ALPHA-LACTALBUMIN: DEVELOPMENT PATH FOR A VALUABLE DAIRY PROTEIN INGREDIENT

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Global R&D
Mead Johnson Nutrition

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Organized by
Davisco®, Agropur, Inc.
Milk is a complete nutritional food designed by nature to support the growth of infant mammals.

<table>
<thead>
<tr>
<th>NUTRIENT</th>
<th>% (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>88.3</td>
</tr>
<tr>
<td>Protein</td>
<td>3.2</td>
</tr>
<tr>
<td>Total lipid (fat)</td>
<td>3.3</td>
</tr>
<tr>
<td>Ash</td>
<td>0.7</td>
</tr>
<tr>
<td>Carbohydrate, by difference (Lactose)</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Milk is synthesized and secreted by mammary glands upon hormonal stimulation (*Prolactin*).

Ref:
- Nutrient Data Lab. USDA
milk proteins

A model of premium quality food protein:
• All essential amino acids are present in appreciable amounts.

CASEINS: The portion of the milk proteins that coagulate into a curd after adding rennet or after acidifying milk to pH 4.6.

CASEINS constitute about 80% of total bovine milk proteins

Three major type of caseins that self assemble into porous spheres known as micelles

WHEY PROTEINS: The portion of the milk proteins that remain in solution in the liquid portion.

WHEY PROTEINS constitute about 20% of total bovine milk proteins.
**milk proteins**

Ratio of milk proteins changes with lactation period.

<table>
<thead>
<tr>
<th></th>
<th>HUMAN MILK</th>
<th>WHEY PROTEINS</th>
<th>CASEINS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Milk protein (mg/mL)</strong></td>
<td><img src="#" alt="Graph" /></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Milk composition changes within species**

<table>
<thead>
<tr>
<th></th>
<th>HOLSTEIN</th>
<th>JERSEY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PROTEIN, %</strong></td>
<td>3.1</td>
<td>3.7</td>
</tr>
<tr>
<td><strong>FAT, %</strong></td>
<td>3.7</td>
<td>5.1</td>
</tr>
<tr>
<td><strong>LACTOSE, %</strong></td>
<td>4.9</td>
<td>5.0</td>
</tr>
<tr>
<td><strong>OTHERS, %</strong></td>
<td>0.7</td>
<td>0.8</td>
</tr>
</tbody>
</table>

**Infant mammal growth rate and milk composition**

<table>
<thead>
<tr>
<th></th>
<th>HUMAN</th>
<th>COW</th>
<th>DOG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WEIGHT x2, days</strong></td>
<td>180</td>
<td>47</td>
<td>8</td>
</tr>
<tr>
<td><strong>PROTEIN, %</strong></td>
<td>1.6</td>
<td>3.3</td>
<td>7.1</td>
</tr>
<tr>
<td><strong>FAT, %</strong></td>
<td>3.7</td>
<td>4.0</td>
<td>8.3</td>
</tr>
<tr>
<td><strong>LACTOSE, %</strong></td>
<td>7.0</td>
<td>5.0</td>
<td>3.7</td>
</tr>
</tbody>
</table>

**Ref:**
milk proteins

### Table: Alpha-Lactalbumin and B-Lactoglobulin Properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Alpha-Lactalbumin (a-lactalbumin)</th>
<th>B-Lactoglobulin (b-lactoglobulin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight, kDa</td>
<td>14</td>
<td>36.8 (dimer)</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>4.2 - 4.5</td>
<td>5.2</td>
</tr>
<tr>
<td>Disulfide bonds</td>
<td>4 (no SH)</td>
<td>2 (1 SH)</td>
</tr>
<tr>
<td>Presence in (% total prot):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human milk</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Bovine milk</td>
<td>3.7</td>
<td>9.8</td>
</tr>
</tbody>
</table>

α-LACTALBUMIN: DEVELOPMENT PATH FOR A VALUABLE DAIRY PROTEIN INGREDIENT
ALPHA-LACTALBUMIN: DEVELOPMENT PATH FOR A VALUABLE DAIRY PROTEIN INGREDIENT

milk ingredients

MILK PROTEIN INGREDIENTS FOR INFANT AND CLINICAL NUTRITION PRODUCTS

Drivers for food ingredient development and functionality

- Physical-chemical
- Sensory
- Nutritional
- Health
- Law - Economics
- Enabler

✓ Food Ingredient development could be traced across inter-related drivers

✓ These drivers are a conjunction of Societal – as its regulations and market forces - and Science & Technology advances

