Milk Proteins Ingredients: Functional Properties & How to Maximize Use in Formulating Foods

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Outline

1. What do we mean by functional properties? Why functional properties are important?

2. Discuss selected functional properties of milk proteins

3. Recent Developments: Examples
What is important to unlock full potential of proteins?

**Successful formulated food and beverages using protein**

- **Type of protein**
  - Chemical composition, structure, amino acid profile and sequence

- **Functional properties**
  - Solubility, Heat stability, emulsification, viscosity/gelling, foaming

- **Applications/end use**
  - Formulation, secondary processing e.g. UHT / Retort, order of processing and Ingredient addition, interactions

- **Physicochemical properties**
  - Iso-electric point, Molecular wt., hydrophobicity

- **Primary processing**
  - Pretreatments, type of processing-MF/UF/IX, Extraction, drying, process-induced interactions

- **Cost and environmental sustainability**

- **Benefits /claims**
  - Nutritional quality
  - BCAA, Bioavailability, PER/PDCAAS/DIAAS,
  - Health claims

- **Flavor and texture**
  - Consumer acceptability
  - Smell and taste,
  - No texture defect e.g. Gritty/chalky
<table>
<thead>
<tr>
<th>Highly functional ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete dairy protein – both whey and casein</td>
</tr>
<tr>
<td>Mild, dairy flavor Smooth mouth-feel</td>
</tr>
<tr>
<td>Unique combination of fast and slow proteins</td>
</tr>
<tr>
<td>Excellent solubility and hydration properties</td>
</tr>
<tr>
<td>Sold in various protein levels, including 34, 42, 60, 70, 80 and 90%</td>
</tr>
<tr>
<td>Adds viscosity through water binding capabilities</td>
</tr>
<tr>
<td>Ideal for high protein products and various product formats</td>
</tr>
</tbody>
</table>
Nutritional Benefits of Whey Proteins

- The best source of essential amino acids required by the body
- High PDCAAS (Protein Digestibility Corrected Amino Acid Score) / DIAAS score
- Proven to stimulate muscle protein synthesis – helps build & maintain muscle
- High in branched chain amino acids – leucine, isoleucine, and valine
- Ongoing research shows benefits in weight management, Lean Body Mass, lowering blood pressure, preventing Sarcopenia and healthy aging
- Delivers satiety as a part of a higher protein diet

Source: National Dairy Council/DMI, Inc.
Functional Properties of milk proteins
Consumer is looking for specific attributes (e.g. texture, melt, taste) in the final products.
What do we mean by functional properties?

• The functional properties of the ingredients are those properties, which provide specific function in the final products. Functional properties determine the overall behavior of ingredients in foods during processing, applications, storage and consumption.

• The functional properties of dairy ingredients may be defined in relation to their performance as ingredients in final product. In other words, functional properties of a particular ingredient determine their end-use in the final product.

• The functional properties of dairy ingredient typically dependent upon:
  • Type and composition of ingredients (e.g. WPC/WPI/MPC/caseinates)
  • Processing conditions- Primary processing (during ingredient making) and secondary processing (during formulations)
  • Formulations/ Final product (e.g. beverage, soup, sauces, ice cream, bakery)
## Functional properties of protein determine their end-use

<table>
<thead>
<tr>
<th>Functional properties</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solubility</strong></td>
<td>An ability of ingredient to readily dissolve and remain in solutions under different processing and formulation conditions</td>
</tr>
<tr>
<td><strong>Heat stability</strong></td>
<td>Heat stability is an ability to withstand severe heat treatments (typically UHT sterilization (140-145°C/4-5s) or retort temperatures (120°C/20 min)) without coagulation, precipitation or excessive thickening, gelation or viscosity increase. Caseins are very heat stable, but whey proteins are not.</td>
</tr>
<tr>
<td><strong>Emulsification</strong></td>
<td>Emulsification is an ability to keep two immiscible liquid (e.g. water and fat/oil) into stable solution. Milk proteins are excellent clean label emulsifier.</td>
</tr>
<tr>
<td><strong>Gelation</strong></td>
<td>Ability of an ingredient to form heat-induced or cold-set gels.</td>
</tr>
<tr>
<td><strong>Whipping/foaming</strong></td>
<td>Ability of an ingredient to make stable foam (e.g. in whipping cream, ice-cream)</td>
</tr>
<tr>
<td><strong>Water binding</strong></td>
<td>Ability of an ingredient to bind water, increase viscosity or thickening effect and retard syneresis</td>
</tr>
<tr>
<td><strong>Flavor and texture</strong></td>
<td>Dairy ingredients provide rich flavor, mouthfeel and desirable texture in many products</td>
</tr>
</tbody>
</table>

