Improvement of functionality of cereal protein ingredients

Dr. Dilek Ercili-Cura

Seminar on New Protein Mining Technologies

The Research View on Wheat, Rice, Soy, Pea and Lupin

17 April 2018, Rotterdam, the Netherlands

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Protein challenge is one of the most powerful driving forces for increased utilization of plant proteins in food products

- Global protein supply security
- High environmental cost of animal proteins
- Consumer trends

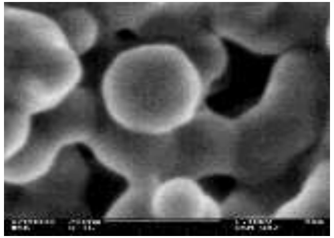
Plant sources and agro side streams must be utilized more efficiently

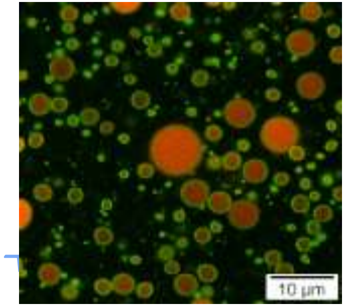
Challenges with plant protein utilization

- Efficient enrichment of protein from the matrix
- Technological functionality
- Sensory quality
- Nutritional quality
  - A.a. composition
  - Anti-nutritional factors
  - Allergenicity

Techno-functional properties of proteins

Proteins are key parts of human diet but they are also functional food ingredients affecting texture, mouth feel and flavor of the product.



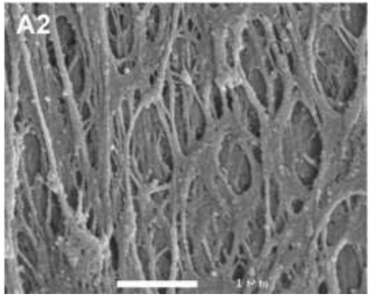


- Solubility
- Foaming
- Emulsifying
- Gelling
- Heat stability
- Water binding
- Fat binding

Dominated by egg and milk protein ingredients

- Texturizing
  - Network formation
  - Microfibrillation
  - Particulation
  - Water absorption

Dominated by meat, milk proteins, wheat and soy



Manski et al., 2008, Food Hydrocolloids, 22, 587-600

WHY Plant proteins have poor techno-functional properties?

- Protein structural features
  - When compared with egg or dairy proteins: Plant proteins are not truly soluble but form colloidal or non-colloidal suspensions.
- Biologically complexed with other molecules in the plant matrix (cell wall structures, lipids, phytate etc.)
- Extraction and drying conditions can cause heavy denaturation and aggregation
- Sensory attributes
  - Flavor or aroma originating from the source (beany, turnip-like etc.)
  - Bitterness,cardboard-like flavor (fat oxidation, processing, endogeneous enzymes)
  - Astringency, coarse mouthfeel (structure, processing)






Contents

Strategies to improve technological functionality of plant protein ingredients

I. Disintegration of biological structures

- Dry fractionation as a means of improvement
  - Case examples: rice and wheat brans

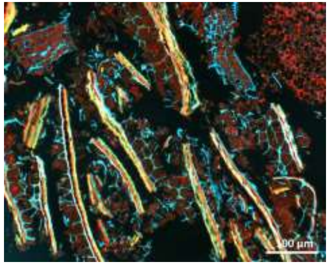
II. Bioprocessing

- Enzymes
  - Case examples: wheat bran and oat protein
- Fermentation
  - Case examples: rye bran and faba bean flour
- Co-processing with enzymes and fermentation

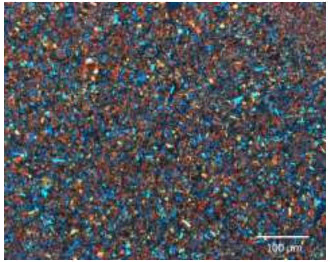
- Summary

Strategies to improve technological functionality of plant protein ingredients (1/2)

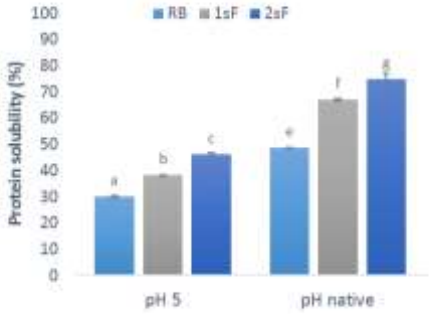
I. Disintegration of biological structures

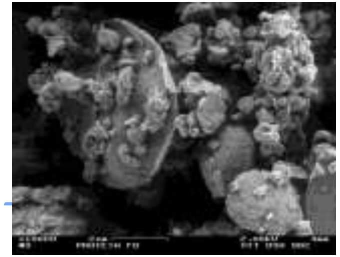


Disintegration by milling followed by air classification



Dry fractionation can be considered as a means of modification because the protein composition of the end products may be different from the raw material.

