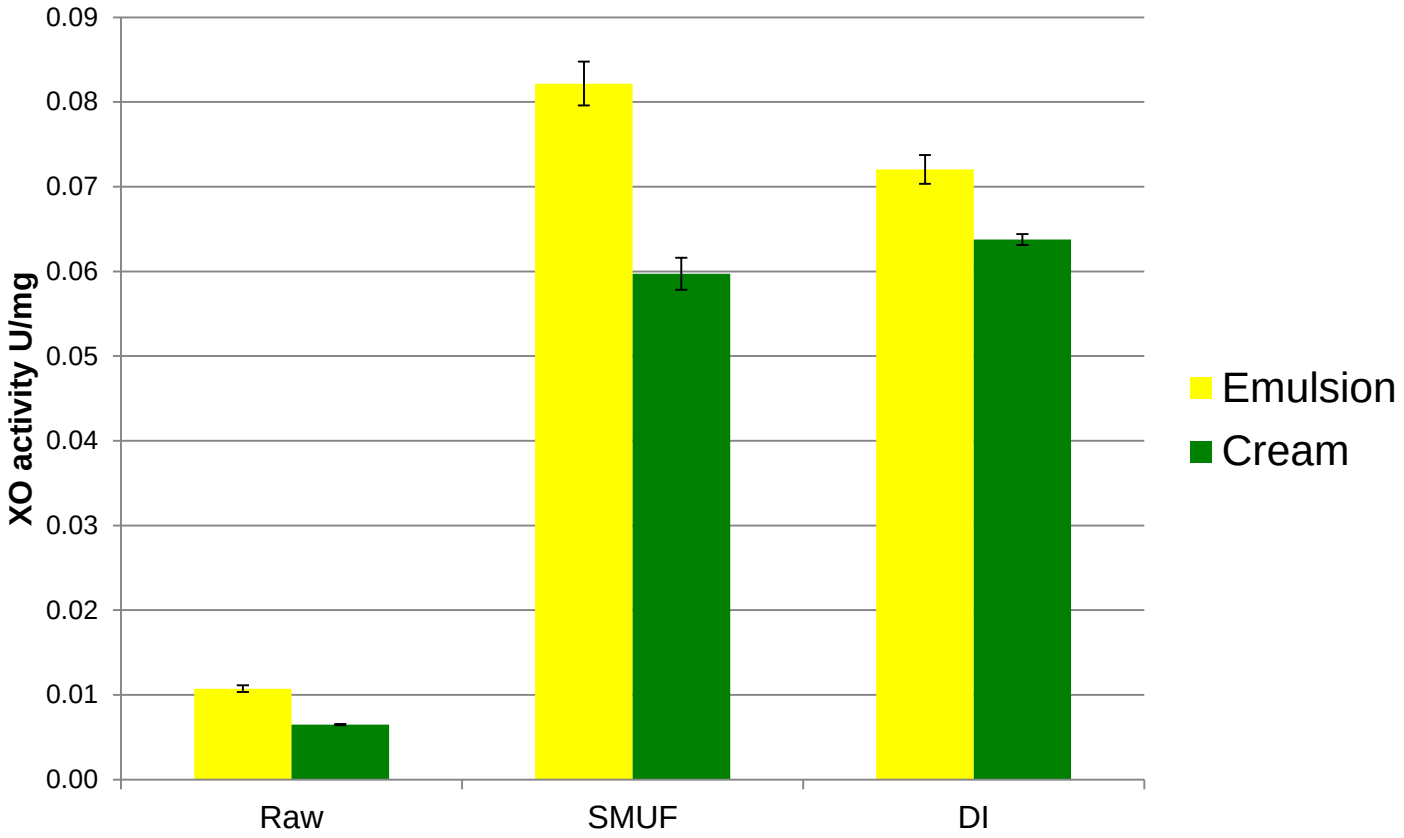
Impact of washing cream

(with simulated milk ultrafiltrate)



Centrifugation conditions: 15000*g*, 20 min

Xanthine oxidase activity on emulsion surfaces



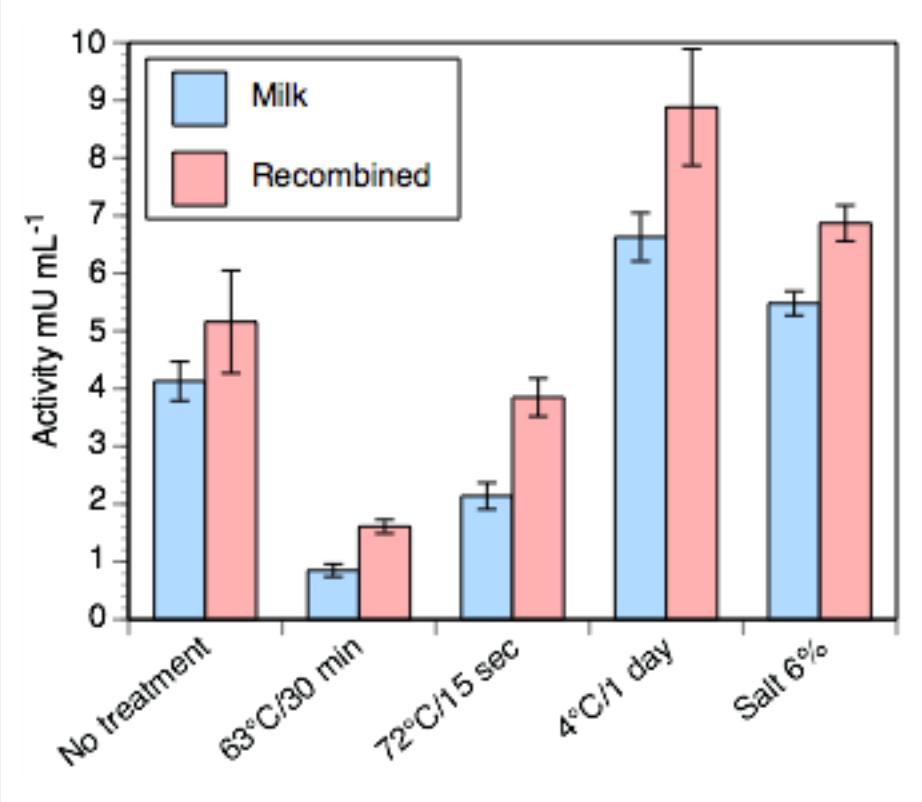
* Specific activity of XO (mU mg^{-1} of protein)

Kathriarachchi, K., PhD thesis, University of Otago (2015)

Glutamyl transferase activity in processed milk

5-L-glutamyl peptide + amino acid ⟶ peptide + 5-L-glutamyl-amino acid

Optimal pH range 8.0 - 9.0
Glutamylization of hydrophobic amino acids
Potential for reducing bitterness and increasing sour taste and astringency
Kokumi flavour in Gouda cheese from γ-glutamyl dipeptides



Conclusions

- Structure is generated by the association of food components (macro-structure). This will impact upon:
  - Breakdown of components under digestive conditions
  - Release and absorption of nutrients
- Digestibility is not just impacted by firmness or structural density of a food product
- Structural interfacial engineering of food products can have a profound effect on digestibility, release of nutrients, and health:
  - Controlling globule-matrix interactions
  - Globule interfacial composition (surface tension effects)
  - Globule size
- Requires interdisciplinary research by food physicists, manufacturing technologists, and gastrointestinal physiologists

Time for questions