✓ Ingredient functionality is identified in the physical-chemical, sensorial, nutritional, and health impact drivers

drivers for food ingredient development and functionality

**NUTRITIONAL PROPERTIES**
- Digestibility
- Compositional Profile
- Absorption Mechanism

**PHYSICAL-CHEMICAL PROPERTIES**
- Structure
- Surface
- Hydration
- Reactivity

**SENSORY PROPERTIES**
- Flavor
- Texture
- Aroma
- Color

**HEALTH**
- Obesity
- CV disease
- Immunological
- Allergy
- Life-stage

**LAW /ECONOMICS**
- Market: Need-Want, Price, Supply-Demand
- Regulation: Law, Safety
- Sustainability: energy, environment, pollution

**ENABLER**
- Processes- make ingredient
- Products - ingredient make
- Attributes – define ingredient/ product
  (Convenience, Portable, Ready-to-eat, Shelf-life, Life Style)

drivers for protein ingredient development and functionality

**PHYSICAL-CHEMICAL** property of proteins is the result of the interaction between *intrinsic* and *extrinsic* factors:

- **Intrinsic** - pertain to the protein itself:
  - Structure, Size, Shape, Charge, Hydrophobicity, Flexibility

- **Extrinsic** - pertain to the environment where the protein interacts:
  - pH, Temperature, Pressure, Ionic Strength, Components (Water, Fat, Salts), Energy used in the system.

Functional properties may be:

**Structural** - relate to how the protein molecule is folded, cross-linked, linked to other subunits, or to other non-protein molecules.

Example of those:
- Viscosity
- Gelation
- Film formation
- Antimicrobial
- Metal chelation

**Surface** - relate to how the bulk solvent – water – interacts at the interface with the protein molecule.

Example of those:
- Solubility
- Wettability
- Dispersibility
- Emulsification
- Foaming
- Fat and Flavor binding

**Hydration** - relate to how a water-bound protein unit interacts with itself and other components present in the bulk.

Example of those:
- Cohesion
- Elasticity
- Water binding
- Thermal denaturation
Denaturation and aggregation of α-lactalbumin at neutral pH

- Heating (95°C) pure α-lactalbumin produced distinct monomers and dimer aggregates at pH 7.0
- α-lac monomers are formed via intra-molecular disulfide interchange, while dimers are formed via inter-molecular disulfide linkages.
- Hydrophobic interactions may be responsible for these interactions.
- Presence of other whey protein impurities requires ionic screening (via higher phosphate or calcium presence) to produce similar aggregates.
- α-lac aggregates dissociated with cooling below 95 °C.

**PHYSICAL-CHEMICAL PROPERTIES**

- Structure
- Surface
- Hydration
- Reactivity

**Alpha-lactalbumin**: A new carrier for vitamin D3 food enrichment

**α-Lactalbumin structure:**
- A large helical domain connected by a loop to a small beta-sheet domain.
- Four disulfide bridges and one hydrophobic pocket.
- A calcium ion is bound to a high affinity binding site in the loop connecting two domains.
- Oleic and palmitic acids bind to the α-lac-apo but not to the α-lac-holo form.

Apo α-lac has one binding site for vitamin D3.
The binding is mostly hydrophobic, with one hydrogen bond.

The binding of vitamin D3:
- Induces α-lac conformational changes (random coil increase).
- Increases α-lac surface hydrophobicity.
- Decreases α-lac heat stability.
- Induces formation of α-lac aggregates (125 nm) that encapsulate the vitamin D3.
- α-lac complexes (aggregates) are stable in presence of high vitamin D3 concentration.

ALPHA-LACTALBUMIN: DEVELOPMENT PATH FOR A VALUABLE DAIRY PROTEIN INGREDIENT

drivers for protein ingredient development and functionality

ENABLER relates to:

- A technology – that allows ingredients to be developed
- An ingredient – that allows products to be developed

Enabling technologies allow for the development of enabling ingredients or products with needed or wanted attributes.

Seek for Enabling Innovations !!!!