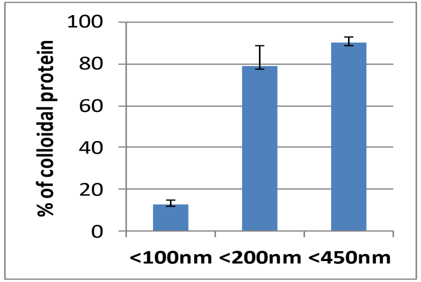




Disintegration by shearing

Formation of colloidal particles by microfluidization of protein fractions and process adjustment

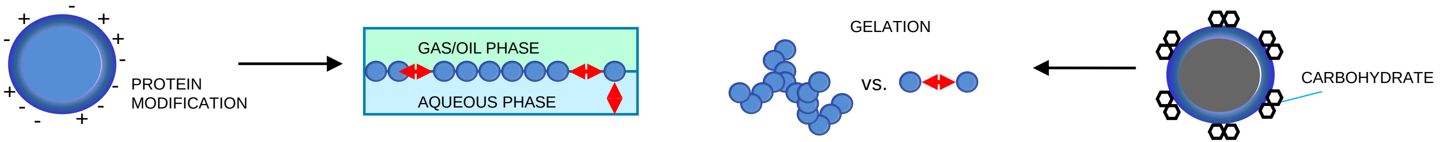
(role of other components)



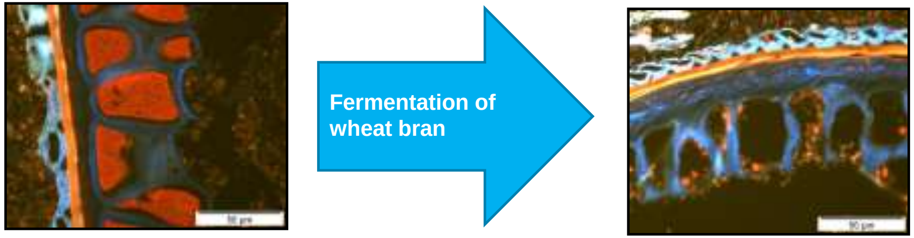
Strategies to improve technological functionality of plant protein ingredients (2/2)

II. Bio-processing

Enzymatic hydrolysis or cross-linking to change particle-particle interactions or surface engineering



Fermentation



Carbohydrates and proteins are modified thereby changing the technological and nutritional functionality.

Co-processing

I. Disintegration of biological structures - Dry fractionation

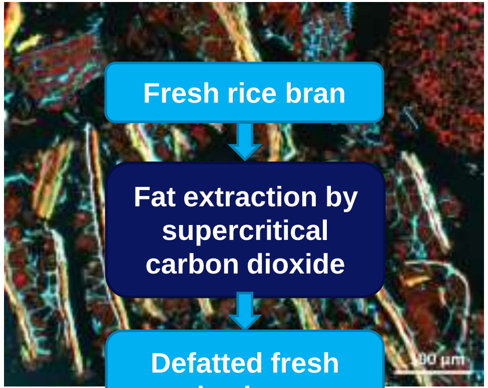
- Dry fractionation does not allow production of pure fractions, but fractions enriched in desired components like protein and fibre
- Possibility to exploit the properties of different components present in the fractions
- Technological and nutritional benefits from all the components

Hybrid ingredients

- **Advantages of dry fractionation over wet fractionation**
  - No addition and removal of water
  - No use of chemicals
  - Native functionality of proteins and other components are better retained
  - More of the raw material utilized ---------► less side-stream is produced

Dry fractionation Case:

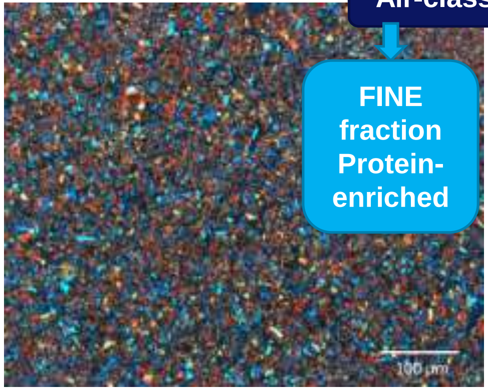
Rice bran One-step air classification



Fresh rice bran
↓
Fat extraction by supercritical carbon dioxide
↓
Defatted fresh rice bran
↓
Dry milling
↓
Air-classification
↓
FINE fraction Protein-enriched | COARSE fraction

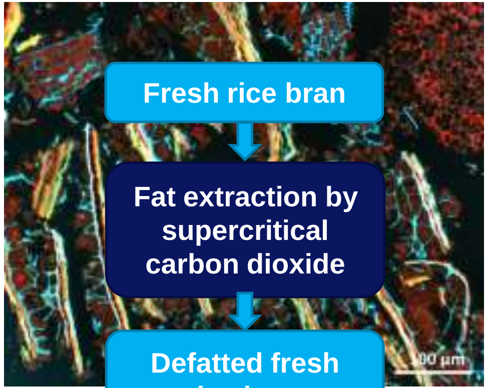
Protein: 18.5%
Starch: 23.5%
Soluble dietary fibre: 6.5%
Insoluble dietary fibre: 30.5%

Mass yield: 27.2%
↑ Protein: 25.7%
Protein yield: 38.0%
Starch: 7.9%
Soluble dietary fibre: 7.1%
↓ Insoluble dietary fibre: 14.2%



Dry fractionation Case:

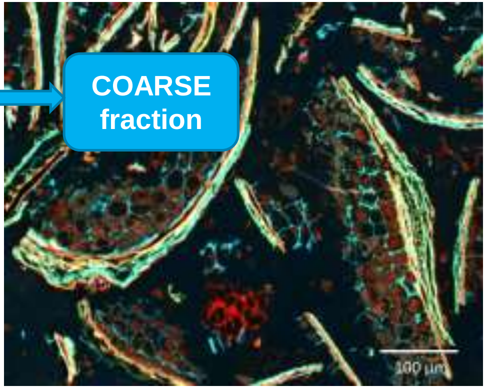
Rice bran Two-step air classification



Protein: 18.5%
Starch: 23.5%
Soluble dietary fibre: 6.5%
Insoluble dietary fibre: 30.5%

