Davisco Whey Protein Processing

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What is whey?

By product of cheese making?
Approximate composition of whey

<table>
<thead>
<tr>
<th>Component</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>93.5</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.9</td>
</tr>
<tr>
<td>Protein</td>
<td>0.8</td>
</tr>
<tr>
<td>Minerals</td>
<td>0.5</td>
</tr>
<tr>
<td>Fat</td>
<td>0.3</td>
</tr>
</tbody>
</table>

- Primarily water and lactose
- To produce whey protein isolate the whey protein is separated from the other components using filtration or ion exchange based processes
Whey pre-treatments

Cheese manufacture

- whey
- Fines removal
- Fat separation
- Bleaching
- Pasteurization

cheese
Cheese fines
Whey cream
(optional – applied to colored whey)
WPI production utilizes two primary separation technologies

**Membrane Filtration**
- Separation of non-protein components and concentration
- Primarily based on molecular weight/size
- Advanced filtration technology can further optimize selectivity through charged membranes
- Most cost effective up to 80-90% protein contents

**Ion-Exchange**
- Isolation of whey proteins through charge-affinity to custom resins
- Best for high purity, low denaturation fractions
Spiral wound membranes
Complete filtration system
Types of filtration

- **Bacteria**: 0.1 - 10.0 µ
- **Fat**: 0.01 - 0.1 µ
- **Protein**: 0.001 - 0.01 µ
- **Lactose**: <0.001 µ
- ** Minerals**: 0.001 - 0.01 µ
- **Water**: <0.001 µ

**Molecular Weight Cut-Off (Daltons):**
- Bacteria: 1,000,000 Daltons
- Fat: 10,000 Daltons
- Protein: 1,000 Daltons
- Lactose: 50 Daltons
- Minerals: 50 Daltons
- Water: 50 Daltons

**Operating Pressure (PSI):**
- Bacteria: 5-30 PSI
- Fat: 15-150 PSI
- Protein: 100-500 PSI
- Lactose: 250-1500 PSI
- Minerals: 250-1500 PSI
- Water: 250-1500 PSI
Ultrafiltration
Microfiltration

Water, Lactose, Whey Protein -> Fat, Aggregated Protein

Flow Direction

Retentate

Membrane

Permeate
WPI - filtration based

Whey → Ultrafiltration → retentate → microfiltration → permeate → permeate → Ultrafiltration → retentate

Whey protein isolate → retentate

Water (dia-filtration)
Ion-exchange

Negatively Charged Analyte [Anion] (Attracted to Positive Surface)

Positively Charged Analyte [Cation] (Attracted to Negative Surface)
WPI - ion-exchange

Step 1 - binding
whey

Unbound components

Step 2 - elution
salt or pH adjustment

Elution of bound components
WPI - ion-exchange

Step: 3 – ultrafiltration of eluent

eluent

Ultrafiltration

Retentate (pure whey protein)

Permeate (salts)
**Whey protein fractions**

**Whey protein nomenclature and relative amounts**

- Raw whey only contains about .8% protein
- Most abundant protein is $\beta$-lactoglobulin

<table>
<thead>
<tr>
<th>Fraction</th>
<th>% of protein in whey</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$ - Lactoglobulin</td>
<td>50</td>
</tr>
<tr>
<td>$\alpha$ - Lactalbumin</td>
<td>25</td>
</tr>
<tr>
<td>Glyco-macro-peptide (GMP)</td>
<td>16</td>
</tr>
<tr>
<td>Blood serum albumin</td>
<td>5</td>
</tr>
<tr>
<td>lactoperoxidase</td>
<td>trace</td>
</tr>
<tr>
<td>Lactotransferrin</td>
<td>trace</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>trace</td>
</tr>
</tbody>
</table>
Major whey protein fractions