Different type of protein interactions contribute to specific functionality

Native proteins

- S-S-

Denaturation and interactions

Energy

e.g. Heat treatment

Appropriate conditions
e.g. pH, heat, ionic strength

Non-covalent Interactions
(e.g. hydrophobic bond)

Covalent Bonding
(e.g. disulphide bond)
### Casein and whey proteins have very different structure and properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Casein</th>
<th>Whey Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility at pH 4.6</td>
<td>Insoluble at pH 4.6</td>
<td>Soluble at pH 4.6</td>
</tr>
<tr>
<td>Coagulation by limited proteolysis</td>
<td>Caseins can be coagulated by specific, limited proteolysis (e.g. rennet coagulation)</td>
<td>Can not be readily coagulated by enzyme or limited proteolysis</td>
</tr>
<tr>
<td>Heat stability</td>
<td>Very heat stable (can withstand very high temperature)</td>
<td>Heat labile (denatured at 90 °C for 10 min)</td>
</tr>
<tr>
<td>Amino acid composition</td>
<td>Low in sulphur containing amino acids, high in proline</td>
<td>Relatively high in sulphur containing amino acids and low in proline</td>
</tr>
<tr>
<td>Physical State</td>
<td>Exist as a large colloidal aggregates called casein micelles</td>
<td>Exist as globular proteins in form of monomer –octamers depending on pH</td>
</tr>
</tbody>
</table>
Different whey protein fractions exhibit different functional properties

<table>
<thead>
<tr>
<th>Protein</th>
<th>Foaming properties</th>
<th>Emulsifying properties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Capacity</td>
<td>Stability</td>
</tr>
<tr>
<td>B-LG</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>α-AI</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Immunoglobulin</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>GMP</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>
Different types of protein interactions are possible depending on the food system.

Processing
- Heat
- pH
- Ionic Strength
- Concentration
- Other factors

Covalent/non-covalent interactions
- Glycosylation
- Lactosylation
- Binding hydrophilic molecules (e.g., Sugar)

- Electrostatic interactions
- Self-assembly
- pH-dependent interaction/swelling
- Gel formation
- Surface hydrophobicity
- Binding hydrophobic molecules
Final product is a result of three way interactions

Formulation parameters

- Protein concentration, pH, type and amount of added minerals/ionic strength, presence of other components (e.g., sugar, fat)

Ingredient functionality

- Protein of type, composition, structure, amount of minerals

Processing Parameters

- Severity of heat treatment (UHT/retort), direct/indirect heat treatment, homogenization

Final product
Various formulations and process parameters affects the performance of proteins in the final products.

Performance of Dairy Protein Ingredients

- Storage conditions and storage related changes
- pH of the product
- Processing (UHT, retort, Pasteurization) Direct/Indirect
- Added minerals/ionic strength
- Type and amount of minerals (e.g. mono vs. divalent)
- Type of proteins (e.g. casein/whey proteins, other proteins)
- Concentration of protein
- Other component present
Solubility
**What is Solubility?**

It is an ability of dairy ingredients to readily go in the solution and remain soluble under different processing conditions such as heat treatments or in different formulations such different pH or mineral levels.

**Why is it important?**

During reconstitution or while making beverages, complete solubalization or rehydration of dairy ingredients is very important:

- To avoid defects (e.g. chalkiness/Gritty texture)
- To avoid settling of floating of particles
- To provide desired nutritional and functional benefits
Dissolving protein Ingredients: Different scenario

Scenario 1
Completely Soluble (excellent solubility)

Scenario 2
Partly soluble (Poor solubility)
Solubility of various protein at different pH
What is the impact of ingredients with poor solubility?