Air-classification

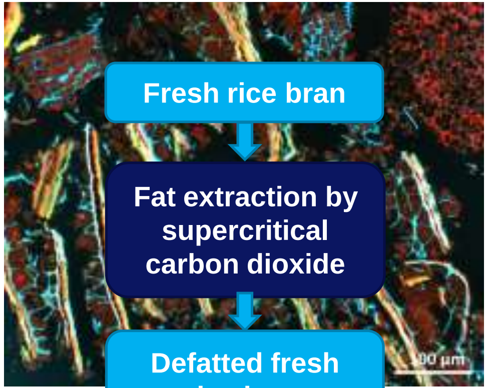
Mass yield: 18.5%
Protein: 19.7%
Protein yield: 19.7%
Starch: 12.9%
Soluble dietary fibre: 7.7%
Insoluble dietary fibre: 13.2%



Mass yield: 77.8%
Protein: 18.3%
Protein yield: 76.7%
Starch: 23.4%
Soluble dietary fibre: 1.8%
Insoluble dietary fibre: 30.4%

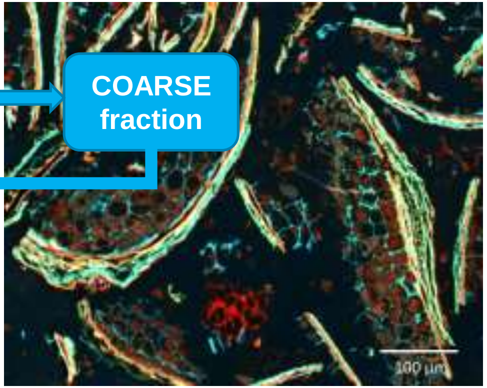
Dry fractionation Case:

Rice bran Two-step air classification

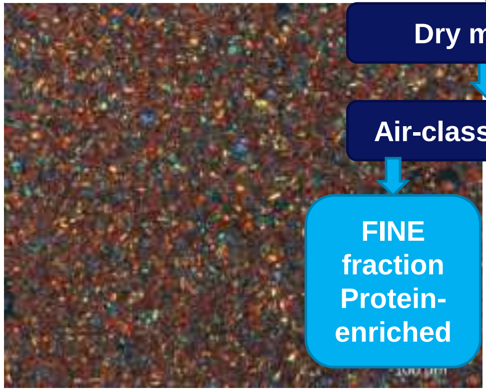


Fresh rice bran
↓
Fat extraction by supercritical carbon dioxide
↓
Defatted fresh rice bran
↓
Air-classification → FINE fraction / COARSE fraction

Protein: 18.5%
Starch: 23.5%
Soluble dietary fibre: 6.5%
Insoluble dietary fibre: 30.5%



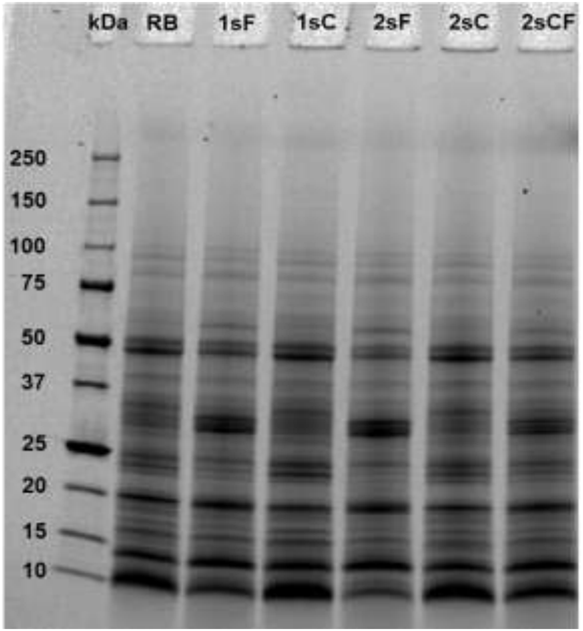
COARSE fraction
↓
Dry milling
↓
Air-classification → FINE fraction Protein-enriched / COARSE fraction



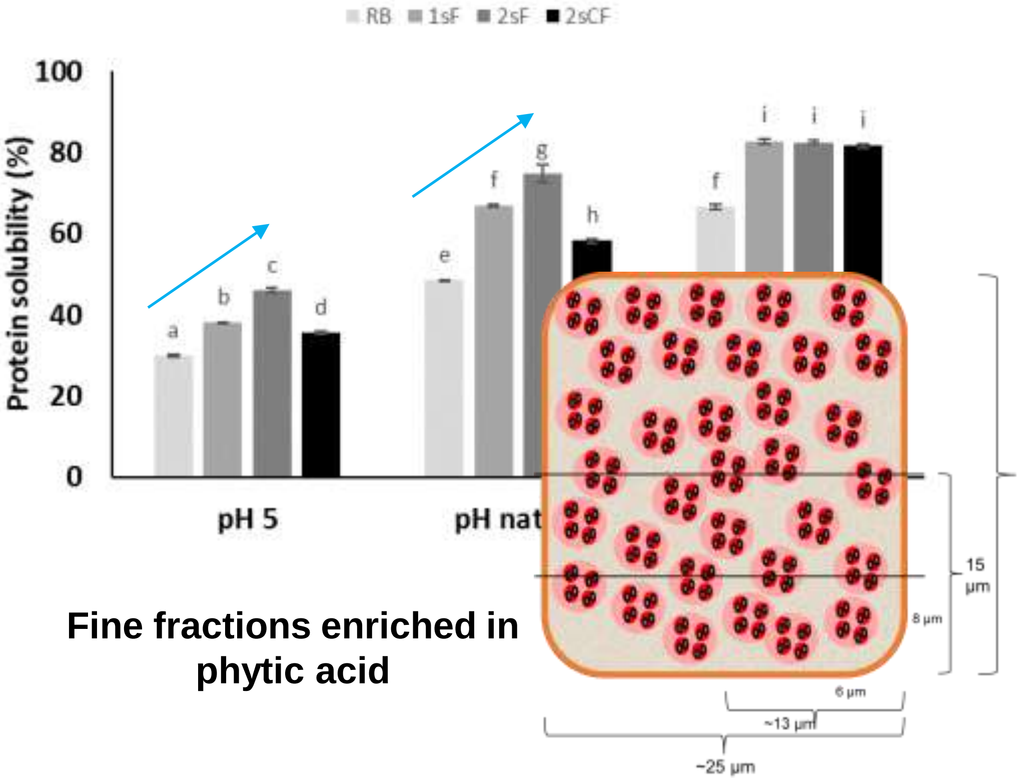
Mass yield: 13.9%
Protein: 27.4% ↑
Protein yield: 20.2%
Starch: 6.8%
Soluble dietary fibre: 6.8%
Insoluble dietary fibre: 27.8%

