Emerging Protein Technologies for Formulation Solutions

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Introducing NIZO

- Independent, private research company (CRO)
  - Confidential, contract based research for the food industry
- 200 expert employees recruited from academia and industry
- Founded by the Dutch dairy industry in 1948
  - Roots in dairy science and processing

Some clients we work with (and who allow us to tell...)

- solanic
- Coca-Cola
- Heinz
- CSM
- FrieslandCampina
- MeadJohnson Nutritionals
- Givaudan
- Insinkelt
- Unisan
- Lipton
- Tetra Pak
- Unilever
- Symrise
- Danone
- Avebe
- Nissui
- Gouda
- GM
- SaraLee
- D.E.
- Purac
- Vion
- CSK
- Sime Darby
- Stork
- Barry Callebaut
- Sympol
- Darby
NIZO food research HQ – Food Valley

Ede, the Netherlands

Processing Centre & Food Application Centre

Research Centre (offices and laboratories)
Expertise areas

Health & Fermentation

Processing Centre

Processing & Safety

Flavor-Texture
NIZO Protein Centre

To bring health and well being through food

Using basic science to design healthy, attractive and sustainable foods
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50+ proud years!

Dairy

1960
start milk protein research

1970
Milk protein analysis

1980
Concentration of milk

1990
β-lactoglobulin aggregation model

2000
Complex coacervation

CaP-nanoclusters

2010
Extraction RuBiSco

α-lactalbumin nanotubes

Cold gelling of whey proteins

Non-dairy

Structural studies

Bioactivity

genetic variants

Casein sub-micellar structure

Analytical methods

Separation technology

Physical/ enzymatic modification technology

Functionality in applications

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NIZO Protein Centre
50+ proud years!

Dairy
- Control of viscosity yoghurt
- Fresher taste
- Tailor-made caseinate
- Stable acid soy drink
- Less fouling
- Well-tasting recovery drink

Non-dairy
- Soluble keratins

Whey powder
- Analytical methods
- Separation technology
- Physical/enzymatic modification technology
- Functionality in applications

NIZOpremia, process control

NIZO Protein Centre
Our protein expertise
from source to benefit and vice versa

PROTEIN FUNCTIONALITY

Protein extraction
Protein processing
Protein modification
Flavour and texture attributes of proteins
RuBisCO is…
… the most abundant protein in the world

• RuBisCO is the main protein in green plants
• Green leaves are everywhere
• Green leaves contain 2-3% protein on a fresh weight basis
• Main enzyme for CO$_2$ fixation (Calvin cycle)
• Highly conserved over evolution
• Amino acid composition is in line with FAO recommendations
Processing

Basic Process To Rubisco Protein Isolate: RuPI

- **Leaves**
  - Maceration and separation

- **Juice**
  - Purification

- **Protein extract**
  - Concentration

- **Protein concentrate**
  - Formulation

- **Product**

- **Decantation**
  - Pressing

- **Aggregation**
  - Precipitation

- **Affinity-separation**

- **Membrane filtration**
  - Evaporation

- **Spray drying**
  - Fiber formation

Traditional extractions are often based on use of organic solvent resulting in insoluble and green powder.

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Processing
Upscaling

Large scale alternatives have been developed for...
- Juice collection
- Discoloring
- Drying

Large scale Proof of Principle is currently running
RuPi Processing

Result

- High protein content
- High solubility (>95%)
- Neutral color and flavor
RuPI has unique properties:
high foam capacity and stability

2% protein solution, foam volume after 30 minutes at room temperature. RuBisCO stable up to 2 hrs!
RuPI has unique properties:
high gelling capacity

➢ RuBisCO preparation starts gelling at 2% ○ Lower concentration than WPI

Conditions: protein dissolved in water
Heating: 90°C for 10 min
Texture analyser
Optimized amino acid profiles

- Find alternative protein sources which can be used in food
- Different target groups have different needs
- Design ingredient blends that match the required amino acid profile