- Functionality in final products may be affected (e.g. viscosity, heat stability, emulsification, foaming)
- May affect sensory aspects (e.g. taste, texture) and lead to defects (e.g. Chalky, sedimentation)
- May affect nutritional properties and nutritional claims And shelf-life stability
- Secondary processing considerations (e.g. time to dissolve/hydrate, fouling, product yields and/or losses)
**Solubility: Various steps involved in reconstitution/rehydration of ingredients**

1. **Wetting**
   - **Wettability**: Ability of powder particles to absorb solvent (e.g. water) at its surface (“get wet”)

2. **Sinkability**
   - **Sinkability**: Ability of powder particles to go below the water surface

3. **Dispersibility**
   - **Dispersibility**: Ability of wetted particles to disperse in the solution

4. **Solubility**
   - **Solubility**: Dissolution of soluble agglomerates into solvent
Several factors can affect reconstitution of dairy ingredients

• Reconstitution temperature (4°C vs. 20°C, 50°C)
• Water quality (Hardness, mineral content etc.)
• pH of the solvent
• Other component present in the solvent (e.g. sugar, competition with other components, availability and mobility of water)
• Particle chemistry surface composition e.g. fat on surface, surface charge (hydrophilic or Hydrophobic, denaturation and aggregation of proteins)
• Particle morphology-particle size and shape, agglomeration, porosity, surface area, etc.
Hydration profile of MPC 85 in milk/water at different temperature (example)

Source: KJ Burrington, Clean label presentation
Solubility of milk proteins at different pH (10% solutions)

Whey Protein Isolate

- pH 6.2
- pH 4.6
- pH 3.0

Milk Protein Isolate

- pH 7.0
- pH 4.6
- pH 3.0
Heat stability
What is heat stability?

Heat stability can be defined as an ability of a given sample to withstand UHT sterilization (140-145°C/ 4-5s) or retort temperatures (120°C/ 20 min) without coagulation, precipitation or excessive thickening/gelation/viscosity increase.
Why heat stability is important?
Heat stability of dairy ingredients is very important for their applications in high protein beverages. Native whey proteins undergo heat treatment, leading to unfolding and aggregation, which results in viscosity increase and further gel formation. Stable and instable states are illustrated by the images. de Kort (2012) and Saglam (2011) studied the heating effect on viscosity, with a graph showing the increase in viscosity with solids content.
Applications of dairy ingredients in the beverage applications

Meal Replacements

Sports Drinks
- Recovery Drinks /Isotonic beverages
- Body Building/Muscle Building

Energy Drinks

Juice Drinks

Protein waters

Healthy Aging
Performance and storage stability of the protein in beverages is further affected by various factors:

- Type protein used
- Too much un-adsorbed proteins (for emulsions)
- Type and amount of minerals present in the system
- Other components present in the system
Various factors can affect the heat stability of milk and food systems (formulations)

Factors affecting heat stability

Factors related to milk composition e.g. TS, protein, salt balance

- Seasonal variations
- Natural variations

Related to formulations e.g. Additives, sugar, salts, phosphate, citrate

Related to processing conditions e.g. pre-heating/ heating, homogenization

Related to interactions of various components

Interactions with carbohydrates, fats, minerals, concentration effects, pH, Ionic strength
Emulsification
Emulsification is an ability to keep two immiscible liquid (e.g. water and fat/oil) into stable solution.

In the process of making oil-in-water emulsions, oil/water interfaces are created. These interfaces are protected by adsorption of surfactants and partially lost again by recoalescence of those emulsion droplets that are not protected quickly enough by surfactants.

The proteins in milk (whey protein and caseins) and phospholipids present in the cream and butter milk can successfully act at oil/water interfaces to form and stabilize emulsions (clean label emulsifiers).
Milk protein is an excellent, clean label emulsifier

The unfolding of the dairy protein exposes hydrophobic amino acid that facilitate the ability of the protein to orient at the oil/water interface and stabilize emulsion.

Native proteins

Unfolding to expose hydrophobic groups to surface

Interactions

Oil / Air Phase

Aqueous Phase

Hydrophobic groups at core molecule

Stabilise droplets against coalescence

Consumer is demanding clean label products:
Milk ingredients can play big role in clean label products

Image source: Google image
Gelation

Gelation is a two-step mechanism that involves an initiation step, the unfolding or dissociation of protein molecules, followed by an aggregation step in which association or aggregation reactions occur, resulting in gel formation.

Dairy proteins, have the ability to form rigid, heat-induced irreversible gels that hold water and fat, and provide structural support.

