Unlocking the potential of alternative proteins for application

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NIZO food research

*good food needs good science*

- Independent, private contract research company for the food industry
- HQ in The Netherlands
  - Food Valley
- 130 professionals
- From lab to practice
  - Food-grade pilot plant

Together to the next level
Agenda

• Nutritional quality

• Mild protein extraction
  • Effect on protein functionality

• Protein blends

• Plant proteins: Flavor & taste
Protein Functionality
unlocking the full potential of proteins

- New sources
- Discovery
- Selection

- Hydrolysis
- Downstream processing
- Extraction
- Fractionation
- Drying

- Characterisation
- Size, Mw
- Chem. Composition
- Peptide profile
- Tailor-made structures

- Product preparation
- Process design
- Formulation
- Interactions

Dairy, Plant, Animal, Algae, Insect Proteins and Hydrolysates
Proteins for the future

- World population is expected to grow above 9 billion people in 2050
  - Increasing demand for high nutritional foods
  - Protein supply is most critical (meat production is expected to double, poultry on top)
Biomass cascading
applied to proteins

A Infant, Sport & Clinical
B Food - functional ingredient
C Food - bulk nutrition
D Pet food
E Feed
F Energy, Bioplastics & Chemicals

Valuable proteins are valorised
Alternatives come from lower step
Ingredient flexibility

developing new protein ingredients

- One-to-one protein or ingredient replacement is not always possible

- Nutritional quality
  - Proteins from different sources have different amino acid profiles (essential amino acids) and digestibility

- Technical functionality
  - Solubility, gelling, emulsifying, foaming
  - Interaction with hydrocolloids

- Taste and smell

Understanding functionality is crucial
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Protein sources

- Pea
- Soy
- Lupin
- Rapeseed or Canola
- Corn
- Rice bran
- Rice
- Potato
- Insect
- Pulses
- Green leaves
- Quinoa, amaranth
- Micro-algae
- Duckweed
- Sunflower
Optimized amino acid profiles

- Find alternative protein sources which can be used in food
- Design ingredient blends that match the required amino acid profile

NIZO developed tools to search for proteins compared to target amino acid profile

- Mixing ratio of proteins delivers the required amino acid profile
- Ranked list of commercial available proteins/hydrolysates

Together to the next level
Digestibility
FAO discussion on methods

• PDCAAS: Protein Digestibility Corrected Amino Acid Score
  → Established method according to AOAC
  → Measurement in the faeces
  → Bioavailability of single amino acids is not taken into account

FAO expert consultation 2012: Dietary protein quality evaluation should be performed by DIAAS

• DIAAS: Digestible Indispensable Amino Acid Score
  → True ileal digestibility -> sampling at terminal ileum
  → Measure digestibility for individual essential amino acids
  → Preferably determined in humans > if not possible: pigs > if not possible: rats

• No established AOAC method yet

Need for fast screening methods
Simphyd
SIMulation of PHYsiological Digestion

Basic “static” procedure
- Digestibility
- Bioavailability
- Probiotic survival
- Etc.

Effects on host physiology in vitro
- Mucosal barrier
- Decoy activity
- Immunity
- Satiety signalling

Optimized mixing
In-line viscosity measurements
- Structure breakdown
- Gastric gelation
- Gastric behaviour
- Etc.

“Texture and satiety”
- Bacterial survival
- Lipid/protein digestion

High-throughput fecal slurry culturing (colonic fermentation)
- Microbiota profiling
- Metabolomics
- Etc.
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Protein functionality: solubility is key

Crop composition
- Downstream processing
- Heat treatment
- Evaporation
- Drying

Solubility

Storage conditions
- Color
- Digestibility
- Emulsification
- Heat stability
- Flavor/taste
- Viscosity
- Gelling
Plant proteins: solubility matters

Solubility at various pH

[Graph showing solubility at various pH levels for different plant proteins, including rice 1, rice 2, potato, soy 1, soy 2, pea, lupin, and WPI.]
Pea protein
*improved processing to prevent unfolding*

