Novel protein ingredients for infant milk formula

Alan L. Kelly, James A. O’Mahony and Shane V. Crowley

School of Food and Nutritional Sciences
University College Cork, Ireland
Human vs Bovine Milk Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Bovine Milk</th>
<th>Human Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Solids</td>
<td>12.5%</td>
<td>12.9%</td>
</tr>
<tr>
<td>Protein</td>
<td>3.5%</td>
<td>1.0%</td>
</tr>
<tr>
<td>Whey:Casein Ratio</td>
<td>20:80</td>
<td>60:40</td>
</tr>
<tr>
<td>Fat</td>
<td>3.8%</td>
<td>4.1%</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>4.8%</td>
<td>7.2%</td>
</tr>
<tr>
<td>Ash</td>
<td>7.0%</td>
<td>2.0%</td>
</tr>
</tbody>
</table>

Challenge for infant formula development is how best to reproduce the composition and health benefits of human milk.
# Human milk vs bovine milk proteins

<table>
<thead>
<tr>
<th><strong>Bovine milk</strong></th>
<th><strong>Human milk</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>3-3.5 % protein</td>
<td>~1 % protein</td>
</tr>
<tr>
<td>Mix of four caseins ($\alpha_{s1}^-, \alpha_{s2}^-, \beta^-, \kappa^-$) - <em>dominant</em> protein family</td>
<td>Mix of three caseins ($\alpha_{s1}^-, \beta^-, \kappa^-$) - <em>minor</em> protein family; major casein is $\beta$-casein</td>
</tr>
<tr>
<td>Two major whey proteins ($\alpha$-lactalbumin, $\beta$-lactoglobulin)</td>
<td>Major whey protein is $\alpha$-lactalbumin, high levels of lysozyme, lactoferrin</td>
</tr>
<tr>
<td>Caseins found in micelles</td>
<td>Caseins found in micelles</td>
</tr>
<tr>
<td>Plasmin is predominant native protease</td>
<td>Plasmin is predominant native protease</td>
</tr>
</tbody>
</table>
Two dimensional studies of bovine milk proteins

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

- Wet-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 $\alpha$-lac:$\beta$-lg (human milk has no $\beta$-lg)
- Increased $\beta$-casein:$\alpha_s$-casein (human milk casein is mostly $\beta$-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.
Differences in milk from pre-term and term mothers

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

<table>
<thead>
<tr>
<th>Protein</th>
<th>Lipid</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim Milk Powder (SMP)</td>
<td>Palm Oil</td>
<td>Lactose</td>
</tr>
<tr>
<td>Demineralised Whey</td>
<td>Coconut Oil</td>
<td>Sucrose</td>
</tr>
<tr>
<td>Whey Protein Concentrate (WPC)</td>
<td>Soybean Oil</td>
<td>Maltodextrin</td>
</tr>
<tr>
<td>Milk Protein Isolate (MPI)</td>
<td>Sunflower Oil</td>
<td>Corn Syrup Solids</td>
</tr>
<tr>
<td>Lactose-Reduced/Free WPC</td>
<td>Arachidonic Acid</td>
<td>Fructo-oligosaccharides</td>
</tr>
<tr>
<td>Soy Protein Isolate</td>
<td>Docosahexanoic Acid</td>
<td>Galacto-oligosaccharides</td>
</tr>
<tr>
<td>Casein/Whey Protein Hydrolysates</td>
<td>Structured Vegetable Oils</td>
<td></td>
</tr>
<tr>
<td>α-Lactalbumin enriched WPC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein</th>
<th>Carotenoids</th>
<th>Human Milk Oligosaccharides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactoferrin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-casein</td>
<td>Conjugated Linoleic Acid</td>
<td></td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>Phospholipids</td>
<td></td>
</tr>
<tr>
<td>Defensins/Casecidins</td>
<td>Gangliosides</td>
<td></td>
</tr>
<tr>
<td>Milk Basic Protein</td>
<td>Milk Fat Globule Membrane</td>
<td></td>
</tr>
<tr>
<td>Osteopontin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## The case for enriching $\beta$-casein

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein</th>
<th>MW (kDa)</th>
<th>Bovine milk (g/L)</th>
<th>Human milk (g/L)</th>
<th>IMF formulae</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Casein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha_{s1}$</td>
<td>23</td>
<td>12-15</td>
<td>0.6</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>$\alpha_{s2}$</td>
<td>25</td>
<td>3-4</td>
<td>0.0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>$\beta$</td>
<td>24</td>
<td>9-11</td>
<td>2.7</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>$\kappa$</td>
<td>19</td>
<td>3-4</td>
<td>0.9</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td><strong>Whey protein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha$-lac</td>
<td>14</td>
<td>1.0-1.5</td>
<td>2-3</td>
<td>1.4-2.3</td>
<td></td>
</tr>
<tr>
<td>$\beta$-lg</td>
<td>18</td>
<td>3.0-4.0</td>
<td>0.0</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>LF</td>
<td>80</td>
<td>0.01-0.1</td>
<td>1-2</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>
The case for enriching $\beta$-casein