Andoyo et al. (2015). Food hydrocolloids, 51, 118–128
## Comparative overview of functionality of dairy ingredients

<table>
<thead>
<tr>
<th>Functionality</th>
<th>WPC</th>
<th>WPI</th>
<th>MPC</th>
<th>MPI</th>
<th>Micellar casein</th>
<th>Sodium caseinates</th>
<th>Calcium caseinates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solubility (60°F)</strong></td>
<td>****</td>
<td>****</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td><strong>Solubility (120-130°F)</strong></td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>***</td>
<td>***</td>
<td>****</td>
<td>***</td>
</tr>
<tr>
<td><strong>Viscosity (10% protein solutions in water at 60°F)</strong></td>
<td>*</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td><strong>Gelling (10% protein solutions, heated 195°F/10 min)</strong></td>
<td>****</td>
<td>****</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td><strong>Heat stability (5% protein solution, 285°F in oil bath)</strong></td>
<td>*</td>
<td>*</td>
<td>**</td>
<td>***</td>
<td>****</td>
<td>****</td>
<td>****</td>
</tr>
</tbody>
</table>

**Keys:**
- * Poor
- ** Good
- *** Very Good
- **** Excellent
Multi-disciplinary approach to make tailored ingredients to deliver desired functionality

Science / Fundamental understanding of milk components

Combined with

New process Technologies (e.g. Membrane processing, others)

To deliver

Ingredients that meet consumer need

Applications

12g protein
15g protein
9g protein
Recent Developments in milk protein ingredients
Dairy Landscape 2017 and beyond

2017 & beyond

- Advancement in Science and Technology
- Consumer awareness and education
- Market pull

Source: National Dairy Council/DMI Inc.
Membrane Fractionation Technology is helping to develop wide range of Dairy Ingredients

Source: National Dairy Council/DMI Inc.
Whey based ingredients across the value-chain

- Lactoferrin, fractions
- Alpha-Lac
- Micellar casein, WPIs
- WPC80
- WPC34
- Sweet whey,
- Lactose (various grades), demin whey
- Permeate, blends
- Feed grade ingredients

*Source: USDEC, from Industry sources, November 2015
Native Whey is filtered directly from fresh milk; not a co-product of the cheese-making process.
### Unique features of Native whey protein compared to traditional/cheese whey

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Cheese whey</th>
<th>Native whey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starter culture</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Rennet/residual</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Glycomacropeptide</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Color</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Fat</td>
<td>Yes</td>
<td>Negligible</td>
</tr>
<tr>
<td>Pasteurization Steps</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

- Native whey has better clarity and cleaner flavor
- It has higher content of Cysteine and Leucine

A. Kelly (2014)
Micellar casein

- New casein ingredient: Has FDA GRAS status (included with MPC/MPI)

**Unique properties**
- Cleaner flavor compared to caseinates as it is not made by precipitation
- Has gone through minimum processing compared to casein/ate
- Has better heat stability compared to WPC, WPI and MPC

**Unique Applications**
- Shelf-stable nutritional, meal replacement, sports beverages, medical foods, coffee whitner
- Retorted/UHT RTE/RTD food and beverages, soup and sauces
- Can replace rennet casein in process cheese applications
- Can replace sodium or calcium caseinates

Source: US Dairy Export Council/DMI, Inc.
Composition of whey proteins

- Beta-lactoglobulin
- Alpha-lactalbumin
- Immunoglobulin G
- Bovine serum albumin
- Lactoferrin
- Non-protein nitrogen

Sources: Swaisgood (1982), Walstra and Jenness (1984), and Fox (1989)
Proteins at the natural pH of milk (pH 6.7) has net –ve charge.

At different pH, proteins have different charges:

- Increase pH (add more OH⁻)
- Lower the pH (Add more H⁺)
- Further lower the pH (add more HCl)

* Iso-electric point of proteins (no net charge) *
- pH 4.6
- pH ~9.0 (negative charge)
- pH ~3.0 (positive charge)
Different Milk Protein has different Iso-electric point

<table>
<thead>
<tr>
<th>Protein</th>
<th>Concentration in milk (g/kg)</th>
<th>Isoelectric point (pI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>28.0</td>
<td>4.60</td>
</tr>
<tr>
<td>(\beta)-lactoglobulin</td>
<td>3.3</td>
<td>5.13</td>
</tr>
<tr>
<td>(\alpha)-lactalbumin</td>
<td>1.2</td>
<td>4.2-4.5</td>
</tr>
<tr>
<td>Bovine Serum Albumin</td>
<td>0.4-0.5%</td>
<td>4.7-4.9</td>
</tr>
<tr>
<td>Immunoglobulin (depending on type of Ig)</td>
<td>0.7</td>
<td>5.5-8.3</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>3-0.4%</td>
<td>8.70</td>
</tr>
</tbody>
</table>
Application of positively charged UF membrane