VTT

Dry fractionation: Protein composition in the fractions is altered



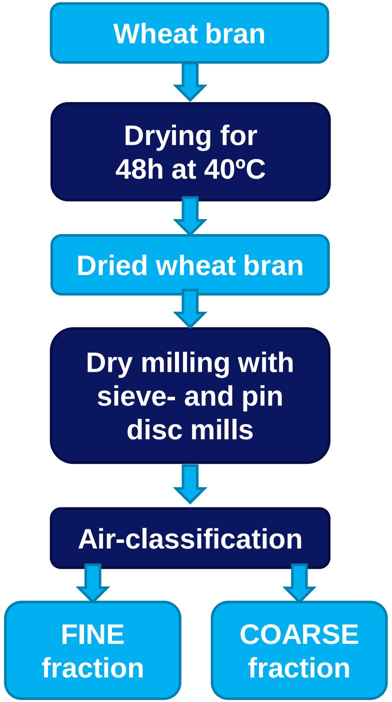
SDS-PAGE of rice fractions under reducing conditions.
RB: raw material rice bran, 1sF: fine fraction from the one-step air classification, 1sC: coarse fraction from the one-step air classification, 2sF: fine fraction from the first classification step of the two-step air classification, 2sCF: fine fraction from the second classification step of the two-step air classification



Fine fractions enriched in phytic acid

Dry fractionation Case:

Wheat bran one-step air classification



Protein: 14.9%
Starch: 14.4%
Soluble dietary fibre: 2.2%
Insoluble dietary fibre: 51.5%

Drying imroved mass and protein yields of the fine fraction obtained during air classification

Mass yield: 11.1%
↑ Protein: 32.2%
Protein yield: 24.1%
Starch: 12.7%
Soluble dietary fibre: 5.3%
↓ Insoluble dietary fibre: 12.0%

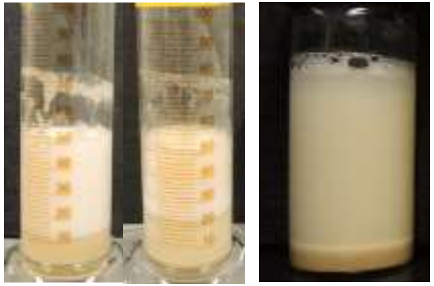
Protein-fibre hybrid-ingredients from rice and wheat brans show improved techno-functional properties compared to milled raw materials

Rice bran hybrid ingredient

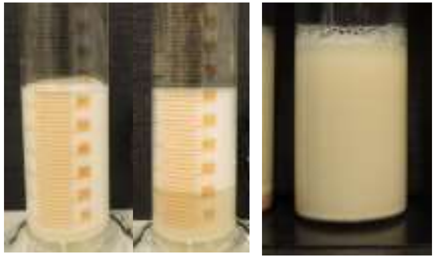
Foaming at pH 7

Foaming capacity (0 min) | Foaming stability (30 min) | Colloidal stability (30 min)

Raw material Rice bran, (SC-CO2, milled)



Rice bran hybrid ingredient

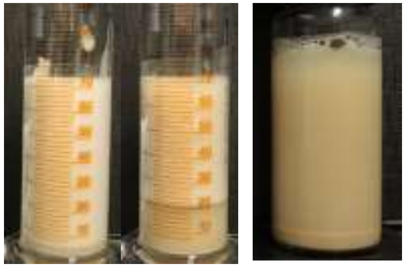


Wheat bran hybrid ingredient

Foaming capacity (0 min) | Foaming stability (30 min) | Colloidal stability (30 min)

Raw material wheat bran

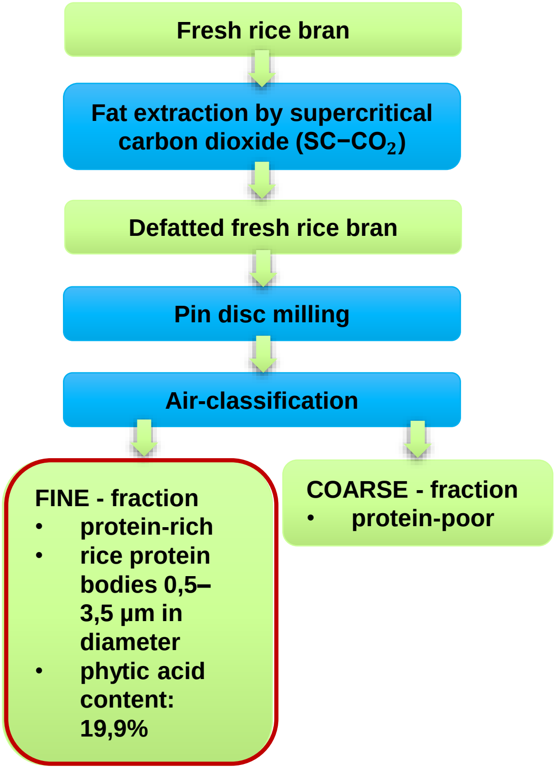
Wheat bran hybrid ingredient



Gelation of Rice Bran Hybrid Ingredient (Bioprocessing)