Technologies:
- Membrane Filtration
- Chromatography
- Extrusion
- Encapsulation
- Hydrolysis

Ingredients:
- Protein fractions
  - WPC
  - WPI
- Isolated proteins:
  - α-lactalbumin
  - Lactoferrin
- Texturized proteins
- Encapsulated oils, probiotics
- Bioactive peptides

Products:
- Infant formula
- Sport beverages
- Geriatric foods
- Premature foods
- Medical foods
- Snacks
- Beverages
ALPHA-LACTALBUMIN: DEVELOPMENT PATH FOR A VALUABLE DAIRY PROTEIN INGREDIENT
drivers for protein ingredient development and functionality

Evaluation of ultrafiltration membranes for production of α-lactalbumin–enriched whey protein concentrate

Process produces a WPC80 enriched α-lactalbumin with:
- 63% purity
- 1.4 ratio α-lactalbumin/β-lactoglobulin
- 21% yield α-lactalbumin

Selective hydrolysis increases α-lactalbumin content in model infant formula

Increases ratio α-lactalbumin/β-lactoglobulin

The increase in α-lactalbumin:

- Reduced the viscosity of wet-mixes
- Created stable emulsions
- Reduced fouling during processing

drivers for protein ingredient development and functionality

The Importance of \( \alpha \)-Lactalbumin in Infant Nutrition

**WILLI E. HEINE, PETER D. KLEIN AND PETER J. REEDS**

Infant formulas with increased concentrations of \( \alpha \)-lactalbumin

© 2003 American Society for Clinical Nutrition

Olof Sandström, Bo Lönnnerdal, Gitte Graverholt, and Olle Hernell

Effects of \( \alpha \)-lactalbumin–enriched formula containing different concentrations of glycomacropeptide on infant nutrition


**Alpha-lactalbumin-rich infant formula fed to healthy term infants in a multicenter study: plasma essential amino acids and gastrointestinal tolerance.**

Davis AM\(^1\), Harris BJ, Lien EL, Pramuk K, Trabulsi J.


**Effect of an \( \alpha \)-lactalbumin-enriched infant formula with lower protein on growth.**

SENSORY PROPERTIES define the sensory experience of the consumer and influence the interaction with the product.

It relates to:

- Liking
- Repeat purchase
- Convenience

Sensory properties may become enablers as they may:

- Satisfy consumer expectations
- Create new experiences
- Supports life-style
- Supports compliance
- Supports quality of life
NUTRITIONAL properties of proteins relate to the digestibility and the absorption mechanisms that allow the protein to be utilized by the body for growth, maintenance or medical purposes.

Nutritional properties also relate to **intrinsic** and **extrinsic** factors:

- **Intrinsic** – relate to the protein itself, such as:
  - amino acid composition (protein quality)
  - Amino acid sequence (peptides)
  - Conformation (domains)

- **Extrinsic** – relate to the external environment where the protein interacts:
  - Processing
    - Heat treatment (denaturation)
    - Hydrolysis (digestibility, bioactivity)
  - Interaction with other food components:
    - Complexation
    - Glycosylation
  - Hydration (enzyme accessibility)
Lactoferrin and α-lactalbumin for improving energy balance and glucose tolerance

Study effect of whey with α-lactalbumin and lactoferrin on:
- Weight
- Body composition
- Food intake
- Energy expenditure
- Glucose tolerance
- Meal-induced hormone response

α-lactalbumin and Lactoferrin seem to be more beneficial than just whey itself in improving:
 - energy balance
 - glucose tolerance

At 65 days:
α-Lactalbumin is more effective than whey in:
• increasing energy expenditure

Lactoferrin is more effective than whey in:
• inducing hypophagia
• promoting fat loss

Diet-induced obese rats:
• 65 day study.
• Feed - isocaloric and isonitrogenous
  √ 30% calories - protein.
  √ 40% calories – fat.

Diets:
• Whey
• α-lactalbumin
• Lactoferrin

Ref: Lactoferrin and lactalbumin are more effective than whey protein in improving energy balance and glucose tolerance in diet-induced obese rats. Zapata, R., Pezeshki, A., Singh, A., Chou, M., Chelikani, P. ADSA 2015. #784. Protein & Enzyme Symp
drivers for protein ingredient development and functionality

HEALTH properties of proteins relate to the benefits that the organism derives from consuming the protein food.

