University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Food Ingredients Research Group

Novel protein ingredients for infant milk formula

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Human *vs* Bovine Milk Composition

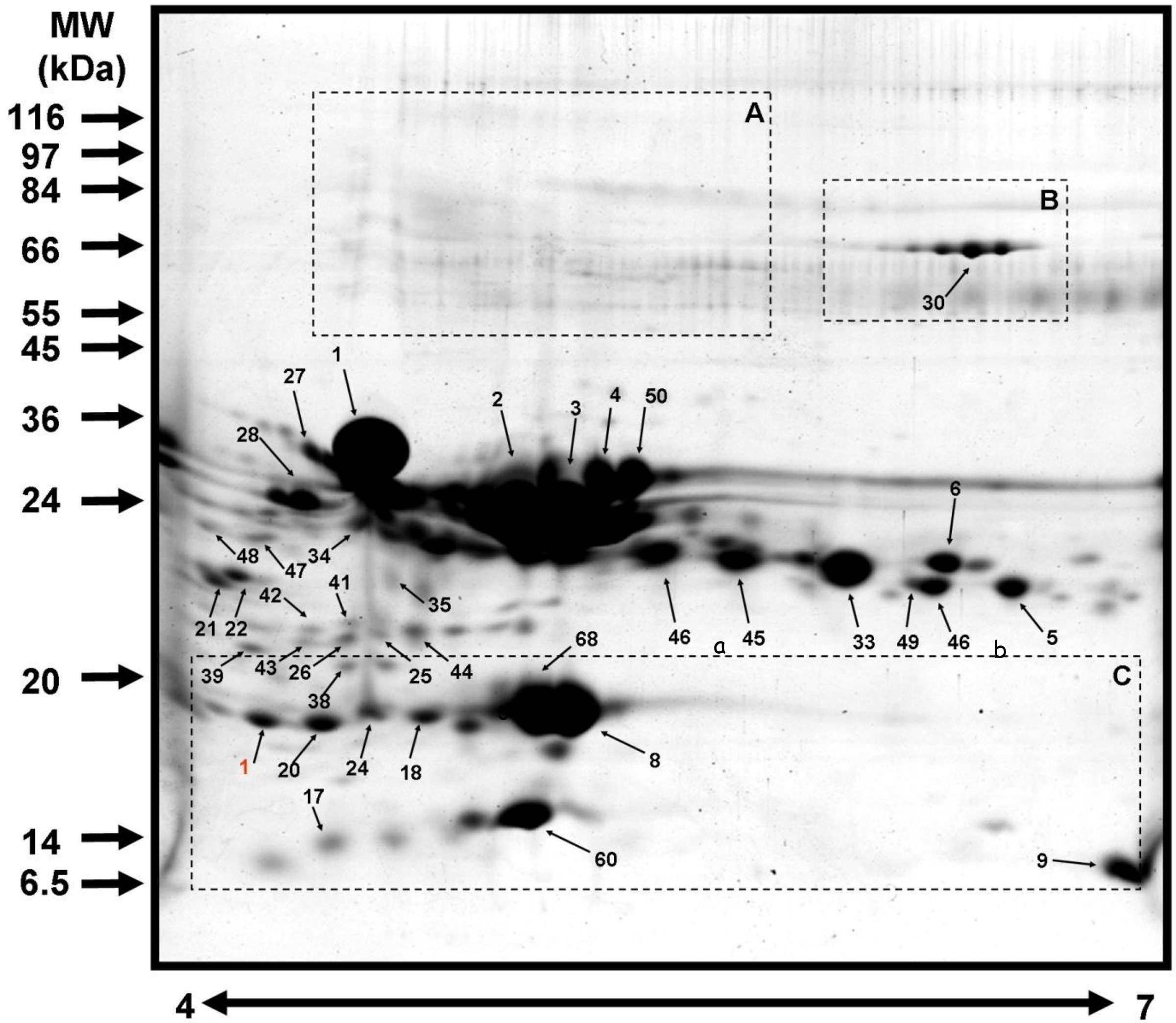
Component	Bovine Milk	Human Milk
	- - - - - - - - - - - - - - - %	- - - - - - - - - - - - - -
Total Solids	12.5	12.9
Protein	3.5	1.0
Whey:Casein Ratio	20:80	60:40
Fat	3.8	4.1
Carbohydrate	4.8	7.2
Ash	7.0	2.0

Challenge for infant formula development is how best to reproduce the composition and health benefits of human milk

Human milk vs bovine milk proteins

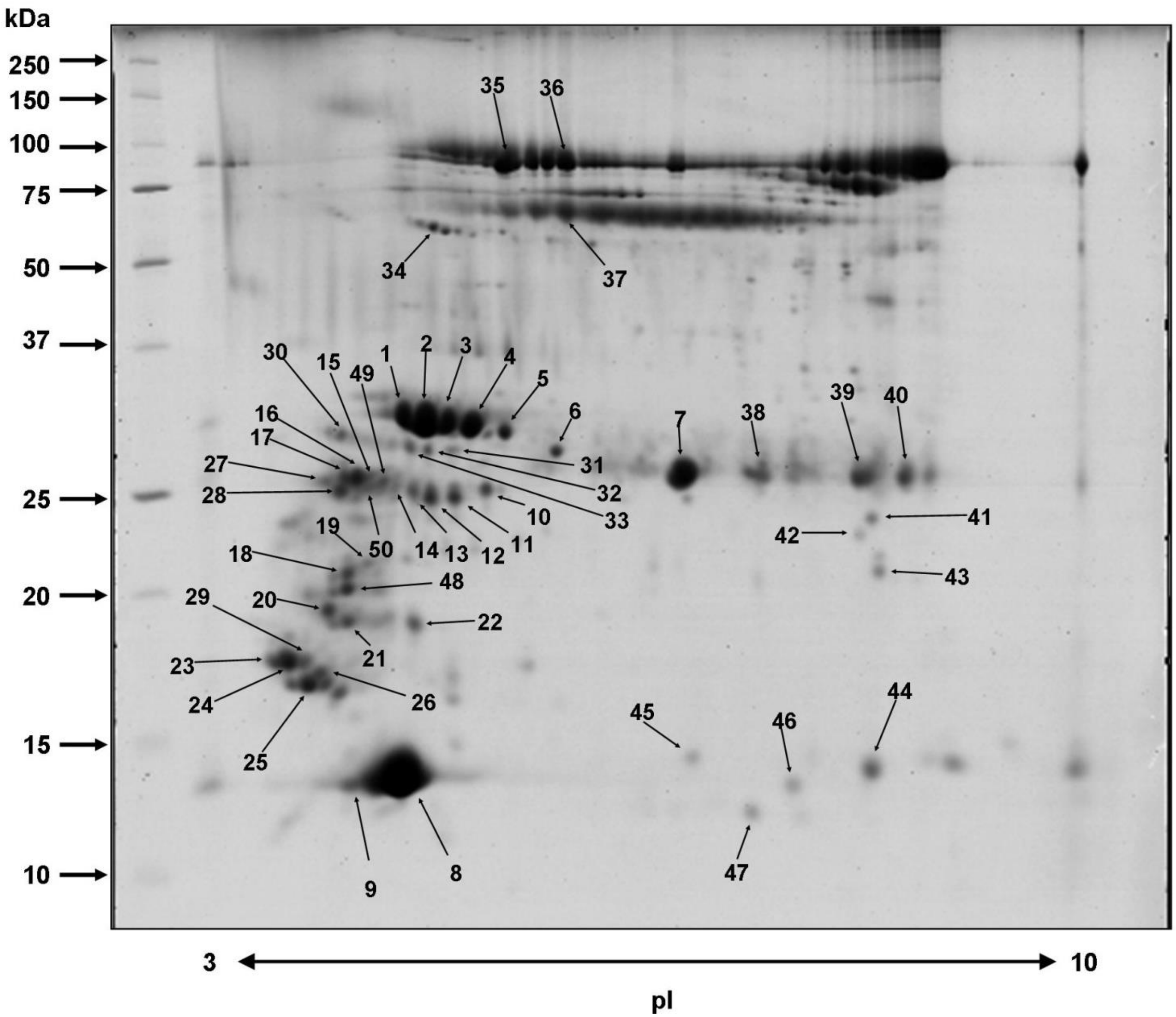
Bovine milk	Human milk
3-3.5 % protein	~ 1 % protein
Mix of four caseins (α_{s1}-, α_{s2}-, β–, κ–) - **dominant** protein family	Mix of three caseins (α_{s1}-, β-, κ-) - **minor** protein family; major casein is β-casein
Two major whey proteins (α-lactalbumin, β-lactoglobulin)	Major whey protein is α-lactalbumin, high levels of lysozyme, lactoferrin
Caseins found in micelles	Caseins found in micelles
Plasmin is predominant native protease	Plasmin is predominant native protease

Two dimensional studies of bovine milk proteins



1: as1-casein; 2-3: β-casein; 4: as2-casein; 5: κ-casein; 8: β-lactogloblulin; 9: β-casein; 17: as1-casein; 18: unknown; 20: β-casein; 21: as1-casein; 22: as1-casein; 24: β-casein; 25-28; as1-casein; 30: serum albumin; 33: κ-casein; 34-35: as1-casein; 38: β-casein; 39: as1-casein; 41-44: as1-casein; 45-46(a,b): κ-casein; 47-48: as2-casein; 49: κ-casein; 50: as2-casein; 60: α-lactalbumin; 68: β-lactogloblulin.