Exploiting differences in isoelectric point of whey proteins to enhance rejection at charged (e.g. positively charged) membrane surface

Arunkumar and Etzel (2013)  
Sep. Purif. Technol., 105, 121-128

Pearce (1987); Maubois et al. (1988)
Isolation of whey protein fractions

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular mass (kg/mol)</th>
<th>Concentration (g/L)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-lactalbumin (ALA)</td>
<td>14</td>
<td>1.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Beta-lactoglobulin*</td>
<td>18</td>
<td>2.2</td>
<td>5.4</td>
</tr>
<tr>
<td>Other proteins (IgG, BSA)</td>
<td>66-150</td>
<td>Trace*</td>
<td>5-8</td>
</tr>
</tbody>
</table>

* Updated

*0.02-0.25 g/L

- ALA is smallest and most acidic protein
- At pH 4.4, ALA is uncharged, other proteins are charged positive

Set pH ≈ 4.4
ALA net charge = 0 (permeates)
Other proteins = + (retained)

Charged membrane can be used to purify α-Lactalbumin and GMP (with 97% purity) and β-Lactalbumin isolates (87% pure) can be obtained without use of Chromatography

Arunkumar and Etzel (2013)
*Sep. Purif. Technol., 105, 121-128*
Modification of functional properties of casein

Non-micellar/dissociated casein due to temporary lowering in pH
Solubility: Impact of temperature and storage time

Source: Metzger, L. (2016)
Tailored casein ingredients for high protein, long shelf-life (retorted/UHT) beverage applications

Solubility at Room Temp (20°C)

- F-MPC
- CtrlMPC

Heat stability at 140°C

- F-MPC
- CtrlMPC

Source: Metzger, L. (2016)

F-MPC= functionalized MPC, CtrMPC=Control MPC
Developing new MPC with better emulsification properties

Fat emulsification, g oil/g pro solution

Source: Metzger, L. (2016)
High pressure has different effects on denaturation of milk proteins


Carroll and Patel et.al., US Patent 20080317823 A1
Summary and Recommendations

• Milk Protein is highly complex and there is structure-functional relationship

• Knowledge of physico-chemical and functional properties allow product developers/end-user to choose right ingredient and optimize its use in final product/applications

• There are some new technological advancements in Processing Technologies in recent years, which helps in the development of new milk protein ingredients with tailored functionality and nutrition
Casein and whey proteins offers unique functionality which can be exploited for different applications

<table>
<thead>
<tr>
<th>Whey Proteins</th>
<th>Caseins</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Whipping/foaming</td>
<td>• Water binding (e.g. sodium caseinates)</td>
</tr>
<tr>
<td>• Solubility over wide range of pH (Acidic and neutral pH)</td>
<td>• Heat stable</td>
</tr>
<tr>
<td>• Heat labile/easily denatured</td>
<td>• Hydrophobic/ emulsification</td>
</tr>
<tr>
<td>• Gelation</td>
<td>• Good solubility at neutral pH</td>
</tr>
<tr>
<td></td>
<td>• Color/opacity</td>
</tr>
</tbody>
</table>

Various milk proteins offer different functionality. Depending on application, ingredients rich in either casein/whey protein can be selected to provide specific functional properties.
Considerations for formulating beverages at different pH

- **pH 2.8-3.5**
  - Isotonic, sports, clear beverages, flavored or protein water

- **pH 3.5-4.5**
  - Creamy Cloudy appearance, Yogurt drink, smoothies

- **pH 6.6-7.0**
  - Mostly milky, cloudy, Meal replacement, Shakes, medical beverages

Low pH beverages

Neutral pH beverages
Thank you for your attention
Protein from different sources are unique

**Nutritional quality:** Protein from different sources has different amino acid profile, different digestibility

**Functional Properties:** Each protein has different functional properties e.g. solubility, heat stability, viscosity, emulsification

**Flavor/taste:** Protein from different sources have their distinct flavor/taste profile

Technologies can help to improve the ingredient profile to certain extent, but there are inherent properties and attributes, that can not be changed.
HPP leads to unique functionality changes in the milk

Untreated  Heated  Untreated  Pressure-treated

High pressure treatment of milk can modify the functionality of milk proteins for yogurt applications

Dissociation of casein micelles, availability of $\alpha_s^2$-casein was responsible for improved functionality of protein in yogurt

Patel et al. (2012)