**NIZO developed text mining to search for proteins compared to target amino acid profile**

- Search over 500,000 proteins and compare the amino acid profiles
- Ranked list of commercial available proteins/hydrolysates
- Selection of alternative protein sources based on search
  - Mixing ratio delivers the required amino acid profile
Quinoa: the healthy choice

• Consumer demand for healthy milk alternatives
• Quinoa is recognised as natural food resource with high nutritive value

NIZO has developed a well tasting, nutritionally balanced Quinoa drink

• NIZO Quinoa milk analog
  • 4 gram protein per serving
  • Less carbohydrates than cow milk
• Produced on pilot scale
  • Quinoa milk analog
  • Refreshing quinoa water
  • Bakery products
## PROTEIN FUNCTIONALITY

<table>
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<tr>
<th>Source</th>
<th>Processing</th>
<th>Protein</th>
<th>Functionality</th>
<th>Application</th>
<th>Benefit</th>
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Protein extraction

**Protein processing**

Protein modification

Flavour and texture attributes of proteins
Properties of caseins

- Little 3D structure
- Partially unfolded open structure
- No denaturation possible
- Casein micelle structure
- Calcium phosphate bridges in the micelle
- Differences in calcium sensitivity between caseins
- Relatively heat-stable
Properties of whey proteins

- Mainly two globular proteins:
  - Beta-lactoglobulin
  - Alpha-lactalbumin
- Well defined 3D structure
  - Internal disulfide bridges
  - Denaturation possible
- Monomeric
- Heat labile
32 commercial MPC’s: the extremes

- Large differences in composition and properties between MPC’s
- Differences attributable to:
  - Desired composition
  - Tailored processing
  - Sub-optimal processing
  - Over-processing
- Control of composition and processing will allow optimized and tailored functionality

<table>
<thead>
<tr>
<th>Protein (%, m/m)</th>
<th>55</th>
<th>85</th>
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<tbody>
<tr>
<td>Moisture (%, m/m)</td>
<td>3.5</td>
<td>6.6</td>
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<tr>
<td>Calcium (mg/g)</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>Sodium (mg/g)</td>
<td>0.3</td>
<td>14</td>
</tr>
<tr>
<td>Lactosylated β-lactoglobulin (%)</td>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td>Denatured β-lactoglobulin (%)</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Dispersibility (%)</td>
<td>38</td>
<td>100</td>
</tr>
<tr>
<td>Nitrogen solubility index (%)</td>
<td>27</td>
<td>87</td>
</tr>
<tr>
<td>Heat stability (min)</td>
<td>0</td>
<td>42</td>
</tr>
<tr>
<td>Gel strength (g)</td>
<td>15</td>
<td>266</td>
</tr>
<tr>
<td>Foam overrun (%)</td>
<td>178</td>
<td>390</td>
</tr>
</tbody>
</table>
Interactions in MPC

- Skim milk powder ➔ continuous matrix of amorphous lactose in which the proteins are suspended
- Membrane filtration removes lactose ➔ less lactose for matrix
- High protein MPC/MCI ➔ protein-protein interactions during processing and storage
- Protein-protein interactions can result in development of insolubility during processing and drying

![Diagram of Skim milk powder, Casein micelle, Lactose, Whey protein, MPC60, and MPC85](image)
• Milk (at natural pH) typically has excellent heat stability but serum phase contains stabilizing
  and destabilizing constituents (lactose, salts, urea)

• Strong reduction in heat stability of MPC with increasing protein content at 3.5% protein
• Increased heat stability for MPC50-70 at 8.0% protein; mostly destabilization for MPC80-90
• Changes in heat stability are the result of removal of serum components
Future plans in protein-mineral interactions in micellar casein concentrates

- Understanding and tailoring protein-protein and protein-mineral interactions through the processing steps → from black box to white box
- Fundamental understanding to facilitate industrial innovation
- Build on platforms of:
  - Physical chemistry and biochemistry of casein interactions and mineral interactions
  - Effects of processing on casein interactions and mineral interactions
  - Influence of protein and mineral interactions on functional properties
Stability of cappuccino foams