The human milk proteome



1-5, 7: β-CN
6:β-casein/immunoglobulin
8-9: α-lactalbumin
10-17: α-casein
18-19: αs1-casein/β-casein 20-22, 24-26, 30:αs1-casein
23: serine protease
27-28: immunoglobulin J 29-31: αs1-casein / β-casein / anti-pneumococcal antibody
32: αs1-casein / β-casein
33: αs1-casein / β-casein / α-lactalbumin
34: α1-antitrypsin / κ-casein
35-36: lactoferrin
37: lacto-transferrin / immunoglobulin
38-40: immunoglobulins
41-44: β-casein
45: fatty acid binding protein
46: β-casein / α-lactalbumin
47: β2-microglobulin
48: lactoferrin / αs1-casein / β-casein
49-50: αs1-casein.

Bringing the two closer: Infant Formula

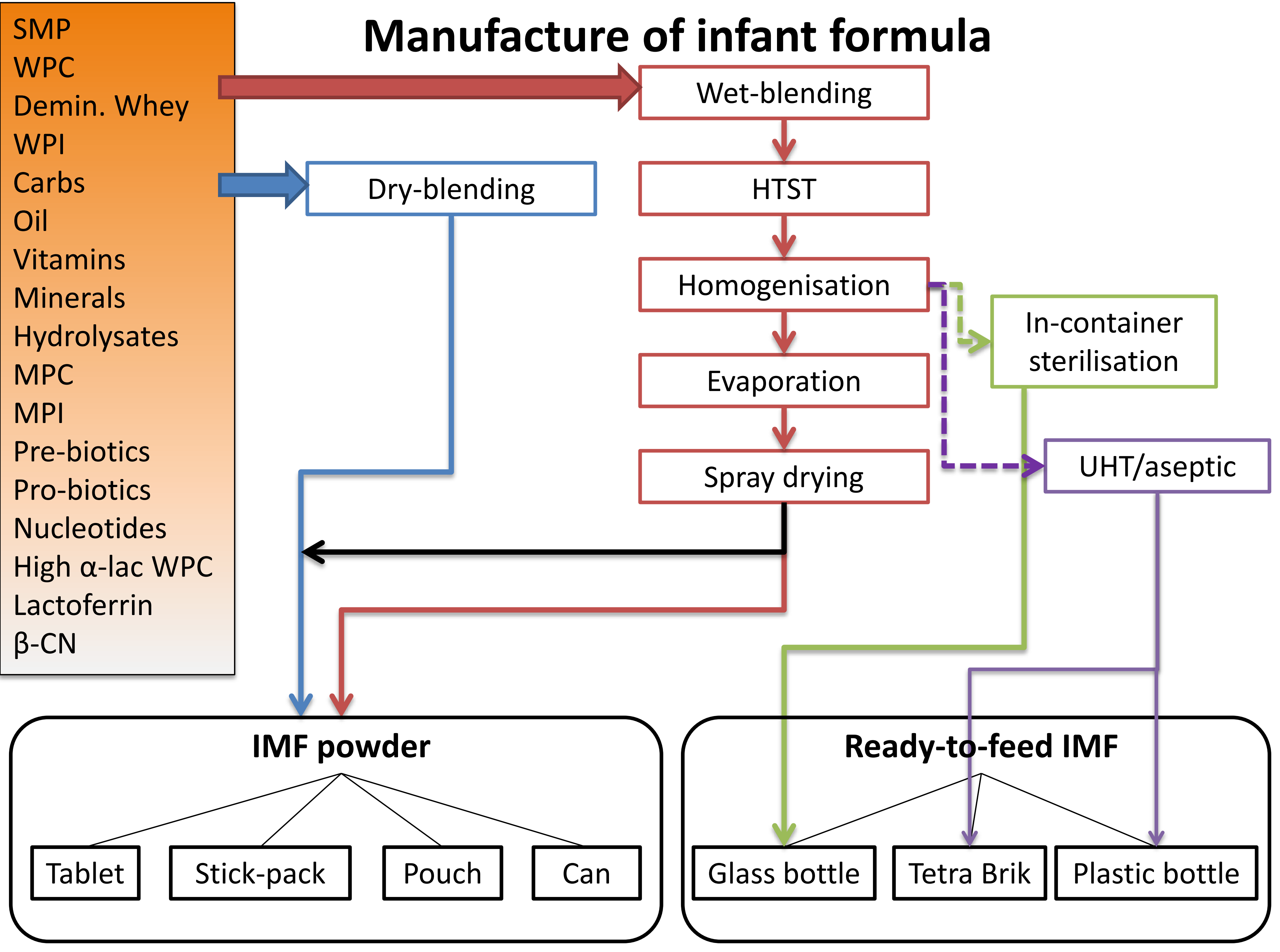
Powder

Tetra Brik

Glass Bottle

Plastic Bottle

Tablets


Manufacture of infant formula
SMP
WPC
Demin. Whey
WPI
Carbs
Oil
Vitamins
Minerals
Hydrolysates
MPC
MPI
Pre-biotics
Pro-biotics
Nucleotides
High α-lac WPC
Lactoferrin
β-CN
Wet-blending
Dry-blending
HTST
Homogenisation
Evaporation
Spray drying
In-container sterilisation
UHT/aseptic
IMF powder
Tablet
Stick-pack
Pouch
Can
Ready-to-feed IMF
Glass bottle
Tetra Brik
Plastic bottle

Protein Ingredients for Humanisation of IMF

- Whey-protein-dominant ($\geq$ 60% whey protein)
- Low/specific mineral content/profile (demin whey, WPC, WPI)
- Increased α-lac:β-lg (human milk has no β-lg)
- Increased β-casein:α_s-casein (human milk casein is mostly β-casein)
- Good protein quality – low levels of non-protein nitrogen (NPN)
- Free of colour (annatto)

Why do the goal posts keep moving?

Understanding of human milk composition and infant growth/development characteristics are constantly evolving

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Proteins and proteolysis in pre-term and term human milk and possible implications for infant formulae