- The aim was to test the gelling ability of a protein-enriched rice bran fraction:
  - acid-induced gelation---------Transglutaminase
  - Heat-induced gelation--------Phytase

- **A product which contains around 3.5% protein and a high fiber claim (3% dietary fiber) was targetted.**
- **Achieved with single Hybrid raw material.**



Protocol of:

Acid-induced gelation

Defatted, milled rice bran raw material
- Dry matter: 13.9%
- protein content: 2.7%
- dietary fibre content: 5.1%

Protein-enriched rice bran fraction
- Dry matter: 13.9%
- protein content: 3.4%
- dietary fibre content: 3%

↓

Hydration at native pH: 6.7

↓

Incubation at 40°C, 2h
- Control (no enzyme)
- Transglutaminase (100 nkat/g protein)

↓

Heat treatment (at pH 8)

↓

Acid-induced gelation
- using glucono-delta-lactone
- 40°C, 4.5h
- 22°C, 22h

↓

Final gel at pH 4.5

Heat-induced gelation

Protein-enriched rice bran fraction
- Dry matter: 13.9%
- dietary fibre: 3%
- protein content: 3.4%

↓

Hydration at native pH: 6.7

↓

pH adjustment to 5

↓

Incubation at 50°C, 2h
- Control (no enzyme)
- Phytase (100U/g sample)

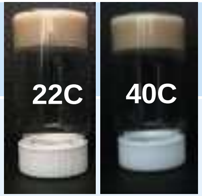
↓ (directly, or via **pH adjustment to 8**)

Heat-induced gelation
- temperature ramp (2°C/min)
  25°C to 95°C (hold at 95°C for 5 min)
  95°C to 25°C (hold at 25°C for 15 min)

↓

Final gel

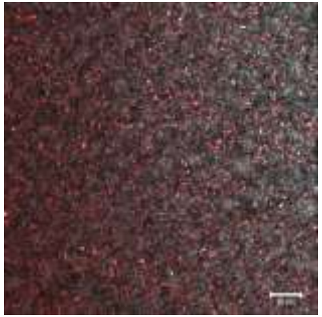
The best gel structure was obtained in heat-induced gelation performed at pH 8 after phytate degradation

Gelation protocol	Pre-treatment	Conditions	G' (Pa)	Water holding capacity (%)	Microstructure of the gel (by CLSM)	Visual appearance of the gel
Acid-induced gelation	No enzyme	22°C	190	57	a particulate gel network with fractal dimensions	
		40°C	107	54	a particulate gel network with fractal dimensions	
Heat-induced gelation	No enzyme	pH 5	90	36	void areas filled with water	
		pH 8	1141	55	a particulate gel network with fractal dimensions	
	Phytase-treated	pH 5	86	38	void areas filled with water	
		pH 8	8216	78	a particulate gel network with fractal dimensions	

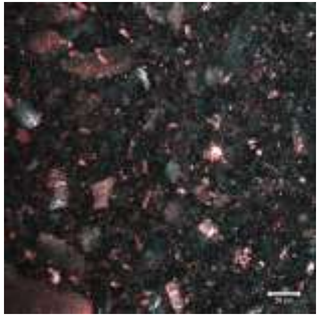
The best gel structure was obtained in heat-induced gelation performed at pH 8 after phytate degradation

Acid-induced gelation

Protein-fraction 22ºC

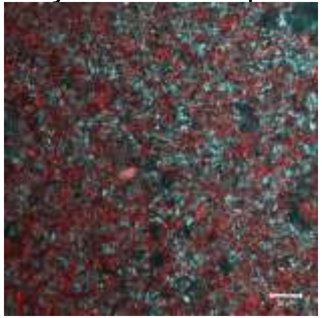


Raw material 22ºC

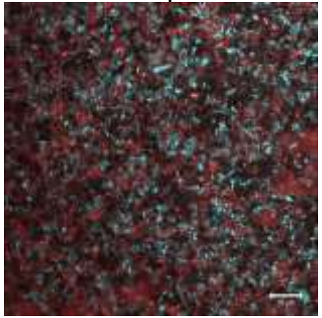


Heat-induced gelation

Phytase-treated pH 8



Control pH 8



Sensory attributes were improved compared to the raw material rice bran

CLSM images of the gels

Case study: Rice bran hybrid ingredient as a source of protein and fiber in commercial wheat bread

	Protein-rich rice bran	Pericarp-free rice bran
Dietary fiber g/100g	20.7	17.1
Protein g/100g	24.3	18

Two rice bran hybrid ingredients

- Protein-rich rice bran
- Pericarp-free rice bran

Commercial wheat based products

- Normal wheat bread / toast

Effect on

- Amount of dietary fiber
  - 3 g / 100 g = source of dietary fiber
  - 6 g / 100 g = high fiber product
- Energy derived from protein (calculated 3 kcal / 1 g protein)
  - 12 % = source of protein
  - 20 % = high protein