Varying fat contents foamed at 20°C

Even a small amount of fat present dramatically reduces stability in protein-stabilized foams
Stability of cappuccino foams

Skim milk of 2.5-10% solids foamed at 20°C

4-fold reduction in solids content does not influence the volume of stability of skim milk foam $\rightarrow$ reduced ingredient cost
PROTEIN FUNCTIONALITY

Protein extraction
Protein processing
**Protein modification of whey**
Flavour and texture attributes of proteins
One raw material...6 novel particle structures

- spray-dry
- heat
- shear
- heat
- heat +pH
- emulsify +heat
- centrifuge
- wash
- wash
- wash
- freeze-dry
- DEP_ST
- freeze-dry
- DEP_LH
- MPP_L
- MPP_S
- aMPP
- wind sift
Applied in three food models

- **sugars, aroma**
- **oil**
- **35% protein**

**dough mixing**

60°C

**overly firm texture**

**hardening during storage**

- **pH 5.7**
- **20% protein**

**stirred gelation**

80°C

**overly firm texture**

- **sugars, aroma**
- **12% protein**

**Pasteurization**

90°C

**gelation during heating**

**phase separation**
Texture control in protein bars

.storage time, days:
- d1
- d7
- d14
- d28

- reduced chemical reactivity prevents ageing

- high water binding for maximum softening

<table>
<thead>
<tr>
<th></th>
<th>REF</th>
<th>MPP_L</th>
<th>MPP_S</th>
<th>aMPP</th>
<th>DEP_ST</th>
<th>DEP_LH</th>
<th>CGP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compression force (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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Texture control in stirred gels

When 50% of protein ingredient is substituted by particles

Adaptable gel textures by protein pre-processing
- A wide variety of food products are protein-based gels
- Increased texture development allows for cost-reduction by ingredient efficiency
- Texture reduction enables high protein products
**Viscosity control in drinks**

When 25% of protein ingredient is substituted by particles

*Low-viscous drinks with 12% intact protein*
- High protein drinks too thick, gelation during processing
- Industrial solutions often include hydrolysis of protein
- Aim: to include high content of intact protein but maintain drinkability
- Particles must remain small enough to not be sensed and to remain in suspension
Optimized processing: yoghurt

**Heat treatment of yoghurt mix required to:**

- Inactivate bacteria
- Induce whey protein denaturation to create desirable texture
- Whey protein denaturation is pH dependent

![Diagram showing pH values for casein micelle and whey protein](Image)
Optimized processing: yoghurt

Formation of soluble denatured whey proteins is crucial for maximizing the firmness of yoghurt gel.

G' (Pa)

Pre-heating pH

% soluble denatured whey protein

pH 6.35  6.45  6.55  6.7  6.9

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Novel processing: cold gelation

Cold gelation of whey proteins

Heat-induced gelation

Native \( \rightarrow \) unfolded \( \rightarrow \) aggregated \( \rightarrow \) network

\( T \uparrow \)

Cold gelation

\( \text{pH, } [I] \uparrow \)
Use cold gelation of whey proteins to:

• Prepare gels of comparable firmness with much less protein
  • 3% whey protein required for heat-set gel
  • 0.5% whey protein required for cold-set gel

• Maximize functionality of all whey proteins:
  • 100% of protein contributes in cold-set gels
  • Only 50-70% of protein contributes in heat-set gels

• Applications in yoghurt, desserts, mayonnaise, sauces.
PROTEIN FUNCTIONALITY

Protein extraction
Protein processing
**Protein modification of casein**
Flavour and texture attributes of proteins
What is high pressure processing?