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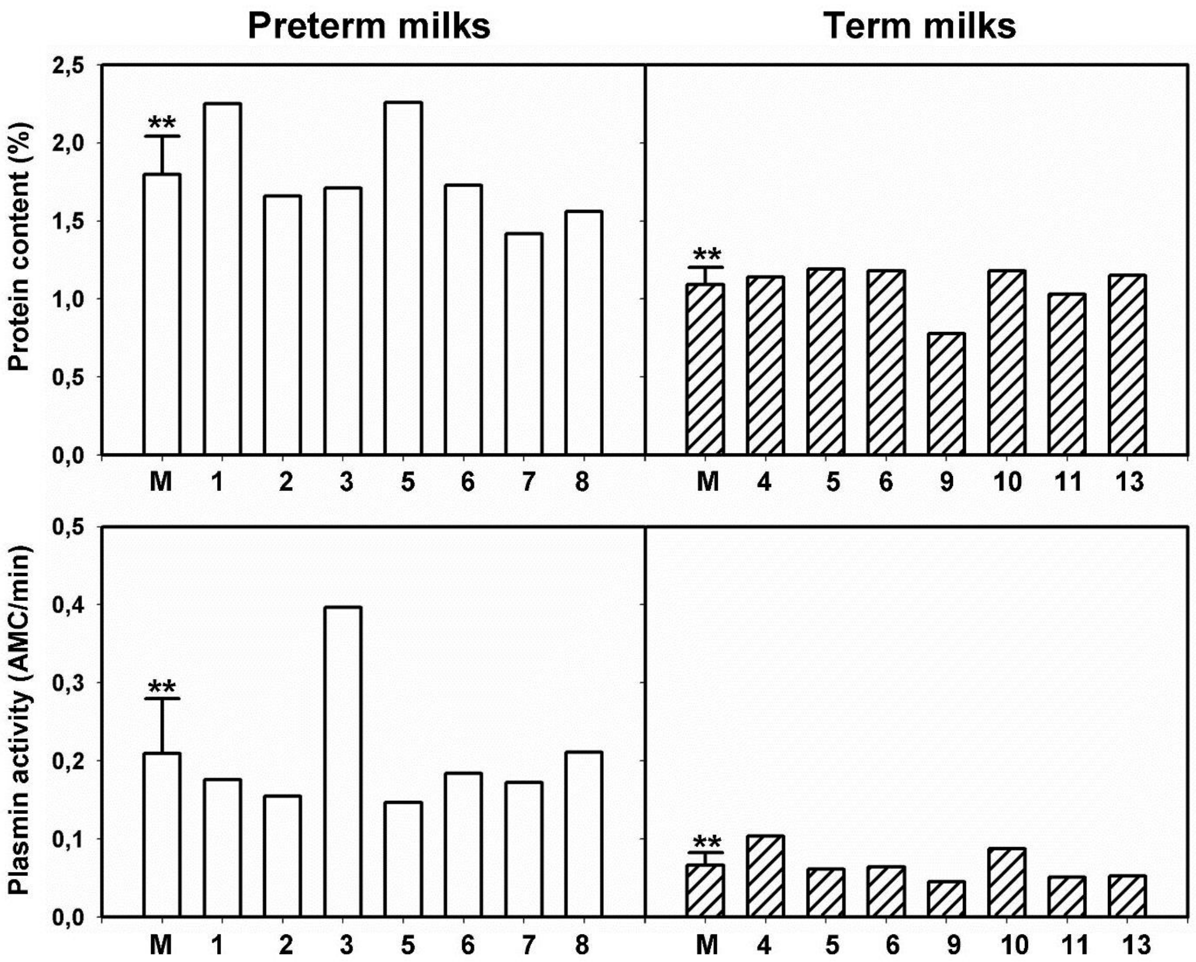
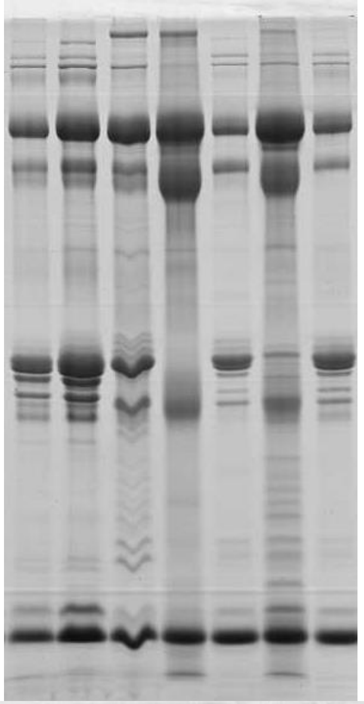
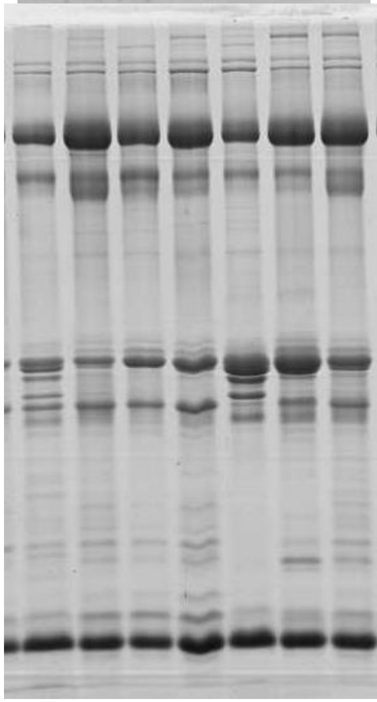
Differences in milk from pre-term and term mothers



Term



Pre-term



Difference in protein level, plasmin activity and the extent of hydrolysis

Age Categories

1st Age 0-6 Months
Nutritionally complete, sole nutrition source

2nd Age 6-12 Months
Follow on formula, complimentary weaning feed

3rd Age 1-3 Years
Growing up milks (GUM's), iron, vitamin D, calcium

4th Age 3-7 Years
Supplimentary nutrition

Specialty Formulas
Lactose free, soy-based, anti-reflux, hypoallergenic

Ingredients used in Infant Formula

Protein	Lipid	Carbohydrate
Skim Milk Powder (SMP)	**Palm Oil**	**Lactose**
Demineralised Whey	**Coconut Oil**	**Sucrose**
Whey Protein Concentrate (WPC)	**Soybean Oil**	**Maltodextrin**
Milk Protein Isolate (MPI)	**Sunflower Oil**	**Corn Syrup Solids**
Lactose-Reduced/Free WPC	**Arachidonic Acid**	**Fructo-oligosaccharides**
Soy Protein Isolate	**Docosahexanoic Acid**	**Galacto-oligosaccharides**
Casein/Whey Protein Hydrolysates	**Structured Vegetable Oils**	
α-Lactalbumin enriched WPC		
Lactoferrin	*Carotenoids*	*Human Milk Oligosaccharides*
β-casein	*Conjugated Linoleic Acid*	
Immunoglobulins	*Phospholipids*	
Defensins/Casecidins	*Gangliosides*	
Milk Basic Protein	*Milk Fat Globule Membrane*	
Osteopontin		

The case for enriching β-casein

Fraction	Protein	MW (kDa)	Bovine milk	Human milk (g/L)	IMF formulae
Casein			**30**	**3.5-4.4**	**4.8**
	α_{s1}	23	12-15	0.6	1.7
	α_{s2}	25	3-4	0.0	0.5
	β	24	9-11	2.7	1.6
	κ	19	3-4	0.9	0.6
Whey protein			**5.0**	**5.3-6.6**	**7.2**
	α-lac	14	1.0-1.5	2-3	1.4-2.3
	β-lg	18	3.0-4.0	0.0	3.5
	LF	80	0.01-0.1	1-2	0.05

The case for enriching β-casein

- Caseins have not been the focus of extensive humanisation work
- The primary goal is to increase the β-CN:α_s-CN ratio in milk
- Few suppliers of industrial-scale volumes of β-CN currently exist
- Processes continue to be researched/developed . . .

A membrane separation process which exploits β-casein

micelle↔monomer equilibria

(12) **United States Patent**
O'Mahony et al.

(10) **Patent No.: US 8,889,208 B2**
(45) **Date of Patent: Nov. 18, 2014**

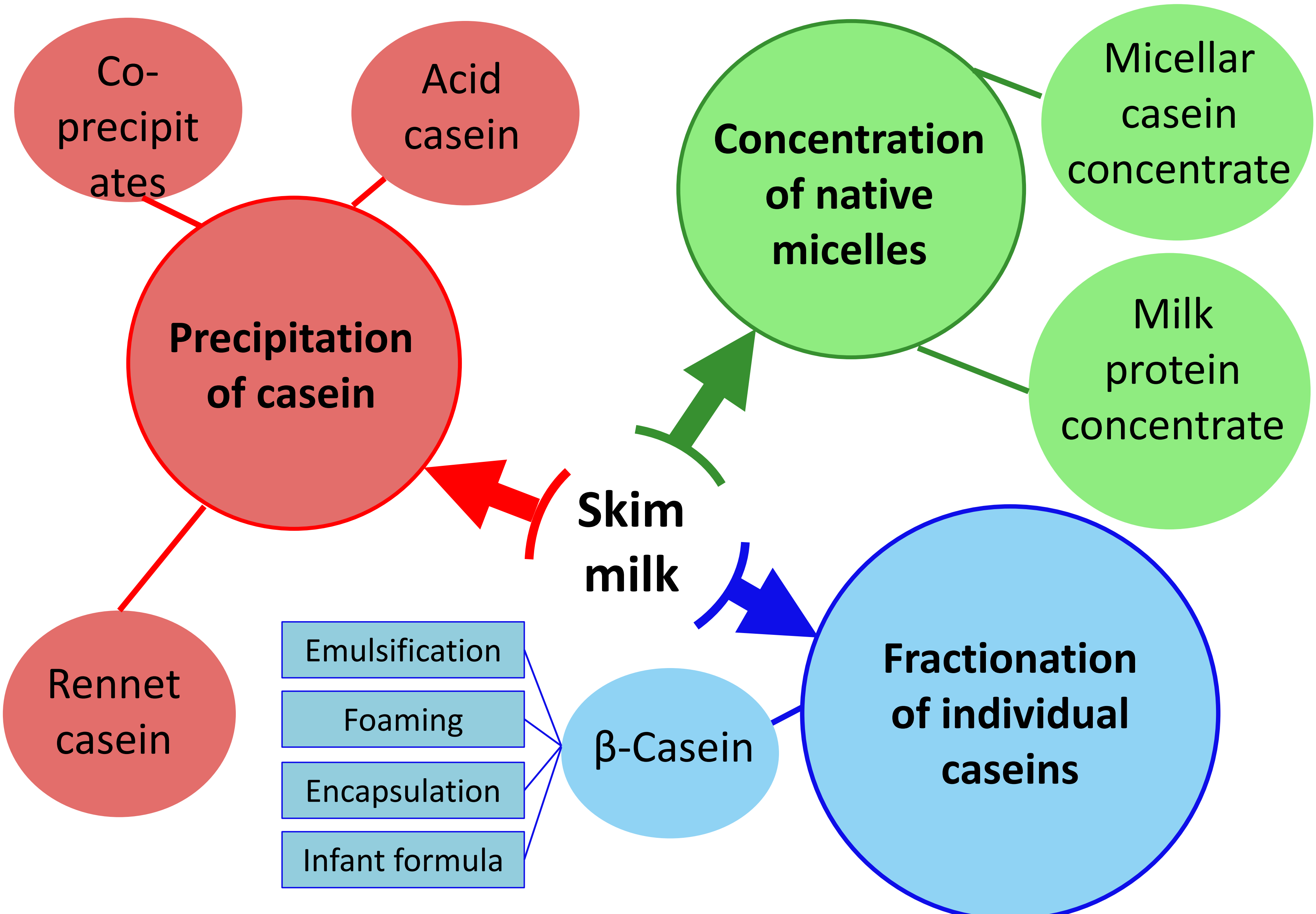
(54) **PURIFICATION OF BETA CASEIN FROM MILK**

