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Impact of different commercial proteases on protein and product quality in a quinoa-based milk substitute

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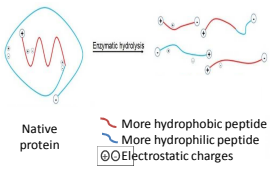
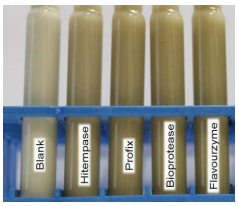
Introduction

In this study, the effect of enzymatic treatment on protein quality in a beverage based on quinoa was investigated. As functionality of proteins is often evaluated in model systems even though food in general is a lot more complex, the assessment of hydrolysis in a food matrix might provide new insights. By the cleavage of peptide bonds, proteases change the initial protein structure. The molecular weight decreases, the amount of ionisable groups increases and hydrophobic groups so far hidden in the inner core get exposed The findings might be of interest to understand and predict product properties in the development of enzymatically modified milk alternatives.

Methodology

Enzymes	Enzyme class	Dosage (rec. dosage)
Profix 100	Sulfhydryl proteases	1, 10, 25, 50x
Bioprotease N100L	Metalloprotease	1, 10, 25x
Flavourzyme 1000L	different enzymes	1, 5, 10x
Hitempase 2XP	Amylase	





Blending water + quinoa flour 12.5%
- Enzymatic treatment, 3 h, 50 °C
- Homogenisation, 180 MPa
- pH adaption, 7

Figure 1. Production of quinoa-based milk substitutes (QBMS) and picture of products

Results

1. Molecular weight

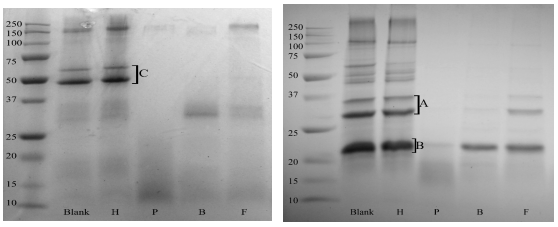


Figure 2: SDS-PAGE gels of QBMS, treated with different enzymes, unreduced (left) and reduced with DTT (right), H=Hitempase, P=Profix, B=Bioprotease, F=Flavourzyme

Bitter flavour and decreased functionality may be caused by small peptides. As Profix consists of a wide range of sulfhydryl proteases (strong nucleophil), this could be a possible reason for the more intense cleavage of peptide bonds by Profix.

Major Findings

- **Typical major bands at 49 and 63 kDa ("C") referred to as chenopodin**
- **Acidic and basic subunits at 30-40 kDa and 20-25 kDa, marked as "A" and "B"**
- **Molecular weight strongly decreased, especially for Profix**
  most proteins were < 25 kDa

2. Protein Hydrophobicity

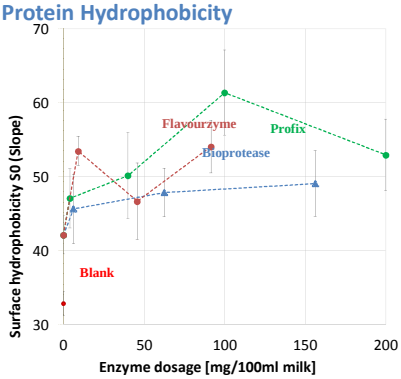


Figure 3: Surface hydrophobicity of QBMS influenced by different dosages of enzymes

Surface hydrophobicity (S_0) describes the extent of hydrophobic parts on the surface of a protein and related to their conformation, function and stability e.g. S_0 can be used to predict the emulsifying properties. Owing to enzymatic cleavage, hydrophobic sites so far hidden get revealed and S_0 increases. Nevertheless, it is determined by the nature of the protein and can decrease or increase due to enzymatic hydrolysis.

Major Findings

- **Effect of proteases is little**
- **In literature it is observed clearer for protein isolate**
- **Conditions possibly not ideal, due to complex QMBS matrix**

3. Secondary structure

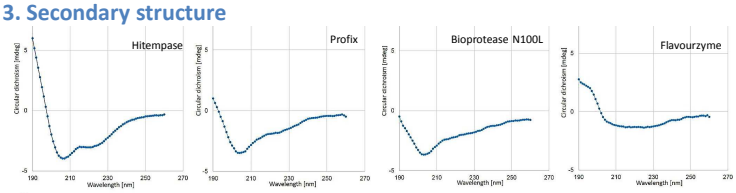


Figure 4: Circular dichroism spectra for QBMS samples

By circular dichroism, structural alteration of proteins can be observed. Flavourzyme contains, different exo- and endopeptidases which may influence the secondary structure in a different manner than the other enzyme preparations with less different modes of action.

Major Findings

- **Protease changed the secondary structure of treated QBMS**
- **Flavourzyme-treated samples changed the most, minimum diminished**
  →Even though is showed the smallest effects on other characteristics

4. Protein Solubility

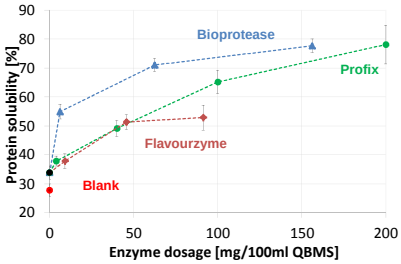


Figure 5. Protein solubility of QBMS samples

Solubility is among the most important properties concerning functionality of food proteins. It is in general accompanied by better functionality for most applications.

Major Findings

- **Significantly increased by all proteases**
- **2.5-fold increase for Bioprotease and Profix**

5. Suspension stability

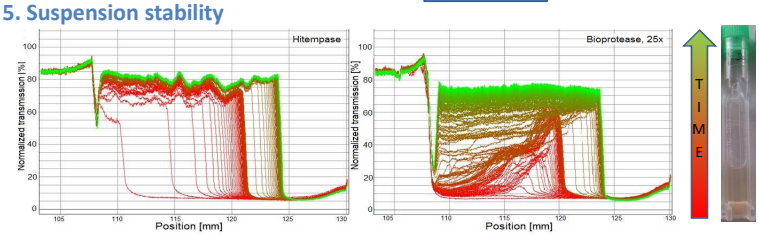


Figure 6: Transmission profiles of Hitempase-treated sample and exemplarily Bioprotease at 25x the rec. dosage and a sample after centrifugation process

The stability of the milk samples was measured using an analytical centrifuge to determine the stability and separation behaviour.

Major Findings

- **Sedimentation occurred, and a clear layer was visible in the upper part**
- **Proteases change sedimentation behaviour**
- **Strategies against sedimentation are necessary, particle size driven → filtration, cellulase treatment**

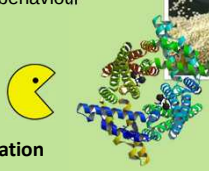
Conclusion

Enzymatic treatment is an interesting tool for tailoring protein and product properties of plant milk substitutes

- ✓ Whiteness decreases
- ✓ Molecular weight decreases
- ✓ Protein surface hydrophobicity not affected
- ✓ Protein solubility increases
- ✓ Changes in secondary structure
- ✓ Changes in sedimentation behaviour

Outlook

Enzymatic treatment
→ individual or combined
→ optimal conditions



Strategies to prevent sedimentation and improve taste & colour

Innovative milk substitute based on a raw material with outstanding properties

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