Case study: Rice bran hybrid ingredient as a source of protein and fiber in commercial wheat bread

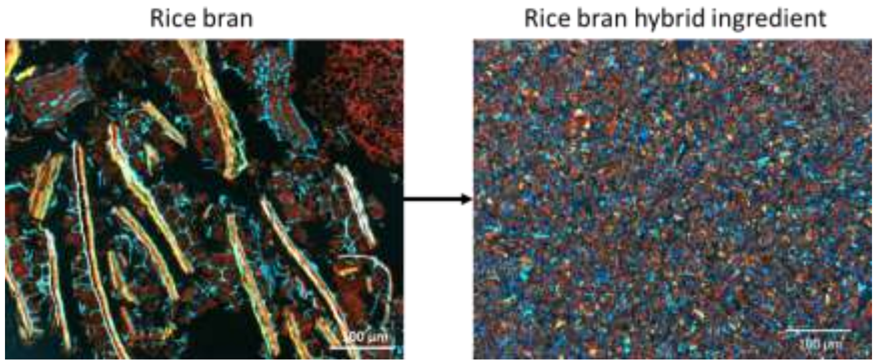
		10 % ingredient addition		20 % ingredient addition		30 % ingredient addition	
100 g	Wheat bread toast	Protein-rich rice bran	Pericarp-free rice bran	Protein-rich rice bran	Pericarp-free rice bran	Protein-rich rice bran	Pericarp-free rice bran
Energy kJ	1134						
Energy kcal	268	268	268	268	268	268	268
Fat	2.3						
Carbohydrates	50						
Dietary fiber	4.2	6.08	5.68	7.96	7.16	9.84	8.64
Protein	9.2	10.98	10.28	12.76	11.36	14.54	12.44
Protein claim (energy) %	10.3	12.3	11.5	14.3	12.7	16.3	13.9
Protein claim (value)	Too low	Source	Too low	Source	Source	Source	Source
Fiber	Source	High	Source	High	High	High	High

<- Note; could be lower with added ingredient

Hybrid Ingredients

Protein concentrates with high amounts of dietary fiber can be customized into high-protein high-fiber foods with a single ingredient.

At VTT, we highly trust on dry separation techniques and tend to name the protein-enriched fractions as 'hybrid ingredients' due to this dual functionality.



II. Bioprocessing – Enzymes

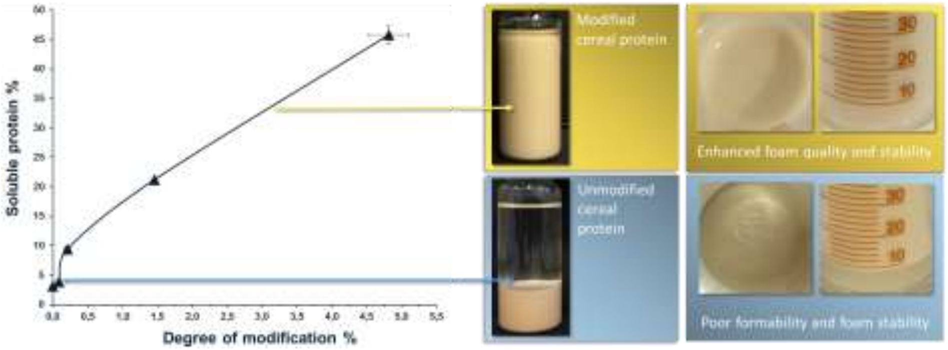
VTT approach for enzymatic protein modification

Approach	Enzymes	Substrate/ Application
Protein crosslinking	• Tyrosinase (VTT) • Laccase (VTT) • Transglutaminase (commercial)	• Dairy proteins and matrices • Meat proteins and products • Cereal proteins and products • Legume proteins
Protein functionalization and grafting	• Tyrosinase (VTT) • Laccase (VTT) • Sulfhyrdyl oxidase (VTT) • Deamidases (commercial)	• Hetero-crosslinking of proteins and carbohydrates • Grafting phenolic compounds to proteins • Mediator induced protein modification
Hydrolysis	• Commerical proteases, xylanases, phytases etc.	• Hydrolysis of proteins or associated compounds to tailor functionality (technical, sensory)

II. Bioprocessing – Hydrolytic enzymes

Controlled hydrolysis of cereal components

Wide range of food application

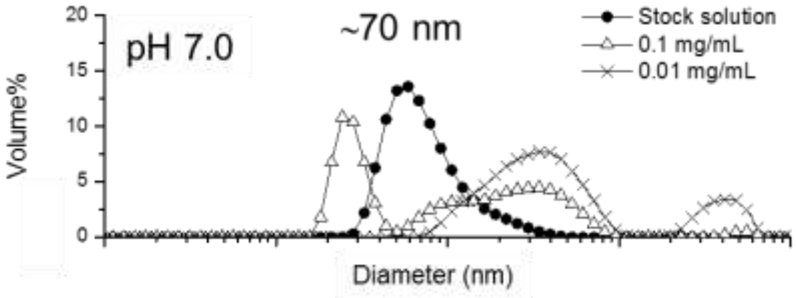


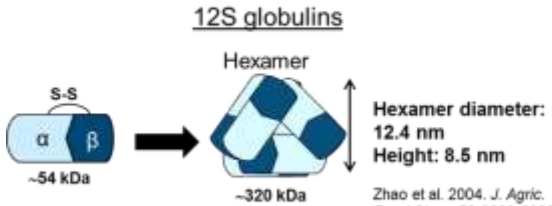
Technological properties of wheat bran can be improved by the use of hydrolytic enzymes at low water content

Santala et al. (2014). J Cereal Sci, 60, 448–456.