(75) Inventors: **James Anthony O'Mahony**, Glenville (IE); **Karen E. Smith**, Madison, WI (US); **John Anthony Lucey**, Madison, WI (US)

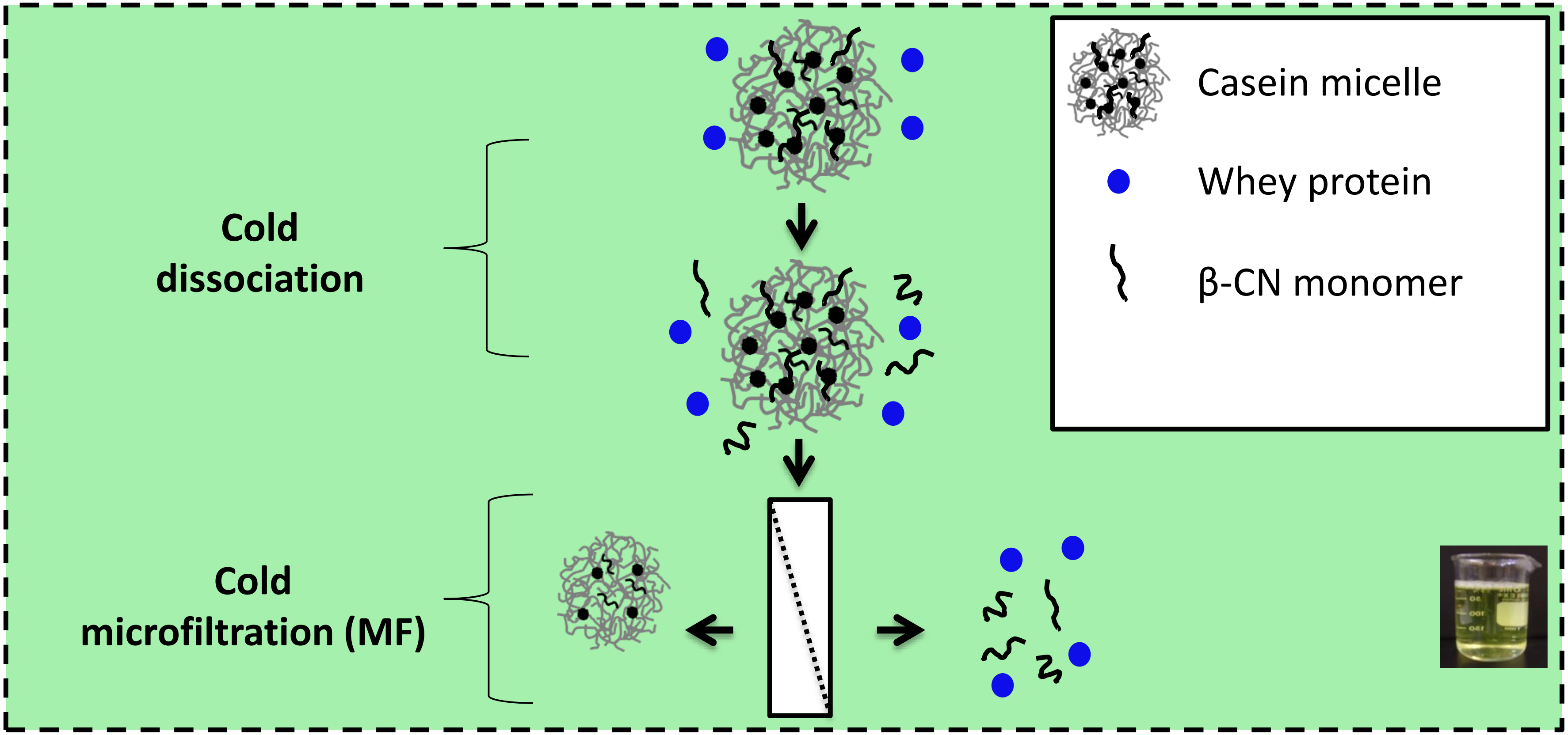
OTHER PUBLICATIONS

Famelart and Surel, "Caseinate at Low Temperatures: Calcium Use in β-Casein Extraction by Microfiltration," *Journal of Food Science*, 59(3):548-587 (1994).
Fauquant, et al., "Microfiltration of Milk Using a Mineral Membrane," *Technique Laitiere*, 1028:21-23 (1988).

