Development of new concepts for the optimization of the structure and sensory properties of reduced-fat food products by means of protein functionalization and molecular-sensory methods.

Entwicklung neuer Konzepte zur Optimierung von Struktur und Sensorik fettreduzierter Lebensmittel durch Proteinfunktionalisierung und molekular-sensorische Methoden.

M.Sc. Caren Tanger, Prof. Dr.-Ing. Ulrich Kulozik
Background and Motivation

Risk for diet-related illnesses

Demand for new concepts related to a significant reduction of salt, sugar and fat in food products

Current concepts for fat reduction:
- Undesired side effects
- Reduction of product volume
- Negative influence on product texture and flavour perception

Sustainability matters

Demand for new concepts related to the exploration of plant-based alternative food ingredients

Challenges related to plant-based protein alternatives:
- Off-flavour
- Low solubility
- Low techno-functionality
Fat reduction in food leads to loss of consumer acceptance

Research Group I: Food and Bioprocess Engineering

- Compensation of the structural effect of fat by protein functionalization

Research Group II: Food Chemistry and Molecular Sensory Science

- Compensation of sensory perception of fat by flavour optimization

Establishment of a new platform of knowledge and methodology for the development of reduced-fat food products
Concepts for the replacement of fat in food products

Utilization of microparticulated milk proteins to maintain a creamy mouthfeel in fat-reduced products

40% fat cheese

- Casein (100 - 300 nm)
- Native Whey Protein (3 - 5 nm)
- Fat (1 - 10 μm)

reduced-fat cheese

- Microparticulated Whey Protein (MWP) (1 - 10 μm)
Microparticulation of proteins

Influencing factors on aggregation
- temperature
- shear rate
- heating time
- composition (Protein, Lactose, Calcium)
- pH
- pre-denaturation of the proteins
Methods for microparticulation of proteins

- High pressure homogenizer
- Dispersion unit
- Scraped surface heat exchanger

Protein concentration as limiting factor (max. 10%)

Advantages using **extrusion** for production of microparticulates:
- High viscosities and thus, high protein concentrations feasible
- High variation possibilities of process parameters
- Low holding times at high degree of denaturation
- Low amount of caking
Project Outline

WP 1
Chemical-physical and sensory characterization of purchased proteins

WP 2
Functionalization of single and hybrid systems (pea, potato and milk protein)

WP 3
Characterization of surface activity and foam properties of the microparticulates

WP 4
Development of fat-reduced model milk products

WP 5
SENSOMICS- and flavor-protein-interaction-studies

WP 6
Flavor-optimization of the fat-reduced model milk products

WP 7
Project management and transfer of knowledge
# Physico-chemical characterization

<table>
<thead>
<tr>
<th></th>
<th>Pea protein isolate</th>
<th>Potato protein isolate</th>
<th>Whey protein isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein concentration</td>
<td>80% - 90%</td>
<td>80%</td>
<td>90%</td>
</tr>
<tr>
<td>Solubility</td>
<td>low</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>Protein profile</td>
<td>Several protein fractions</td>
<td>Two protein fractions</td>
<td>Two protein fractions</td>
</tr>
<tr>
<td>Nativity</td>
<td>low</td>
<td>high</td>
<td>high</td>
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</table>
Protein profile pea

<table>
<thead>
<tr>
<th>Pea protein isolate</th>
<th>Salt soluble</th>
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</thead>
<tbody>
<tr>
<td>Protein concentration</td>
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<td>Protein profile</td>
<td>Several protein fractions</td>
</tr>
<tr>
<td>Nativity</td>
<td>low</td>
</tr>
</tbody>
</table>

- Salt soluble: IEP: pH 4.5
  - 11S Legumin, Hexamer (360-410 kDa)
    - Basic subunit
    - Disulfide bridge
    - Monomer 60-80 kDa
  - 7S Vicilin, Trimer (150-200 kDa)
  - 7S Convicilin, Tetramer (210-290 kDa)

- Globulin 65-80%
- Glutelin <10%
- Albumin 15-25%
- Prolamin <10%
- Water soluble: Low molecular weight protein