Native character

Functionality: solubility

Solubility remained high after pasteurisation and storage (6 months)
Pea protein

*improved processing to prevent unfolding*

**Improved processing:**
- finer foam (cappuccino etc…)
- stabilized by protein; not by insoluble particles
  - **Particles stabilized foams**: undesired during dissolving
Pea proteins

extraction method and fat content

Fat content depend on extraction and pea type

Free fatty acids were highest in commercial pea
Pulse drink

- Consumer demand for healthy milk alternatives
  - High in protein
  - Low in carbohydrates
  - Low in fat

**NIZO developed a well tasting, nutritional balanced Pulse drink**

- Pulse drink
  - Protein content higher than milk and soy
  - Less carbohydrates than cow milk
- All natural ingredients
  - Water
  - Pulse protein
  - Vegetable oil
  - Natural flavors
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)
- Very conserved over evolution
Abundant protein combines functionalities

- Growing population and increasing living standards boost the demand for proteins
- Green leaves are abundantly present but needed the technology to deliver functional protein

Abundant protein resource to increase ingredient flexibility

- Extraction technology that delivers functional and acceptable protein
  - No green color
  - Highly functional
- Leave proteins combine technical functionality with an excellent nutritional profile

Excellent foam stability

Table:

<table>
<thead>
<tr>
<th>Protein Type</th>
<th>pH 4.5</th>
<th>pH 7.0</th>
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</thead>
<tbody>
<tr>
<td>SoyPI</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td>WheyPI</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td>RubiscoPI</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
</tr>
</tbody>
</table>

Together to the next level
RuBisCO protein isolate has unique properties.

High gelling capacity

Purified RuBisCO starts gelling at 2% w/w

Gelling properties of crude protein
Structuring into fibrous protein

- Consumers demand for
  - Sustainable food production
  - Protein enrichment
  - Meat alternatives with the “bite of meat”

The NIZO fibrous proteins bring “bite” and juiciness

- NIZO fibrous proteins bring texture and “bite” in
  - Meat and meat alternatives
  - Protein enrichment in bread
  - Protein enrichment and texture in beverages

- Technology applies to all sources
  - Dairy: whey, caseinate
  - Vegetable: soy, pea, RuBisCO
  - Egg

Together to the next level
Protein rich smoothie
based on fibrous pea protein

good source of protein
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Protein flexibility/exchangeability

- Protein flexibility/exchangeability
  - Why? What does it mean?
  - Being able to replace one protein by another one without affecting the textural properties (sensory perception)
  - Reason: price, sustainability

- Protein blends
  - Nutritional value,
  - Cost reduction,
  - BUT: Addition of plant proteins may negatively impact the textural properties
Protein flexibility/Exchangeability

*Acidified sodium caseinate and soy protein systems*

- Addition of soy proteins resulted in changes in microstructure,
- Decrease in mechanical property

100% NaCas | No heat treatment | Heat treated soy proteins
---|---|---

Martin, Pouvreau et al. (2016) Food hydrocolloids, 58
Acidified sodium caseinate and soy protein systems

Pre-treatment of proteins

• Pre-treatment of proteins together:
  • Microstructure and mechanical properties recovered
  • pH and protein ratio at heating crucial

70/30 NaCas/SP
100% NaCas
No treatment  Treated NaCas/SP

Martin, Pouvreau et al. (2016) Food hydrocolloids, 58
Soy protein and gelatin mixed systems

Mixing of proteins: opportunity to create new texture
Emulsion capacity/stability with pea proteins

Lack of soluble protein increases emulsion instability

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Clarification rate (μs/s)</th>
<th>Cream layer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na-Cas</td>
<td>1.20</td>
<td>32.0</td>
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<tr>
<td>Na-Cas:PPI = 0.75:0.25</td>
<td>1.39</td>
<td>36.0</td>
</tr>
<tr>
<td>Na-Cas:PPI = 0.50:0.50</td>
<td>1.65</td>
<td>38.5</td>
</tr>
<tr>
<td>Na-Cas:PPI = 0.25:0.75</td>
<td>1.98</td>
<td>42.5</td>
</tr>
<tr>
<td>Na-Cas:PPI = 0:1</td>
<td>2.55</td>
<td>45.3</td>
</tr>
<tr>
<td>Na-Cas:HF-PPI = 0.50:0.50</td>
<td>1.40</td>
<td>32.5</td>
</tr>
</tbody>
</table>