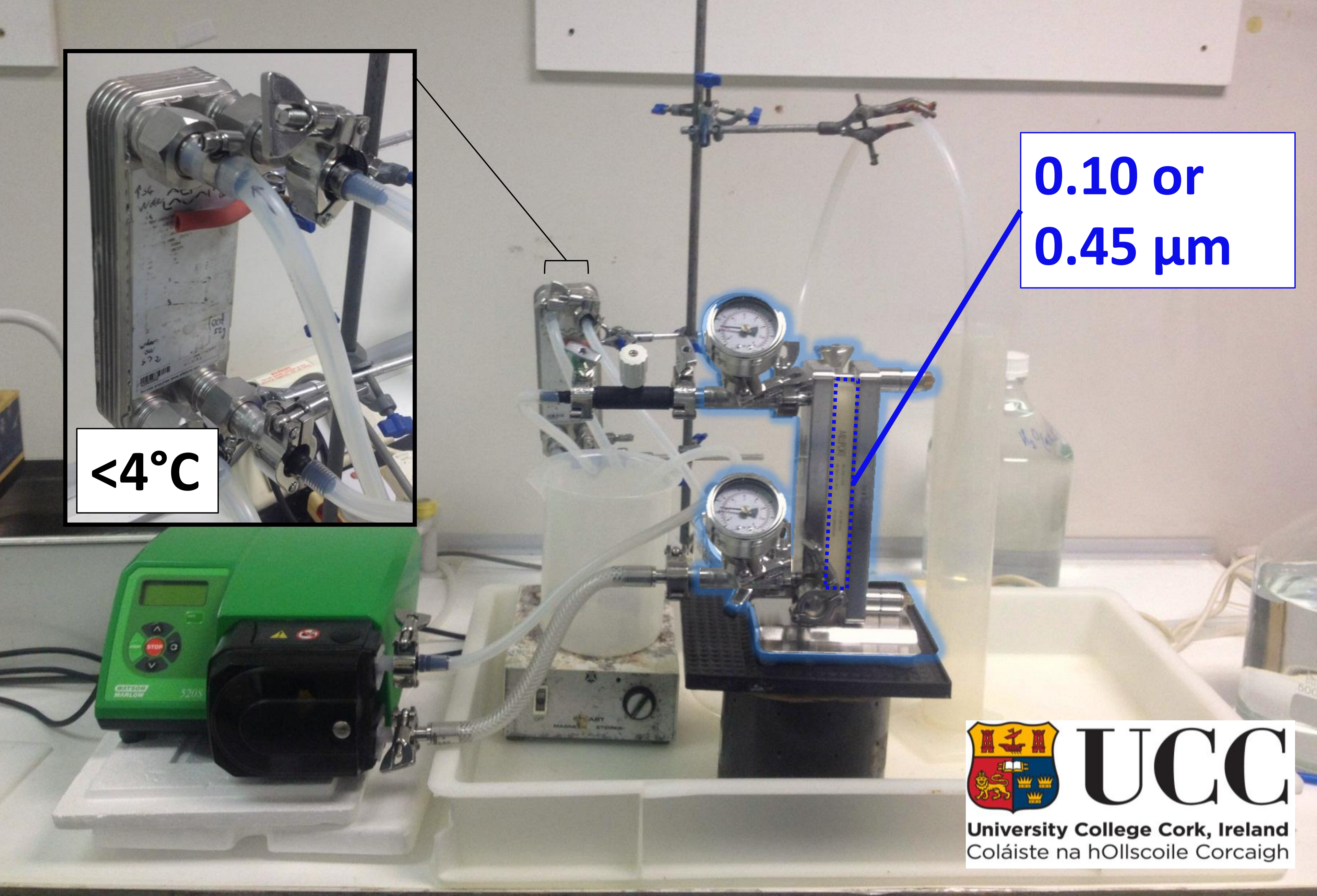
Production of casein-based ingredients



Manufacturing β-Casein - Enrichment



β-casein enrichment (cold MF)



β-casein enrichment

Permeate from 0.1 µm membrane:

β-Casein-enriched

Whey protein-dominant

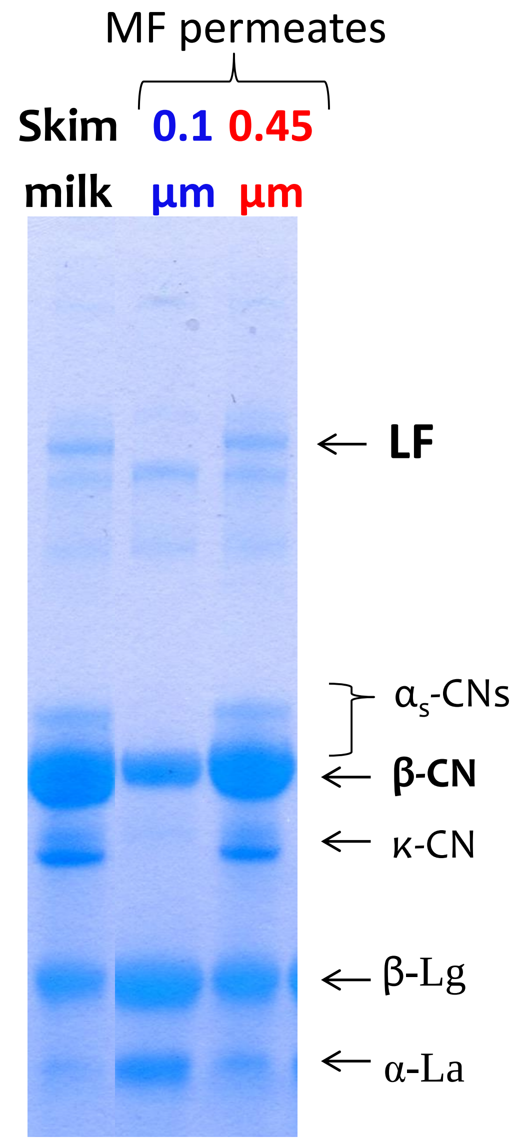
A potential base for infant formula*

Permeate from 0.45 µm membrane:

β-Casein-enriched

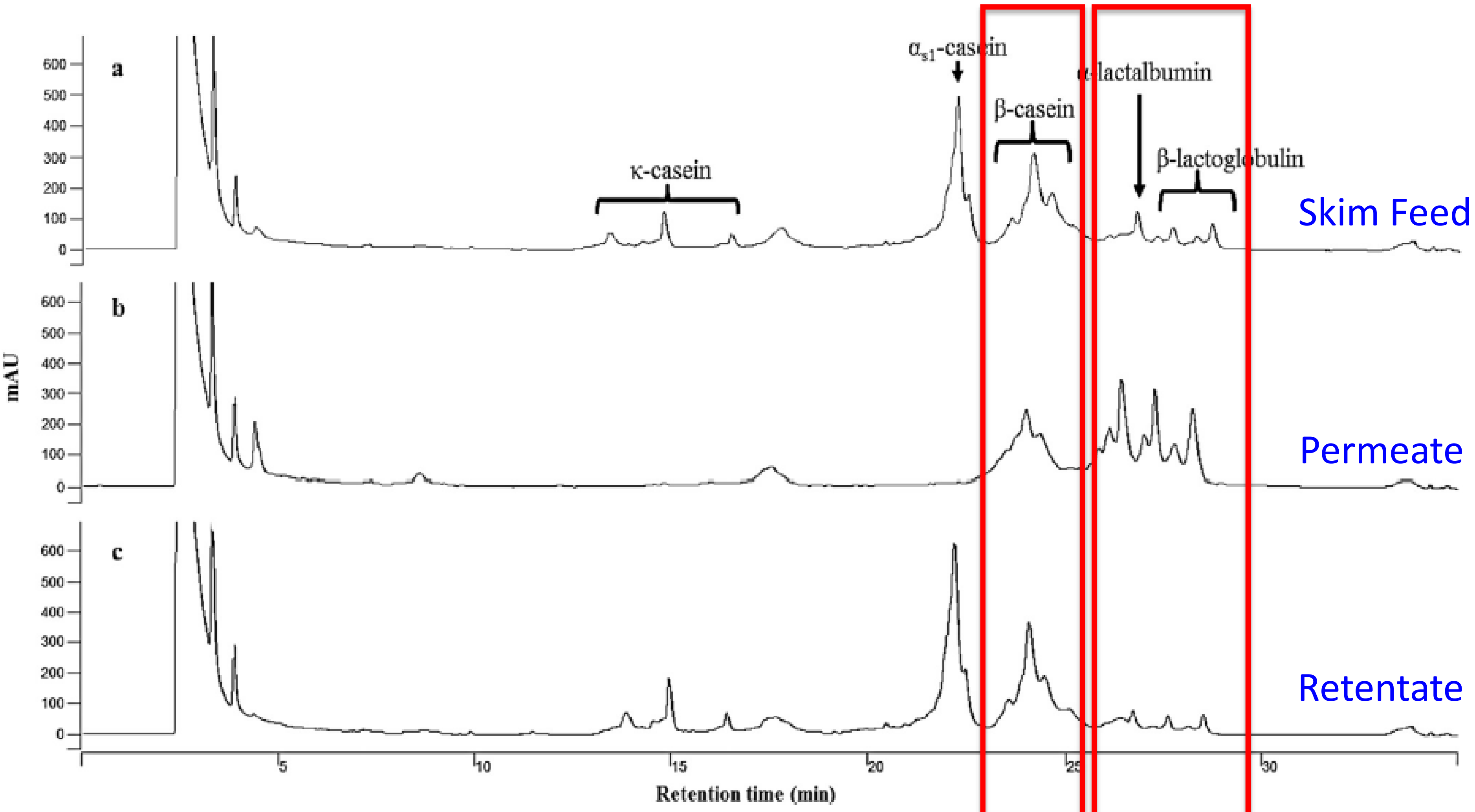
Contains small casein micelles

Not depleted in lactoferrin (LF)



Production of Ideal Whey containing β-casein

Protein profile of feed, permeate and retentate streams



Production of Ideal Whey containing β-casein

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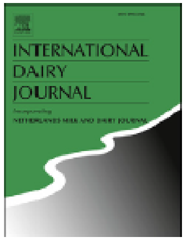

Processing and protein-fractionation characteristics of different polymeric membranes during filtration of skim milk at refrigeration temperatures

