Title: Bioprocessing of protein-rich raw material towards improved functionality

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Bioprocessing of protein-rich raw material towards improved functionality

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Novel multifunctional plant protein ingredients with bioprocessing (BIOPROT)

1. Improve protein and nutritional functionality of bran and faba bean ingredients by tailored bioprocessing with microbes and enzymes

2. Establish technological functionality of modified plant protein sources in several food categories (pasta, bread and extruded snacks)
BIOPROT-team

- University of Helsinki, Finland
  - Elisa Arte, Rossana Coda, Kati Katina (coordinator)

- VTT Ltd, Finland
  - Riikka Juvonen, Anni Karsma, Arja Laitila, Atte Mikkelson, Margherita Re, Natalia Rosa-Sibakov, Nesli Sozer, Irina Tsitko

- University of Bari, Italy
  - Marco Gobbetti, Carlo Rizzello

- Central Research Institute of Food and Feed Control, Turkey
  - Nurcan Aysar Guzelsoy, Orhan Eren
Faba bean is an excellent source of protein

- High protein content (25-35%)
- Vitamins, minerals, dietary fibre and bioactive compounds
- Low cost: Half the price of soy bean and peas (~200 vs 400 €/t)
- Suitable for gluten-free products
- The **limitations** on using faba beans are the presence of antinutritional factors, a bitter taste and poor technological functionality
Bioprocessing as a tool to improve the techno-functional and nutritional profile

Enzymatic treatments
- Fast, controlled, targeted actions

Microbial food cultures
- Bacteria (especially lactic acid bacteria), yeasts and moulds
- Versatile, multifunctional actions

- Structure
- Colour
- Safety and shelf life
- Process performance
- Taste
- Digestibility and bioavailability
This study aimed to improve:

- protein bioavailability
- technological functionality

1. Enzymatic treatment with phytase
2. Lactic acid bacteria, including exopolysaccharide producing (EPS) bacteria

- Fermented faba bean ingredients in extrusion

**Phytic acid**
- Strong binding affinity to important minerals
- Forms complexes with proteins decreasing solubility

**Microbial EPS such as dextrans**
- Selected lactic acid bacteria
- EPS to deliver viscosifying, texturising and emulsifying agents in to foods and beverages
- Effective at low concentrations (0.5-1%, d.w.)
- Health promoting properties
- In situ production: Label-free technology for modifying texture and nutritional properties
Vicia faba L. var major
Dehulling, milling (particle size ca 600 µm)
Raw material characterisation

Enzyme selection
Food-grade phytase (Ultra Bio-Logics Inc)

LAB selection
Indigenous and from other plant sources
L. plantarum, Lc. lactis, W. confusa
10^6 cfu/g

Enzyme treatment
2, 10 and 20 U (33-167-333 nkat)
Ratio (s:l) = 1 solid : 2 water
RT and 55°C; 1,2,4 h

Fermentation
0.5 kg and 10-15 kg
33% flour + 62% water + 5% sucrose*
(*only EPS production)
25°C 24 h

Freeze drying and milling

Extrusion
100 -50 – 25 % mixed with rice flour

Pasta

Baking
LAB = lactic acid bacteria
Treatment with food-grade phytase was effective

Phytic acid content in faba beans was reduced up to 89 %
Phytase treatment increased protein release of faba bean


**Phytase treatment**
- enhanced the solubilisation and release of protein from faba bean in the gastric stage of *in vitro* digestion
- increased Ca, Mg, Fe, Zn availability (data not shown)
Bioprocessing of faba beans with LAB
Faba beans are natural source for exopolysaccharide (EPS) producing LAB

- Intensive LAB growth
  - 100-1000 cfu/g → >10^8 cfu/g

- *Weissella* and *Leuconostoc* bacteria isolated and characterised

- *In situ* production of EPS in protein rich matrix?

- EPS-negative: *Lactobacillus plantarum*
  - Antimicrobial potential
  - Reduction of antinutritive factors and protein modification
Lactobacillus plantarum E76 (EPS-negative) vs Weissella confusa E3403 after 24h fermentation
Intensive dextran production during faba bean fermentation

Control = without added sucrose
**FESEM micrographs after 24h fermentation**

Starch granules

Protein matrix

**EPS negative**
*L. plantarum*

*In situ* produced dextran

Polymeric EPS matrix

**Weissella** bacteria

EPS = exocellular polysaccharide

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**VTT: Field emission scanning electron microscopy (FESEM) / Tsitko & Laitila**

08/09/2015 Laitila
Fermentation releases proteins from the cell wall matrix and significantly increases the amount of free amino acids (AA)

<table>
<thead>
<tr>
<th>Total free amino acids</th>
<th>g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.7</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>7.6</td>
</tr>
<tr>
<td><em>Lc. lactis</em></td>
<td>10.4</td>
</tr>
<tr>
<td><em>W. confusa</em></td>
<td>9.8</td>
</tr>
</tbody>
</table>

Fermentation had a positive impact on amino acid profiles → The amount of essential AAs increased.
Bioprocessed faba bean ingredients in extrusion
Fermentation with *L. plantarum* improved mechanical properties of extruded snacks

**Decreased hardness**

**Increased crispiness (50 and 25% addition)**
In protein-rich material, no additional impacts with EPS producers

<table>
<thead>
<tr>
<th></th>
<th>100%</th>
<th>50%</th>
<th>25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native faba</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>L. plantarum fermented</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>W. confusa fermented (EPS)</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Challenge:** Burning (Maillard reaction), especially with EPS producing LAB

**Future:** Other applications such as in baking
Benefits of faba bean bioprocessing

Enzymatic treatments
- Phytase treatment as a fast tool to increase
  - protein solubility
  - mineral availability

Microbial food cultures
- Release of proteins and modification of amino acid profile by fermentations
- Reduction of antinutritional compounds
- Biocontrol
- Faba beans provides an excellent base for EPS production → functionality in other applications such as baking, vegetable pastes
Take home messages

- Well-characterised microbes and enzymes as a tool for improving nutritional profile and functional properties of protein-rich legume ingredients and products

→ New product innovations
TECHNOLOGY FOR BUSINESS