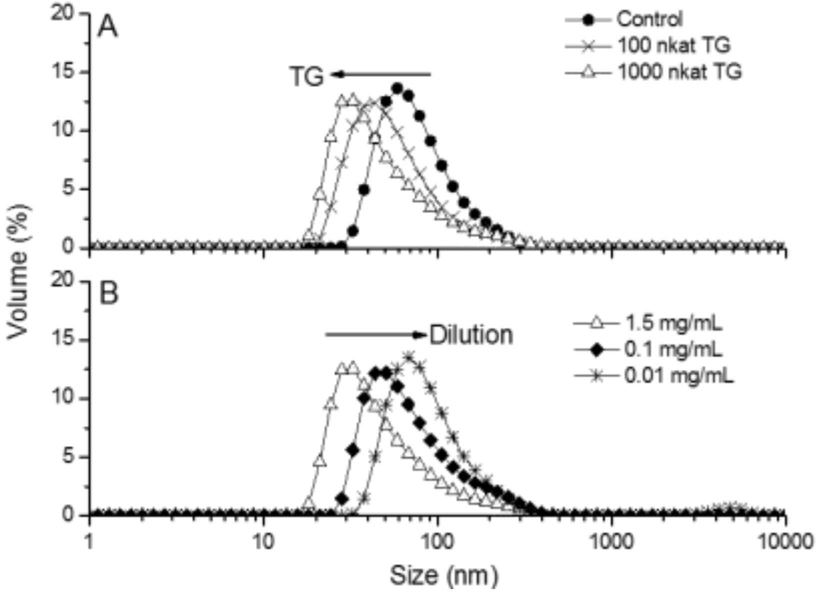
II. Bioprocessing – Crosslinking enzymes

Engineering oat protein colloidal particles by transglutaminase



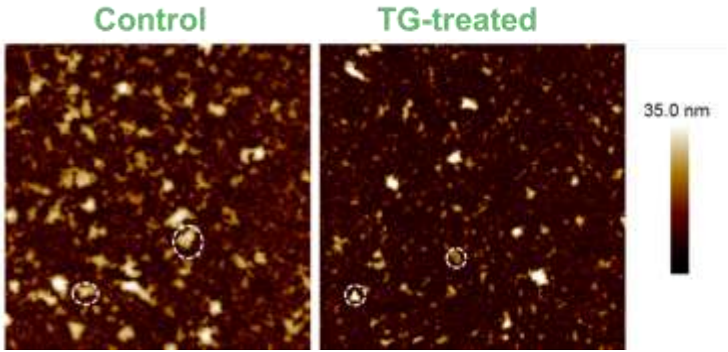


Zhao et al. 2004. *J. Agric. Food Chem.* 52, 3089-3096

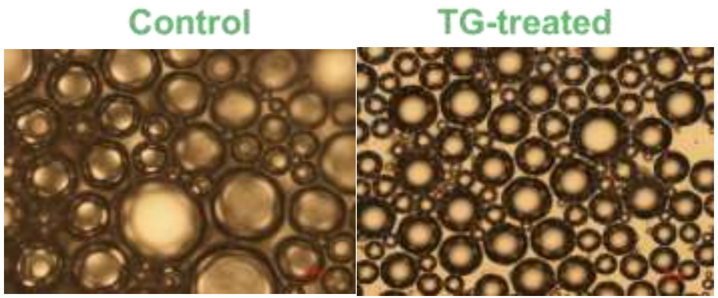


Similar to soy glycinin

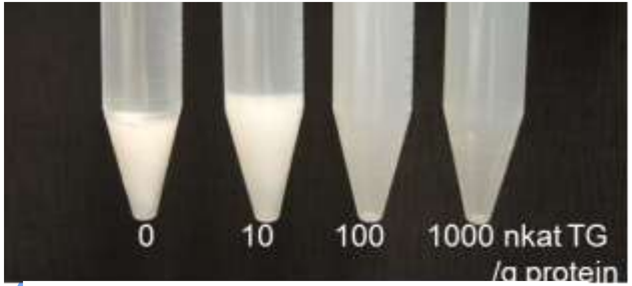
II. Bioprocessing – Crosslinking enzymes



Atomic force microscopy of interfacial films at pH 7.2



Light microscopy of foams from oat protein isolates (control and TG-treated 1000nkat/g protein).



Storage stability of oat protein isolate dispersions (1 month at 4 °C).

The increased dispersion stability and foamability of TG-treated oat protein isolate was achieved likely due to:

- improved electrostatic stability
- intra-particle crosslinking providing stability against dissociation/re-association
- smaller particle size.

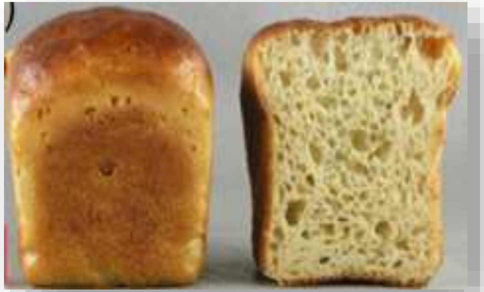
Ercili-Cura et al., 2015, *Food Hydrocolloids*, 44, 183-190
Nivala et al., 2017, *Food Chemistry*, 231, 87-95

II. Bioprocessing - Fermentation

Fermentation as a tool to improve the techno-functional and nutritional profile of faba bean flour