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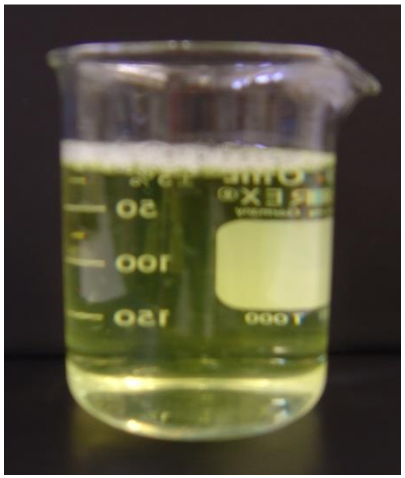
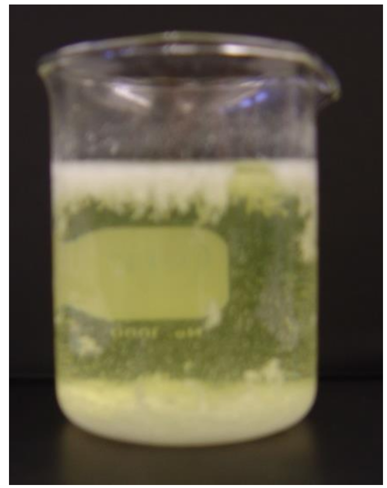
A B S T R A C T

Serum protein concentrates (SPCs) were generated from reconstituted skim milk (3.2% protein) using lab-scale tangential-flow filtration at 3–4 °C. The influence of membrane type on process performance (e.g., permeate flux) and protein-enrichment (e.g., protein profile) was assessed with polyvinylidene-difluoride membranes (0.1 μm and 0.45 μm pore-size), and a polyethersulfone membrane (1000 kDa cut-off). The 1000 kDa membrane exhibited the highest starting flux (6.7 L m^{-2} h^{-1}), followed by the 0.1 μm (5.4 L m^{-2} h^{-1}) and 0.45 μm (4.8 L m^{-2} h^{-1}) membranes. Flux decreased by >40% during filtration with the 1000 kDa and 0.1 μm membranes, while the decrease was lower (<20%) with the 0.45 μm membrane. β-Casein comprised >97% of casein in SPCs from the 0.1 μm and 1000 kDa membranes. SPCs from the 0.45 μm membrane had higher β-casein:α_s-casein ratios than the feed and higher levels of minor whey proteins (e.g., lactoferrin) relative to the other SPCs.

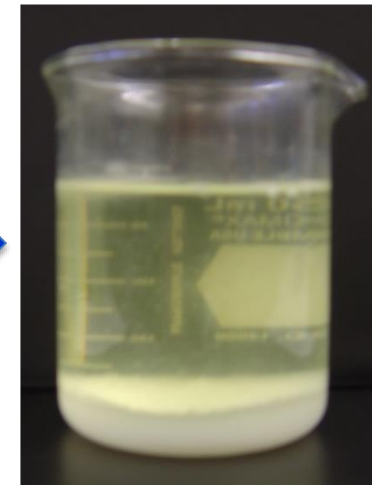
Purification of β-casein from Ideal Whey



Heating
(> 10^{o}C)



Holding
(> 10^{o}C)



β-CN-enriched native whey (un-demineralised)

Irreversible precipitation of β-CN

Sedimentation of highly pure β-CN

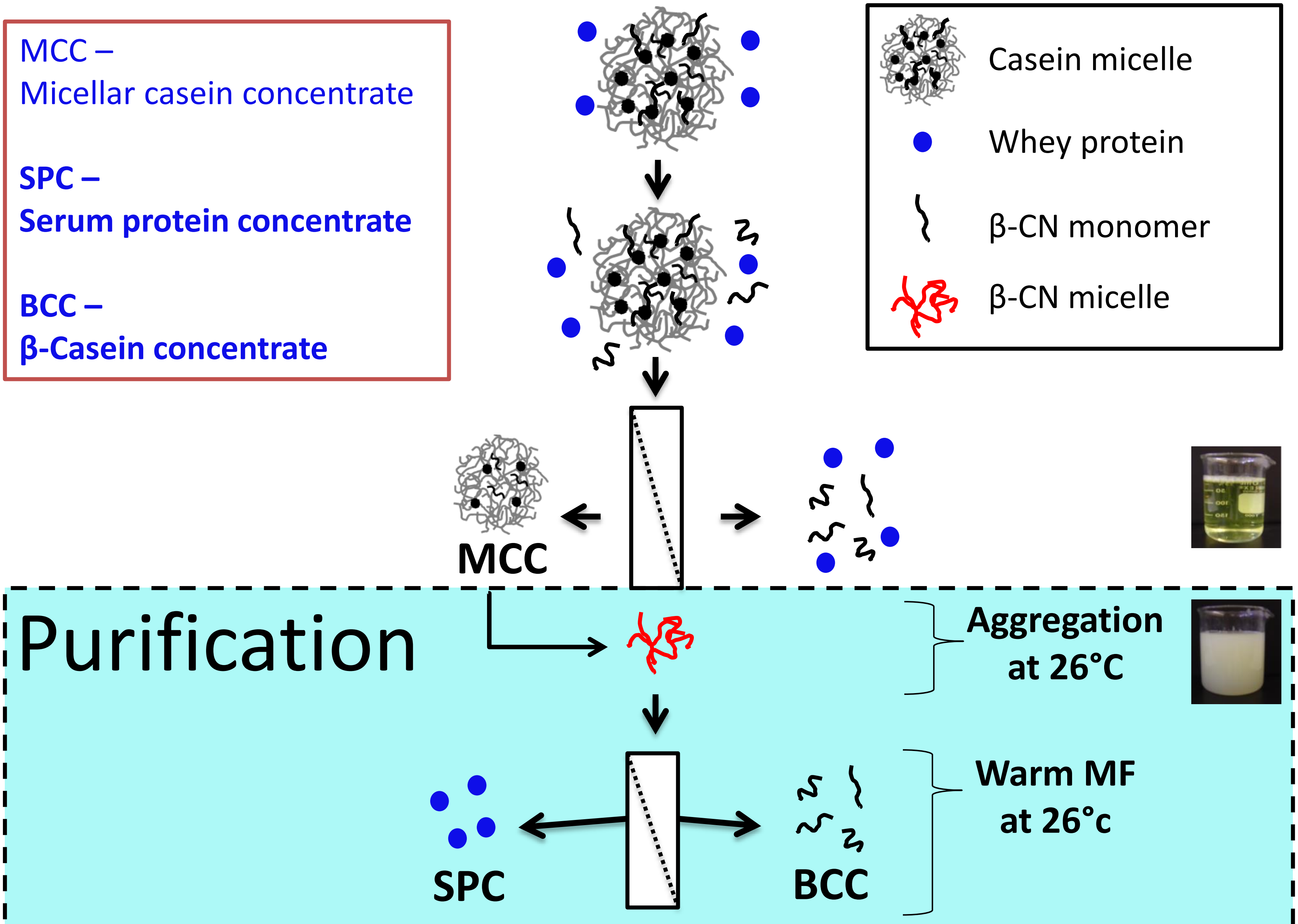
Precipitation-based approaches yield β-casein with poor solubility and functionality