Protein ingredients and foods may be developed to manage particular life stages, life styles, or diseases:

- Muscle building (sports nutrition)
- Muscle maintenance and recovery (elderly or convalescence)
- Allergy management (protein allergy)
- Digestion deficiencies (PKU)
- Weight control

Peptides have been shown effective:

- ✓ ANTI-OXIDANTS
- ✓ ANTI-HYPERTENSIVE
- ✓ ANTI-MICROBIAL
- ✓ IMMUNO-MODULATORY
- ✓ FAST DIGESTION RATE
- ✓ ANTI-TUMOR
- ✓ HYPOCHOLESTEROLEMIC
- ✓ ANTI-THROMBOTIC
- ✓ ENHANCES MUSCLE RECOVERY
- ✓ ENHANCE MINERAL ABSorption
ALPHA-LACTALBUMIN: DEVELOPMENT PATH FOR A VALUABLE DAIRY PROTEIN INGREDIENT

drivers for protein ingredient development and functionality

Evening intake of $\alpha$-lactalbumin increases plasma Trp availability, improves alertness and brain measures of attention

- Brain serotonin function is thought to promote sleep regulation and cognitive processes
- Brain uptake of tryptophan, a serotonin precursor, is dependent on nutrients that influence the availability of tryptophan in blood plasma.

  ✓ Study tested evening consumption of $\alpha$-lactalbumin to increase plasma Trp level, to improve alertness and performance on the morning after on patients with sleep complaints.
  ✓ Evening $\alpha$-lactalbumin intake caused a 130% increase in plasma Trp levels before bedtime; the morning after it reduced sleepiness, improved brain-sustained attention processes and also improved behavioral performance.

Serotonin plays a role in the regulation of human appetite and food intake.
Serotonin is synthesized from its precursor, the amino acid L-tryptophan.
Studies have shown that supplementation with L-tryptophan reduces food intake.

- Patients consuming a breakfast with either α-lactalbumin or casein as the sole protein source, voluntary energy intake of a subsequent lunch was lower after the α-lactalbumin-containing breakfast.
- Trp-rich α-lactalbumin may be useful in controlling appetite or food intake; however, its mechanism of action remains to be elucidated.
ALPHA-LACTALBUMIN: DEVELOPMENT PATH FOR A VALUABLE DAIRY PROTEIN INGREDIENT

drivers for protein ingredient development and functionality

Regulatory Aspects:
- Demonstrate safety and efficacy
- Health claims: Generic vs. Innovative
- Scientific validation – animal and human intervention studies

Quality:
- Strict manufacturing standards
- Strict ingredient specifications
- Strict control microbial and chemical contaminants
- Consistent and validated nutrient levels

Economics:
- Supply and demand balance
- Specialty ingredients: Capital investment for commercial isolation
- Specialty premium ingredients demand premium prices
- Possible low recovery yields – unfavorable pricing proposition
- Sourcing high quality dairy feed
- Investments to meet high quality standards
- Investments to support health claim

LAW /ECONOMICS
- Market: Need-Want, Price, Supply-Demand
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drivers for protein ingredient development and functionality

Knowledge Center  Food Technology  Membership  Meetings & Events  Public Policy & Regulations

Home  Food Technology  Daily News  Davisco increases alpha-lactalbumin production

Davisco increases alpha-lactalbumin production

March 5, 2015

Davisco, a business unit of Agropur Inc., has increased the production capacity of alpha-lactalbumin at its cheese facility in Jerome, Idaho. The potential alpha-lactalbumin volume from Jerome Cheese will be increasing tenfold. Alpha-lactalbumin is the primary protein in human milk and is critical for infant nutrition. It is also the richest source of natural tryptophan, the precursor to the neurotransmitter, serotonin.

“Davisco has always been on the cutting edge of protein separation technology in our Protein Technology Center in Le Sueur, Minn., and to see the successful high volume commercialization of what has become our centerpiece product is very rewarding,” said Jon Davis president, Davisco Foods. “High-purity alpha-lactalbumin will revolutionize our whey protein capability, allowing us to partner with customers in developing new products for the infant formula industry. This will naturally lead to our next generation of products, which are well on their way to commercialization.”

Press release
THANK YOU

α-lactalbumin:
Development path for a valuable
dairy protein ingredient

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Mead Johnson Nutrition