\(\beta\)-lactoglobulin

- Major whey protein (50% of total whey protein)
- 162 a.a., 2 disulfide bonds and one free cysteine, high in branched chain amino acids
- Often blamed for allergic reactions (not present in human milk)
- Undergoes pH dependent self association reactions
- Susceptible to thermal denaturation above 65°C at pH6.7 – caused by exposure of free cysteine
- Binds retinol (vitamin A) – may be involved in Vit A transport to young
- Excellent emulsification, foaming, and gelation properties

- Molecular segments and structure
  - Eight strands of \(\beta\)-sheet that form a \(\beta\)-barrel with the shape of a flattened cone (interior is hydrophobic, but the opening is lined with hydrophilic a.a)
  - Disulfide bonds – one bridge between strands G and H (cys 106 and 109) and one joins flexible loop to the c-terminus (cys 66-160)
  - Free cysteine is buried in the interior
Whey proteins – β-lactoglobulin

Adapted from Food Prot. Applic. – Cayot and Lorient 225-256
Major whey protein fractions

α-lactalbumin

- 25% of total whey protein
- 123 a.a., 4 disulfide bonds and no free cysteine groups
- Very similar to egg white lysozyme
- Necessary for synthesis of lactose
- High affinity for calcium and other metal ions (released below pH 4)
- Heat denatured above 62°C
- Complex of α-lactalbumin and oleic acid causes tumor cells to self destruct (may be critical in infant health and disease prevention)
- Moderate foaming and emulsification properties, synergistic gelation with β-lactoglobulin
- Molecular segments and structure
  - Ellipsoid shape with a deep cleft, calcium binding site is bridged by disulfide bond (73-91) deep in the cleft – functions to stabilize against thermal denaturation
Whey proteins – $\alpha$-lactalbumin

Adapted from Crit. Rev. Food Sci. – 1996, Wong et al. 807-844
Membrane based as compared to ion exchange

- Membrane based WPI contains 18-20% glyco-macro peptide (a protein fragment from casein)
- Ion exchange based WPI primarily contains $\beta$-lactoglobulin and $\alpha$-lactalbuimin

This difference in protein fractions results in important nutritional and functional differences

- Amino acid profile
- Solubility, heat stability, gelation, foaming
Specifications

♦ Compositional based – protein, fat, minerals
  - Fat and mineral content will have an impact on performance (fat is critical for clarity and mineral contact impacts gelation and heat stability)

♦ Performance based – solubility, viscosity, clarity, heat stability, gel strength, foam overrun and foam stability, water holding capacity
  - All of these properties are influenced by pH, ionic strength, and thermal treatment
  - Pay close attention to the details of the testing methods when comparing products from different companies

♦ Instantized versions are available
### α-lactalbumin isolate

#### Major whey proteins: Physico-chemical characteristics

<table>
<thead>
<tr>
<th>Property</th>
<th>α - La</th>
<th>β - Lg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoelec. pH</td>
<td>4.4</td>
<td>5.4</td>
</tr>
<tr>
<td>Mol. Wt, kD</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Conc. In whey, g/L</td>
<td>1.2</td>
<td>3.2</td>
</tr>
</tbody>
</table>

- β – Lg occurs in dimer form with a mol. wt of 36 kD at pH 5.5 to 7.5
- Octomer with 144 kD at pH 3.5 to 5.2
A variety of \( \alpha \)-La enriched products that have a wide range in purity are available.

Why is the market for \( \alpha \)-La enriched products growing?

- Infant formula
- Whey protein based therapeutic formula
- Stress reduction products (tryptophan:LNAA)
- Nutraceuticals applications
- Nutritional supplements
Membrane separation
- Options for low to intermediate purity products

Precipitation
- Intermediate purity products

Ion Exchange
- Only process that produces a highly pure product
Davisco $\alpha$-lactalbumin isolate

- Proprietary production process

- True $\alpha$-lactalbumin isolate in its native form containing >90% $\alpha$-lactalbumin

- Other products are referred to as $\alpha$-lactalbumin enriched and contain less than 65% $\alpha$-lactalbumin
Questions?