β-Casein manufacture

MCC –
Micellar casein concentrate

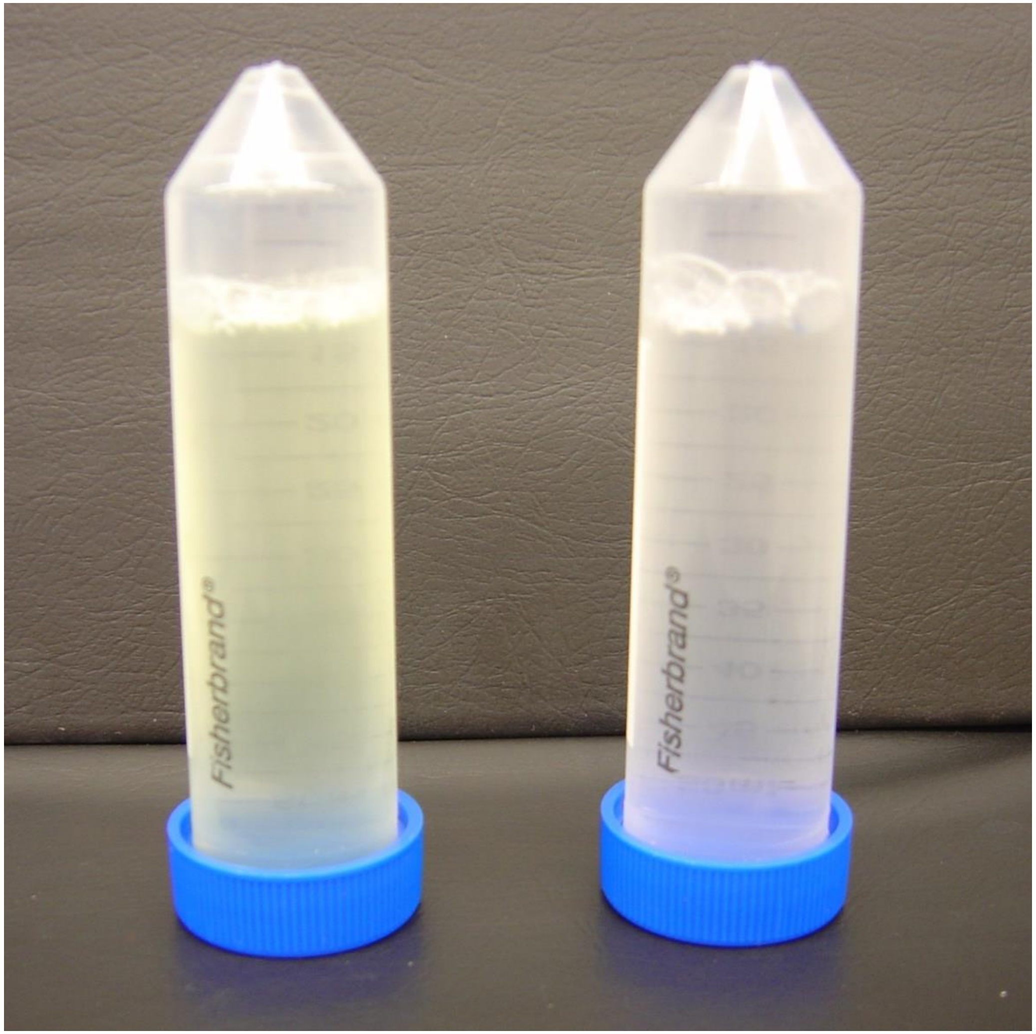
SPC –
Serum protein concentrate

BCC –
β-Casein concentrate



Purification of β-casein from Ideal Whey

Step 1: Demineralisation of β-CN-enriched ideal whey by UF/DF eliminates Ca^{2+}-induced precipitation during heating



Purification of β-casein from Ideal Whey

Step 2: Thermo-reversible micellisation of β-casein in MF permeate

Pre-micellisation (4°C)

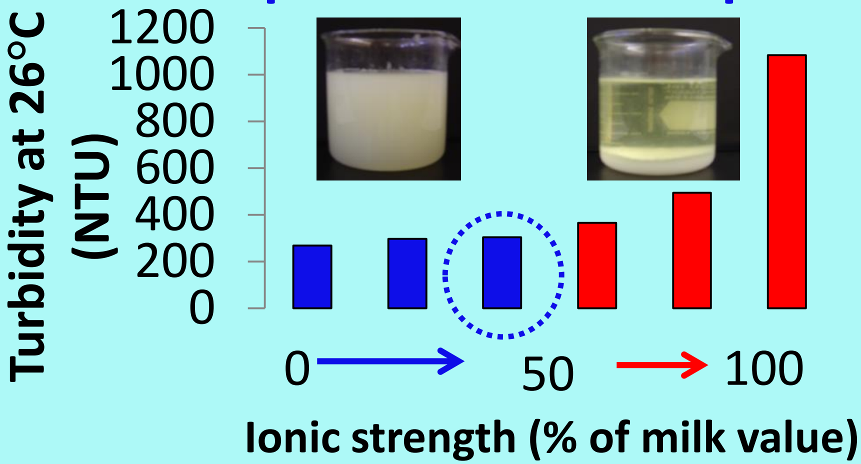
Micellisation (>25°C)

Post-micellisation (4°C)

At low Ca^{2+}, β-casein associates hydrophobically

β-casein purification (warm MF)

Optimum for 1.2% protein



- Minerals **promote aggregation**, which can aid fractionation
- But, too many ions can **cause destabilisation** of β-CN

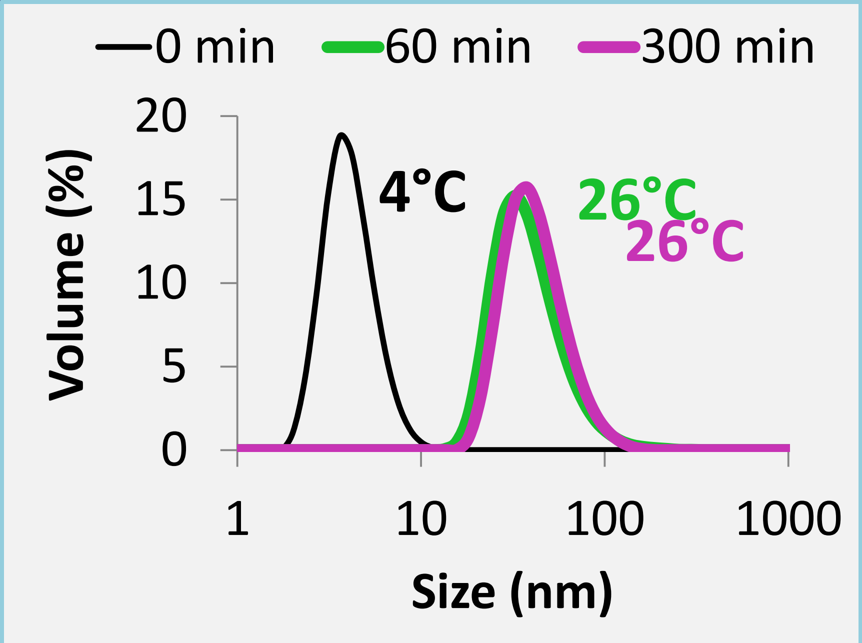
Optimising ionic strength with diafiltration

De-ionised water → **Option 1:** **High ionic strength MF** (100%) - Precipitation ✗

Option 2: **Low ionic strength MF** (<10%) - Stable, good purity ✓

40% milk permeate → ≤ 1.2% protein → **Option 3:** **Medium ionic strength MF** (40%) - Stable **?** Higher purity **??**

β-casein purification (warm MF)



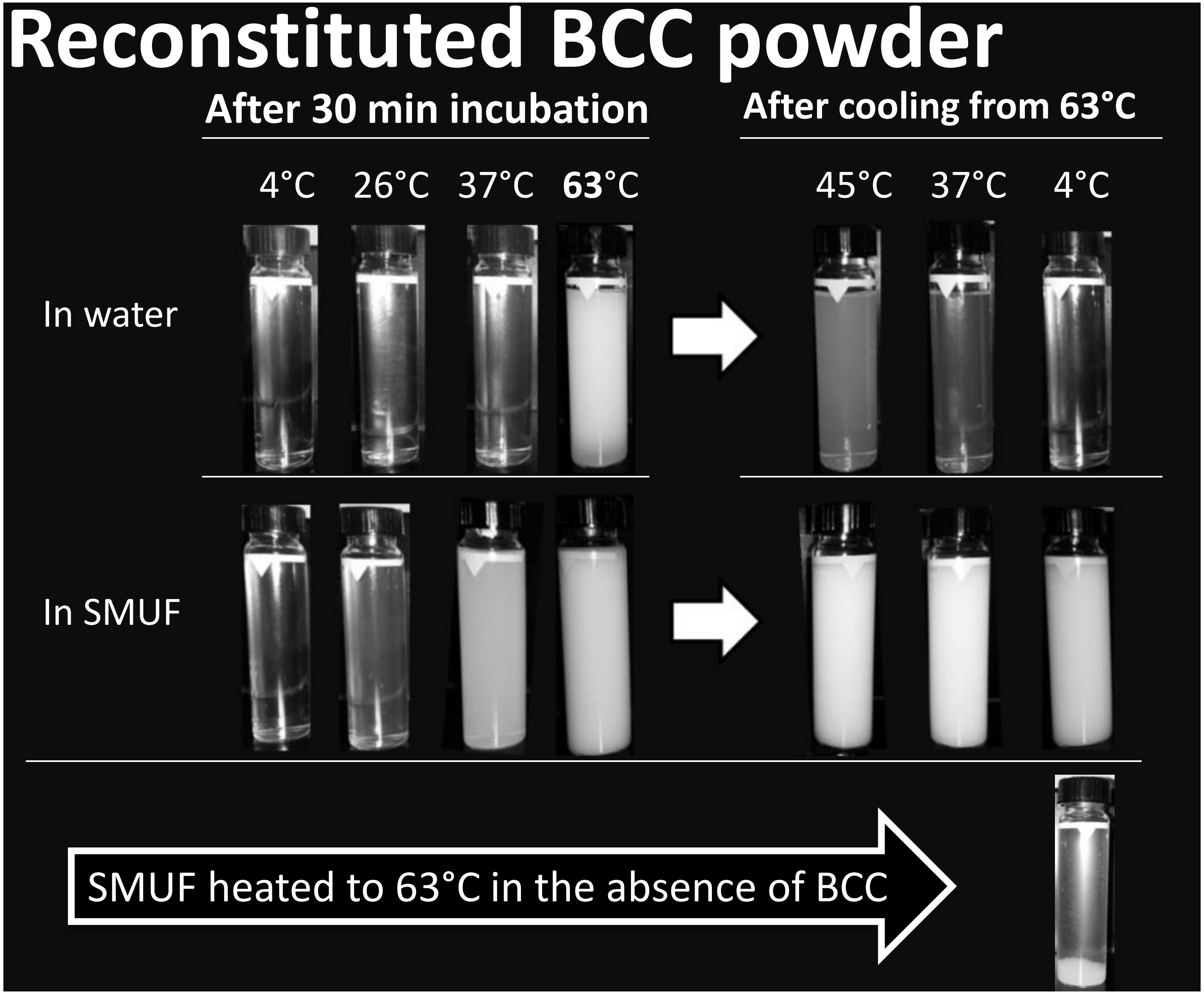
β-Casein Micelles:

