Valorization of microbial protein by proteomics and bioinformatics-assisted targeted hydrolysis

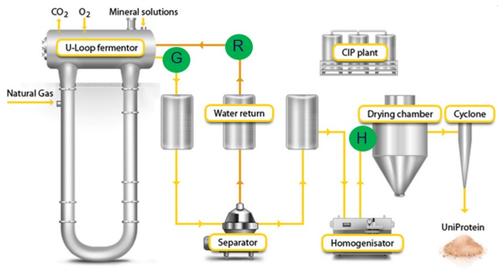
Jesper Skovmark and Jacob Lund Jensen, Simon Gregersen (AAU), Theis Sommer (Unibio)

Helix Lab

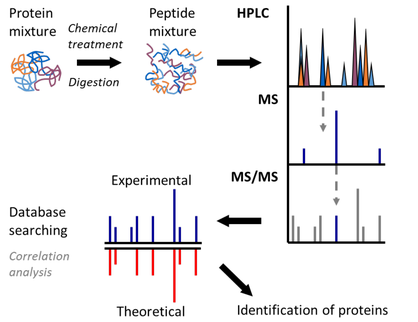
Introduction

As part of the green transition and a more sustainable food sector, the search for alternative protein sources is rapidly developing. A potential solution to accelerate the transition is the use of microbial biomasses. Unibio produces feed protein by fermentation of methane metabolizing bacteria, thereby decoupling protein production from farming and fishing to obtain a more sustainable protein source. The biomass was recently shown to contain abundant proteins in which part of their sequence (i.e., embedded peptides), show great promise as functional and bioactive food ingredients. In this project, the goal is to transform the feed-grade protein product into a food-grade functional ingredient using enzymatic hydrolysis. Using proteomics and bioinformatic big data analysis, the project will develop a targeted approach for release of these peptides and downstream enrichment for improved functionality.

Below is an illustration of the process at Unibio for the production of their product, UniProtein®, with upstream fermentation in their patented U-Loop fermentor and downstream processing of the ferment (G) from the fermenter. Three streams of Unibio's downstream are selected for investigation as illustrated with green circles each representing the streams ferment (G), water return (R) and homogenate (H).



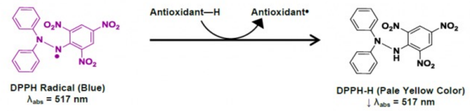
Methods



Computational translation of MS/MS spectra to amino acid sequences using genomic or protein databases

The bioinformatic tool AnOxPePred is utilized to predict possible antioxidant peptides from hydrolysis of *M. capsulatus*.

The DPPH assay is utilized to measure the radical scavenging ability of the hydrolysates. The neutralization of the radical DPPH is illustrated:

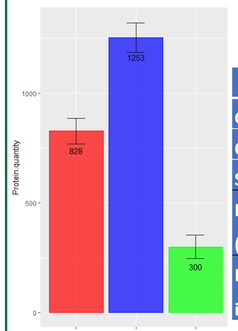


The hydrolysis is evaluated by the method pH-stat, where the pH value is kept constant by addition of alkali, and the degree of hydrolysis is calculated by the following:

$$DH = \frac{B \cdot N_B}{\alpha \cdot M_P \cdot h_{tot}} \cdot 100\%$$

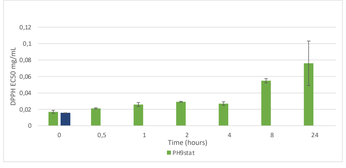
Where DH is degree of hydrolysis, B is the volume of base consumed, N_B is the normality of the base, α is the average degree of dissociation of the α-amino group of the protein substrate specified by the pK of the average α-amino group and the pH. M_P is the mass of protein in the mixture based on the protein concentration from Kjeldahl nitrogen and h_{tot} is defined as: *"h_{tot} is the total number of peptide bonds in the protein substrate (meqv/g protein)"* (Rao *et al.*, 2018).

Outcome



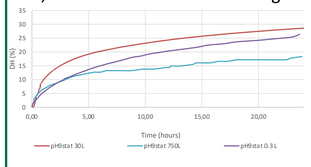
	G	H	R
G1UBD1 (%)	27.4	10.8	25.1
Q60AR6 (%)	11.2	6.38	5.89
Sum (%)	38.6	17.18	30.99
Protein content (mg/mL)	9.69	86.9	0.440
Protein of interest (mg/mL)	3.74	14.9	0.136

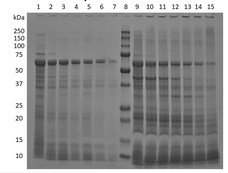
The number of identified protein groups are highest in the homogenate (barplot) and the amount of protein of interest (G1UBD1 - Particulate methane monooxygenase alpha subunit and Q60AR6 - Methanol dehydrogenase protein, large subunit) is highest in the homogenate, which is utilized to pick that stream for hydrolysis. Hydrolysis by autolysis by endogenous proteases present in the homogenate proved more feasible than addition of external enzymes.

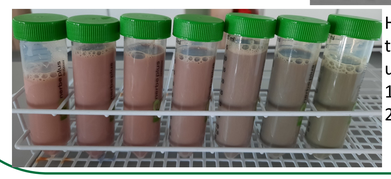


A lower DPPH EC50 is a sign of increased radical scavenging effect, and the blue bar is the untreated homogenate. This indicates that hydrolysis decreases the radical scavenging effect over time, maybe because the proteins are radical scavenging, and hydrolysis of these will destroy the radical scavenging ability.

Upscaling of hydrolysis by autolysis was achieved from 0.3 L to 30 L to 750 L while monitoring DH% by pH-stat. An SDS-PAGE was utilized to visualize the protein profile over time, where a band represent a protein. Lane 1, 2, 3, 4, 5, 6, 7 is soluble protein at timepoints untreated, 0, 1, 3, 6, 12 and 24 hours. Lane 9, 10, 11, 12, 13, 14 and 15 is the same loading order but for the pellet.







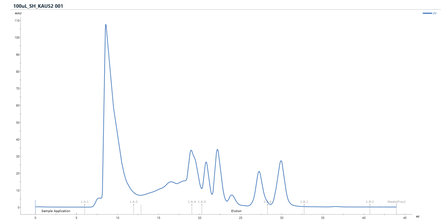
Hydrolysate at timepoint: untreated, 0, 1, 3, 6, 12 and 24 hours.

Perspectives

Further scale-up into an industrial production plant and making the process continuous.

Evaluate the high radical scavenging activity of the untreated homogenate. Does it originate from proteins, metabolites or something different.

Fractionation by size exclusion chromatography at HelixLab was not useful in determining the radical scavenging activity in the fractions:



In the future it would be useful to test membrane filtration as a source to evaluate and concentrate the fraction causing the radical scavenging activity.

Experiments of the produced hydrolysates' solubility and digestibility are next in-line. Solubility will be investigated in several solvents to identify increases or decreases compared to normal Uniprotein®. Digestibility will be tested in rats, where the ratio of essential amino acids and toxicology will be studied. Sensory analysis can also be conducted to uncover the most optimal smell, flavor, texture or others for a given product. An ammonia-like smell intensified during the 24 hours of hydrolysis by autolysis and flavor changed into an umami-rich taste.