(Swanson, 1990; Shand et al. 2007)
Protein profile potato

- **Patatin (30-40%)**
  - 40-45 kDa, glycoprotein,
  - Low heat stability
  - 1 free thiol group

- **Protease inhibitors (40-50%)**
  - Consist out of 7 subgroups, 5-25 kDa
  - Decrease digestibility
  - Inactivated upon heating

<table>
<thead>
<tr>
<th>Potato protein isolate</th>
<th>Whey protein isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>90%</td>
</tr>
<tr>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>Two protein fractions</td>
<td>Two protein fractions</td>
</tr>
<tr>
<td>high</td>
<td>high</td>
</tr>
</tbody>
</table>

- **Minor proteins**

Food and Bioprocess Engineering
Protein profile whey

β-Lactoglobulin

- 40 – 50%
- Amount in whey: 3.5 – 5 g/l
- Globular protein
- 18.4 kDa
- 1 free thiol group
- 2 internal disulphide bonds

α-Lactalbumin

- 10 – 15%
- Amount in whey: 1 – 1.5 g/l
- Globular protein
- 14.2 kDa
- Ability to bind calcium:
  - holo-α-La (+Ca)
  - apo-α-La (-Ca)

Whey protein isolate

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>90%</td>
<td>high</td>
</tr>
<tr>
<td>high</td>
<td>Two protein fractions</td>
</tr>
</tbody>
</table>
## Producing „native“ pea proteins on laboratory scale

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<th>Whey protein isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nativity</td>
<td>low</td>
<td>high</td>
<td>high</td>
</tr>
</tbody>
</table>

### Extraction method

<table>
<thead>
<tr>
<th>Method</th>
<th>Pea protein isolate</th>
<th>Potato protein isolate</th>
<th>Whey protein isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkali extraction – isoelectric precipitation</td>
<td>+</td>
<td>-</td>
<td>Low solubility</td>
</tr>
<tr>
<td>Alkali extraction – isoelectric precipitation modified</td>
<td>High solubility, fast extraction</td>
<td>Possibly damaged protein structure</td>
<td></td>
</tr>
<tr>
<td>Salt extraction</td>
<td>High solubility</td>
<td></td>
<td>Low denaturation peak</td>
</tr>
<tr>
<td>Micellar extraction</td>
<td>Probably lowest damage (clear denaturation peak)</td>
<td></td>
<td>Low solubility</td>
</tr>
</tbody>
</table>
Microparticulation of plant protein in comparison to whey protein on small scale

WPC = whey protein concentrate; WPI = whey protein isolate; Pat = potato protein isolate; PPe = laboratory extracted pea protein; PPC = commercial pea protein isolate
Microparticle characteristic – size and shape

- Plant protein particles are in a suitable size range
  - Extracted and commercial pea protein behave differently!
- Plant protein microparticles are round

**Graph:**
- Different lines represent different protein types:
  - PPe
  - PPC
  - Pat
  - WPC

**Images:**
- Microscopy images of proteins at different shear rates (100 s⁻¹ and 1000 s⁻¹)
  - PPe
  - PPC
  - Pat
  - WPC

**Observations:**
- Extracted and commercial pea protein behave differently.
- Plant protein microparticles are round.
# Thermal stability of whey and plant proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>$T_{\text{denat}} [^\circ\text{C}]$</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patatin</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Whey protein isolate</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Laboratory extracted pea protein</td>
<td>78</td>
<td></td>
</tr>
</tbody>
</table>

* Commercial pea protein did not show any peak by DSC analysis → high initial denaturation
Possible reaction mechanism of thiol groups

Thiol reaction: $R_1\text{-SH}^* + R_2\text{-SH}^* = R_1\text{-S-S-}R_2$

Thiol-disulphide interchange: $R_1\text{-SH}^* + R_2\text{-S-S-}R_3 = R_1\text{-S-S-}R_2 + R_3\text{-SH}^*$