- **Formed at 26 °C**
- **Stable during warm MF**

	β-Casein (% protein)
Low ionic strength MF	
BCC	73
SPC	14
Med. ionic strength MF	
BCC	80⇧
SPC	7 ⇩

Moderate ionic strength:

- **Promoted aggregation**
- **Increased protein purity**



BCC solutions

Clear at low temperature

Can **stabilise** calcium phosphate

More **turbid** in presence of minerals

Gel on extended incubation in mineral solution

Protein aggregation typically a concern at > 70°C

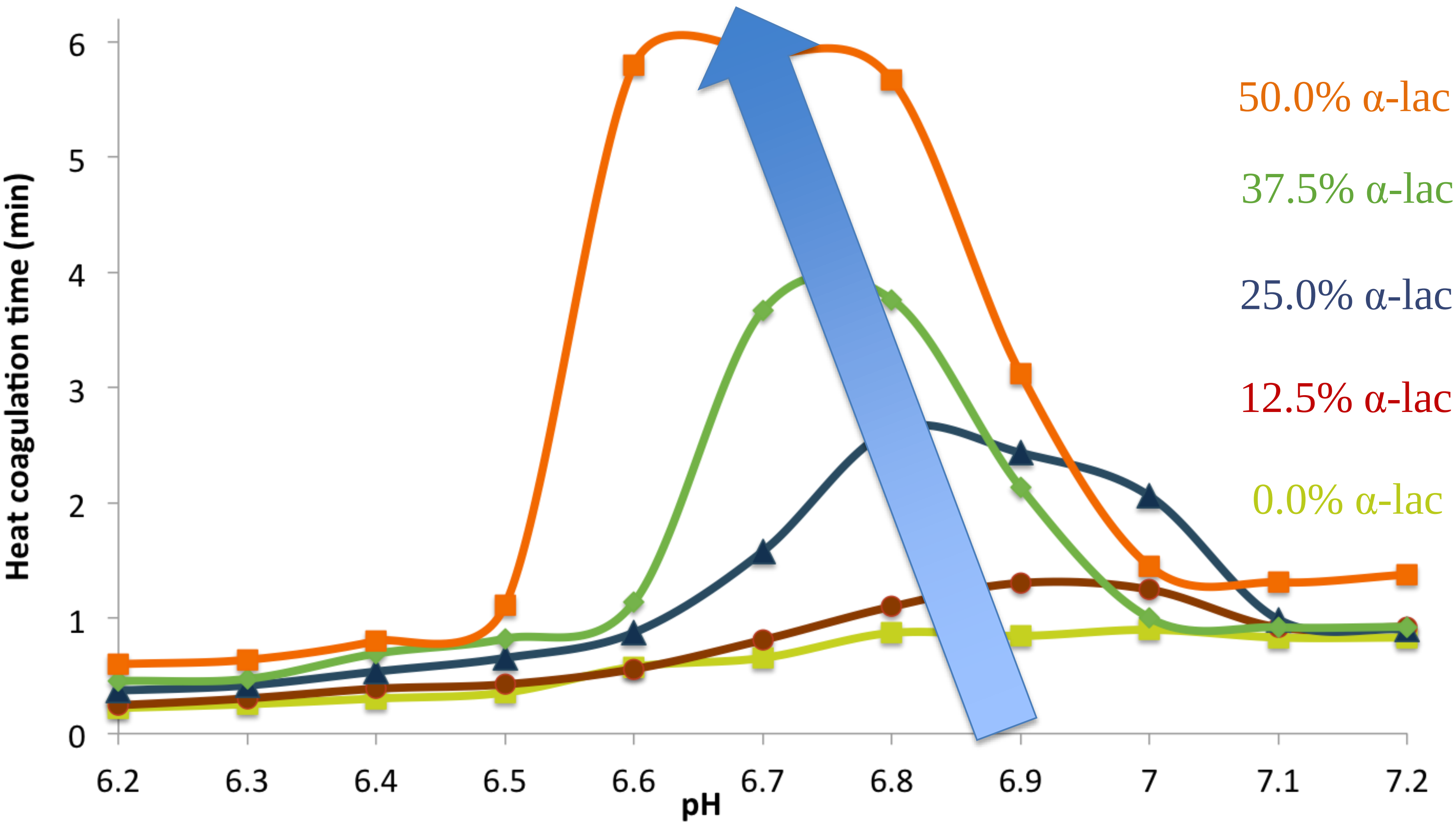
Modifying the whey protein profile

Fraction	Protein	MW (kDa)	Bovine milk	Human milk (g/L)	Formula
Total whey protein			**5.0**	**5.3-6.6**	**7.2**
	α-lac	14	1-1.5	2-3	1.4-2.3
	β-lg	18	3-4	0.0	3.5
	LF	80	0.01-0.1	1-2	0.05

- IMFs with humanised whey protein profiles are commercially-available
- These products are enriched in either α-lac or LF
- The current goal is to increase the α-lac:β-lg ratio in milk
- Little is known about the process-performance of IMFs with humanised whey protein profiles

Processing Implications of Changing Whey Protein Profile

Heat Stability – changing α-lac:β-lg impacts heat stability



Infant Formula UF Permeate – Heat Stability

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ELSEVIER

Use of ultrafiltration to prepare a novel permeate for application in the functionality testing of infant formula ingredients

CrossMark

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ABSTRACT

Ultrafiltration (UF) permeates produced from reconstituted infant milk formula powder (IMF; 1.3%, w/w, protein) and reconstituted skim milk powder (SMP; 3.2% protein) were compared with simulated milk ultrafiltrate (SMUF) in terms of composition, physicochemical properties and impact, as dispersants, on the heat stability of model infant formula systems. Permeates from IMF and SMP were generated at 15 °C using a lab-scale UF unit with a 10 kDa cut-off polyethersulfone membrane. Operation at optimal cross-flow velocity and sub-critical flux allowed 1 L of IMF to be concentrated by a volume concentration factor (VCF) of 3 in 20 ± 2 min, with minimal flux decline and constant trans-membrane pressure (*TMP*); conversely, UF took 33 ± 4 min for SMP, with a decrease in flux and increase in *TMP* over that time. Permeate from IMF (IMF_p) had a markedly different mineral profile to SMP permeate (SMP_p), with the former having considerably lower levels of the major ions (e.g., calcium, phosphorus and sodium). IMF_p, SMP_p, SMUF or deionised water was used to reconstitute milk protein concentrate (MPC)80 and whey protein isolate (WPI) powders in combination to give 5.5% total protein and a 60:40 ratio of whey protein:casein. These model IMFs were assessed for heat stability at pH 6.8 and 140 °C; the type of dispersant used influenced heat stability strongly, with heat stability decreasing in the order water > IMF_p > SMP_p > SMUF. Calcium-ion concentrations of 0.01, 0.71, 1.51 and 1.77 mM L^{-1} were measured for water, IMF_p, SMP_p and SMUF, respectively, indicating that increased heat stability of proteins dispersed in IMF_p, compared to SMP_p or SMUF, may have been due to lower calcium-ion concentration. This study highlights the influence of serum phase composition on the heat-induced destabilisation of infant formula ingredients and outlines a novel approach for the generation of IMF_p, which is of importance in the development of ingredients which remain stable during the processing of IMF products.

Conclusions

- Native whey is a good base for IMFs
- Cold MF can be used to enrich β-CN
- Further purification possible w/ warm MF
- β-CN presents
  - Opportunities: Humanisation of casein fraction
  - Challenges: destabilisation during heat treatment?
- MCC co-products produced may be of interest
- Increasing α-la:β-lg improves heat stability