Fermentation releases proteins from the cell wall matrix

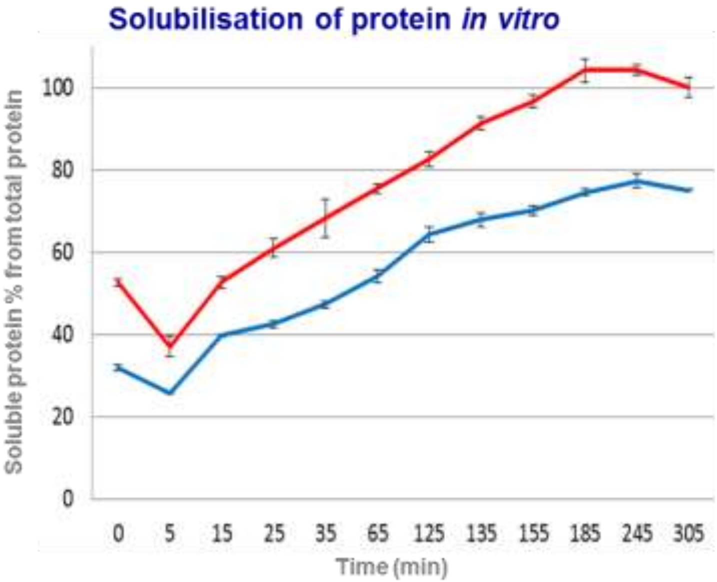
Fermented faba bean flour provided high-loaf-volume bread with improved nutritional profile.



Sozer et al., submitted

II. Bioprocessing – Fermentation and Enzymes Co-processing

Bioprocessing with enzymes and yeast transforms the rye bran protein more bioaccesible

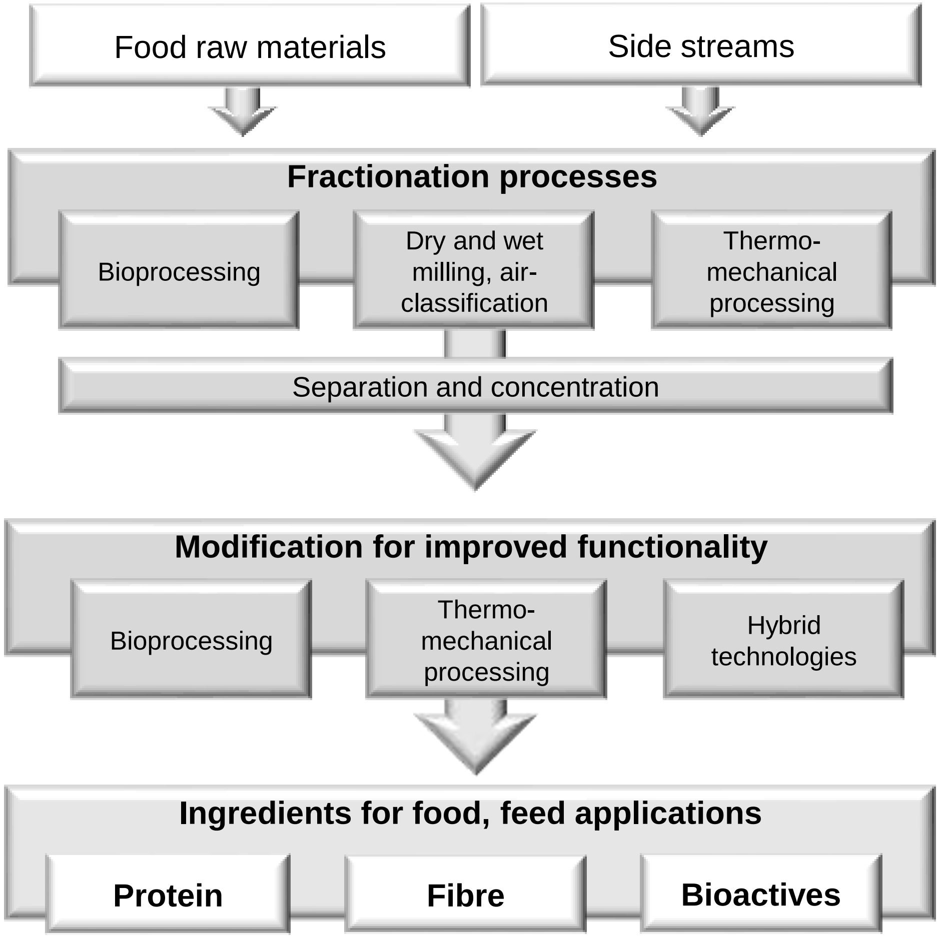


Nordlund et al.2013, J Cereal Sci, 58, 200

Also check: Coda et al., 2014, Food Microbiology, Volume 37, Pages 69–77

Ingredient development at VTT

Summary slide



Acknowledgements

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- **Bio Based Industries Joint Undertaking** under the EUs Horizon 2020 research and innovation programme

- **VTT team on plant protein research**
  - Dr. Emilia Nordlund, Research team leader, Food Solutions
  - Dr. Dilek Ercili-Cura, Colloidal food systems
  - Dr. Nesli Sozer, Solid and semi-solid plant-based food systems
  - MSc Pia Silventoinen, Fractionation technologies
  - MSc Anni Nisov, Techno-functional properties of proteins
  - Dr. Outi Mattila, Bioprocessing of cereal ingredients
  - Dr. Natalia Rosa-Sibakov, Processing and digestibility of plant ingredients
  - Dr. Riikka Juvonen, Fermentation technologies
  - Dr. Anna-Marja Aura, *In vitro* models and research
  - Dr Raija-Liisa Heiniö, Sensory research
  - Dr Kyösti Pennanen, Consumer research
  - Prof. Kaisa Poutanen, Research manager

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