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

A membrane separation process which exploits $\beta$-casein micelle$\leftrightarrow$monomer equilibria
Production of casein-based ingredients

- Precipitation of casein
  - Rennet casein
  - Co-precipitates

- Acid casein
  - Concentration of native micelles
    - Micellar casein concentrate
    - Milk protein concentrate

- Skim milk
  - Fractionation of individual caseins
    - β-Casein
      - Emulsification
      - Foaming
      - Encapsulation
      - Infant formula
Manufacturing β-Casein - Enrichment

Cold dissociation

Cold microfiltration (MF)

Casein micelle
- Whey protein
- β-CN monomer
β-casein enrichment (cold MF)

<4°C

0.10 or 0.45 µm
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)

MF permeates

Skim 0.1
milk 0.45 µm

αs-CN
β-CN
κ-CN
β-Lg
α-La
LF
Production of Ideal Whey containing $\beta$-casein

Protein profile of feed, permeate and retentate streams
Processing and protein-fractionation characteristics of different polymeric membranes during filtration of skim milk at refrigeration temperatures

Shane V. Crowley a, Veronica Caldeo a, Noel A. McCarthy b, Mark A. Fenelon b, Alan L. Kelly a, James A. O'Mahony a, *

a School of Food and Nutritional Sciences, University College Cork, Cork, Ireland
b Teagasc Food Research Centre, Moorepark, Fermoy, Co., Cork, Ireland

ABSTRACT

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-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

β-CN-enriched native whey (un-demineralised) → Heating (> 10°C) → Irreversible precipitation of β-CN → Holding (> 10°C) → 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

Aggregation at 26°C

Warm MF at 26°C

Casein micelle

Whey protein

β-CN monomer

β-CN micelle
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

At low Ca^{2+}, β-casein associates hydrophobically
β-casein purification (warm MF)

**Option 1:**
High ionic strength MF (100%)
- Precipitation ×

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

**Option 3:**
Medium ionic strength MF (40%)
- Stable ? Higher purity ??

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

Optimising ionic strength with diafiltration

Optimum for 1.2% protein

Turbidity at 26°C (NTU)

<table>
<thead>
<tr>
<th>Ionic strength (% of milk value)</th>
<th>0</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>600</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>800</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1200</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- 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
- 40% milk permeate
- ≤ 1.2% protein
**β-casein purification (warm MF)**

**β-Casein Micelles:**
- Formed at 26 °C
- Stable during warm MF

**Table:**

<table>
<thead>
<tr>
<th>MF Strength</th>
<th>β-Casein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low ionic strength MF</td>
<td></td>
</tr>
<tr>
<td>BCC</td>
<td>73</td>
</tr>
<tr>
<td>SPC</td>
<td>14</td>
</tr>
<tr>
<td>Med. ionic strength MF</td>
<td></td>
</tr>
<tr>
<td>BCC</td>
<td>80↑</td>
</tr>
<tr>
<td>SPC</td>
<td>7 ↓</td>
</tr>
</tbody>
</table>

**Graph:**
- 0 min
- 60 min
- 300 min

**Sizes:**
- 4°C
- 26°C
- 26°C
Reconstituted BCC powder

After 30 min incubation

<table>
<thead>
<tr>
<th>Temperature</th>
<th>In water</th>
<th>In SMUF</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>26°C</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>37°C</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>63°C</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>

After cooling from 63°C

<table>
<thead>
<tr>
<th>Temperature</th>
<th>In water</th>
<th>In SMUF</th>
</tr>
</thead>
<tbody>
<tr>
<td>45°C</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>37°C</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>4°C</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>

SMUF heated to 63°C in the absence of BCC

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

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

- 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 $\alpha$-lac:$\beta$-lg impacts heat stability

- 50.0% $\alpha$-lac
- 37.5% $\alpha$-lac
- 25.0% $\alpha$-lac
- 12.5% $\alpha$-lac
- 0.0% $\alpha$-lac
Use of ultrafiltration to prepare a novel permeate for application in the functionality testing of infant formula ingredients

Shane V. Crowley, Tom F. O’Callaghan, Alan L. Kelly, Mark A. Fenelon, James A. O’Mahony

School of Food and Nutritional Sciences, University College Cork, Cork, Ireland
Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland

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 delonised 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