<table>
<thead>
<tr>
<th></th>
<th>$\beta$-LG</th>
<th>Patatin</th>
<th>Legumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disulphide</td>
<td>2</td>
<td>0</td>
<td>1-2</td>
</tr>
<tr>
<td>Thiol group</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Plant protein microparticles are mostly stabilized by hydrophobic interactions

Softer particles compared to whey protein particles

Image: Comparative Assessment of Thermal Aggregation of Whey, Potato, and Pea Protein under Shear Stress for Microparticulation

Authors: Caix Teng, Paola Quintana Ramos, and Ulrich Kubacki
Thermo-mechanical treatment – extrusion set-up

Process parameter
- Case temperature
- Screw speed
- Screw composition
- Mass flow

- Same as used for whey protein microparticulation (Wolz et al. 2016)
Pea and potato protein microparticulation – large scale (extruder)

- Pea protein microparticles have a smooth peanut butter like texture
- Potato protein microparticles are foamy and show big visible particles

> Commercial pea protein isolates is investigated in more detail
Influence of shear rate on particle size – large scale

- Small shear rate is sufficient to limit particle size and prevent gel formation
  - Similar to whey protein -> impact on particle size has only been seen at lower shear rate (< 200 rpm)
  - Below 200 rpm extrusion of pea protein was not continuous
Influence of temperature on particle size

- $D_{90}$ increase at 100° C
- Hydrophobic interaction increase in intensity with increasing temperature
- Reactivity of thiol groups increase at increasing temperatures
  - Shear cannot limit particle growth due to the increase in intensity of protein interaction
  - Temperatures between 75° C and 120° C most suitable
Effect of drying on particle size

- Drying does not increase particle size of microparticulate
- Drying does change protein-flavour binding

Commercial pea protein

Dilute with water

E86, n = 600 rpm, T = 100 °C, m = 4 kg/h

$Q_\beta(x) [%]$

Particle size [µm]
WP 4: Development of a model milk dessert

7% Powdered sugar
47% Skim milk yoghurt (0.1% fat)
1% Stabiliser (HAMULSION)

45% Pasteurized cream

Microparticulates as substitutes (50%)

Non-foamed dessert

Dispersing

Foamed dessert

Dispersing

Whipping
Flavour profile of full-fat and fat-reduced milk dessert

- Sweet
- Sour
- Umami
- Salty
- Bitter
- Ads
- Creamy
- Mouthfulness
- Kokumi
- Fatty
- Sour
- Rancid
- Vanilla-like
- Milk-like
- Grassy, green, bean-like

Intensity

Reference
Fresh MP MD
Spray-dried MP MD
Freeze-dried MP MD
Summary:

• Different functionalities of pea, potato, and whey protein lead to different thermally induced aggregation behaviour

• Aggregates / microparticles could possibly be used as fat replacer

Outlook:

Can aggregates / microparticles also be used for other applications (foam stability, emulsion stability, food structuring)?

Can the microparticulation process also be used for functionalising other plant-based proteins (oat, sunflower, chickpea, etc.)?
Thank you for your attention!

Das IGF-Vorhaben 20197 N der Forschungsvereinigung Forschungskreis der Ernährungsindustrie e. V. (FEI), Godesberger Allee 142-148, 53175 Bonn, wurde über die AiF im Rahmen des Programms zur Förderung der Industriellen Gemeinschaftsforschung (IGF) vom Bundesministerium für Wirtschaft und Energie aufgrund eines Beschlusses des Deutschen Bundestages gefördert.

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... ein Projekt der Industriellen Gemeinschaftsforschung (IGF)

gefördert durch/via

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Backup - slides
WP 2b: Influence of mass flow

- Mass flow influence screw filling
- Biggest particles at 4.5 kg / h
- Powder mass flow of 4.0 kg / h is most suitable (smallest particles) -> same as whey proteins
WP 2 b: Hybrid systems 50:50 WPC and PPI

- In hybrid systems particle size and protein interaction are temperature dependent
  - Whey protein start to denature at 70°C -> start of thiol-disulphide interchange -> more disulphide bonds are built
  - Whey protein only fully denatured at 130°C barrel temperature (Wolz, 2016)