Increasing creaming layer

Pouvreau & van de Velde (2015), World of Food Ingredients
Biomass cascading
applied to proteins

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Plant proteins

• Often described off-notes
  • Beany, hay, cardboard aroma
  • Bitter taste
  • Astringency

• Three strategies to deal with off-notes
  • Prevent occurrence
  • Eliminate by (natural) post-processing
  • Mask perception

What is the cause for the perceived off-note?
  • Aroma
  • Taste
  • Astringency
  • Or a combination of them?
Interactions
volatile organic compounds (VOCs) and proteins

• VOCs are present in most of the seeds (soy, pea, beans,....)
• VOCs are aldehydes, ketones and alcohols.
• VOCs are mainly generated by:
  • Oxidation of unsaturated fatty acids
  • Synthesized by enzymatic reactions
• Hexanal is responsible of:
  • Beany off-flavor in soy protein isolates
  • Hay-like off-flavor of peas
• Factors such hydrophobicity of proteins, pH, temperature, ionic strength may influence the retention of VOC to a protein

Preventing VOC formation is better than curing
NIZO extract is low in oxidation off flavors, but high in pea
Molecular properties of the proteins affection astringency not altered
Example

Soy based products

- Soy based products are perceived as less attractive in the West.
- The most offensive attributes are the “beany” flavour and the astringent mouthfeel.

Solution: Fermentation with specific co-starters
Decrease of hexanal in soy milk

Hexanal is degraded by dehydrogenase enzymes expressed by specific strains.

Beany perception in end product significantly decreased.
Astringency- definition

Astringency perception is defined dry, puckering mouthfeel, rough "sandpapery" sensation in the mouth.

It is a tactile sensation that arises from precipitation of lubricating salivary proteins or from reduced lubrication in the mouth.

Typical examples are wine and tea.
Some proteins show astringency

**saliva: a complex fluid to interact with**

- Oral fluid secreted from different glands
  - pH = 6 - 7
- More than 1000 different proteins
  - Mw 0.8 kDa – 40 MDa
  - Mucins, α-amylase, cystatins, histatins

- Interaction of saliva proteins with some food components results in astringency or rough feeling
  - Proteins or polyphenols
  - Loss of lubrication in the mouth
  - Complexes between ingredient and saliva give rough feeling

Technologies to study complexation
Technologies to reduce complex formation
Astringency reduction

Astringency reduced by modification

Giuseppin, Lambers, et al., 2012
Masking Technologies

Masking:
small molecule – small molecule
aroma or taste compounds which attach to
the same receptors as the responsible off-
note are added to the formulation.
Example: taste binding

Evaluation of the Bitter-Masking Potential of Food Proteins for EGCG by a Cell-Based Human Bitter Taste Receptor Assay and Binding Studies

Maxime C. Bohin,† Wibke S. U. Roland,‡ Harry Gruppen,† Robin J. Gouka,‡ Harry T. W. M. van der Hijden,† Peter Dekker,‡ Gerrit Smit,†‖ and Jean-Paul Vincken*‡

Milk proteins bind to bitter tea compounds → Decrease of bitterness and astringency

Bitter receptor activation by EGCG (250 µM) complexed with increasing concentrations of β-casein (●) or Na-caseinate (○)

→ 93% reduction of receptor activation

Sensory tests: Bitterness of free EGCG vs. complexed EGCG in 2-AFC tests.

(*) significant difference (p<0.05)

→ reduced activation of bitter receptors by addition of food proteins led to a real reduction of bitter perception

Bohin, Roland et al. J. Agri. Food Chem. 61 (42) 2013
Take home messages

• Protein blends allow ‘new’ source proteins to compete with animal proteins in nutritional quality
• Solubility is a MUST for functional proteins
• Mild processing leads to optimal functional properties
• Understanding protein blends enables texture customization
• The largest step in flavor optimization can be achieved by process understanding
• Masking technology and/or modification address final quality issues
Co-creation

Together to the next level!