• Non-thermal technology which uses treatment at 100-1000 MPa (1000-10000 bar)

• Applied commercially to inactivate undesirable micro-organisms and enzymes inactivated without affecting flavour, colour and nutritional value

• **Guacamole** → inactivate polyphenol oxidase without causing non-enzymatic browning

• **Juice** → inactivate micro-organisms without loss of vitamins and colour

• **Oysters** → inactivate *Vibrio spp.* and facilitate opening of shell

Two elephants balanced on a 10 cent coin will create 400 MPa
Meltdown in ice cream

- High pressure treatment of ice cream mix can be used to adapt the microstructure
- After HP treatment, micellar fragments are smaller
- Finer, more continuous protein network formed
- Dramatically reduced meltdown, improved mouthfeel
Protein modification: cross-linking

- **Enzymatic cross-linking of milk proteins:**
  - Formation of covalent cross-links between amino acids
  - Transglutaminase (TGase): cross-link between glutamine and lysine residues
  - Tyrosinase: cross-link between tyrosine and lysine, tyrosine or cysteine residues
  - Food grade and applied in dairy industry

![Diagram of Lysine, Glutamine, and Isopeptide-bond formation]
Cross-linking: cream cheese

Cream → Homogenization → Heat treatment → Cooling

Transglutaminase → Lactic acid culture

Incubation → Cutting & cooking → Separation → Homogenization

Viscosity

Syneresis

Homogenization pressure (bar)

Control
Tgase

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Cross-linking: yoghurt

• TGase treatment results in improved gel firmness
• Comparable firmness with less protein
• Applicable to other acid-coagulated dairy products
• Opportunities for novel ingredients
Design of swollen casein micelles

- Fat reduction requires water binding
  - Increasing the water binding capacity of casein micelles
  - Combining cross linking with calcium depletion

Transglutaminase treatment

 Ion exchange treatment

Swollen micelles

- Micellar solvation
- Viscosity (Pa s)
- Particle size (nm)

Huppertz, Slangen, van de Velde, patent pending
Functionality of casein micelles

zero-fat ice cream with high water binding casein micelles

- Skimmed milk powder as the sole protein source
  - Treatment with Tgase
  - Treatment with chelating agent
- Additional ingredients
  - Sugar, glucose syrup solids
  - Thickeners
- Continuous ice cream freezer

- Zero-fat ice cream with excellent taste
PROTEIN FUNCTIONALITY

Protein extraction
Protein processing
Protein modification

Flavour and texture attributes of proteins
Protein fibers for juicy meat alternatives

- Consumers demand for sustainable food production
- Consumers demand for meat alternatives with the “bite of meat”

Meat alternative with protein fibers have juiciness and the “bite of meat”

- Protein fibers produced with the NIZO technology have a meat-like texture and mouthfeel
  - Juiciness
  - “Bite of meat”
- Technology developed for dairy and vegetable proteins
Structurising proteins (NIZO results)

Whey  
Soy  
Pea  

Chicken breast
Control of juiciness
understanding the role of the microstructure

By manipulating the microstructure (phase separation), NIZO was able to tune juiciness which is depicted in the figure by an increase of serum release in the product.
VION Hack plus:
Less Fat - More Protein

• 30 % reduction of fat
• 30 % reduction of salt

Plant proteins & fibres
Changing the flavour/taste perception
enhanced serum release in cooked sausages

15 to 40% NaCl reduction in sausages without taste loss

Serum release boosts Saltiness and Juiciness

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1. Based on expert knowledge on microbial pathways, organisms that produce specific dehydrogenase enzymes were selected from culture collection.

2. Screen these microorganisms for their hexanal-converting capabilities and choose the most active species (food grade).

3. Apply for improvement of sensory characteristics of fermented soy products.
Flavor

Removal of beany off-flavor (hexanal) from soy drink

Hexanal conc. After incubation in soy milk (2% fat).
Initial hexanal conc. 1 ppm.

Reference

Fermentation can greatly reduce the beany off-flavour and form positive flavours.
Conclusions

More and more alternative protein sources are coming available for use in foods.

New (combinations of) processing methods allow the creation of new functionalities of proteins.

Protein is an excellent candidate to replace other ingredients when needed.
Thank you for your attention