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D 4.5: Protocol for production of protein-rich fraction

Macro Cascade -Project

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Summary

Proteins in seaweed are present as a soluble fraction and as part of the cell wall. Isolation of each type of protein requires different approaches and experimental conditions. In this study both soluble and membrane-bound proteins were isolated from the brown seaweed *Saccharina latissima.* Focus was on an isolation procedure to produce a protein-rich fraction.

The protein content of seaweeds can roughly vary between 5% and 50%, 5-20% for brown seaweed and 20-50% in red and green seaweed. The protein content differs not only between species, but it also depends largely on the seasonal harvesting period. Proteins have complex structures based on their amino acid composition, three-dimensional structures (helices, beta sheets) and the way subunits are linked together. Molecular weights vary from thousands to millions of Daltons.

For the isolation of proteins from seaweed it is important to acknowledge the desired end applications. In case the protein is used as binder in coating or adhesive systems, the proteins have to maintain most of their functional properties. The proteins should be isolated in such a way that the molecular backbone (molecular weight) is kept intact and that major denaturation of the protein is prevented. When protein fragments and/or amino acids are desired, more severe extraction conditions could be used. Most of the native proteins are water soluble at a low or high pH, and proteins can be solubilized in acidic or alkaline solutions. The protein extraction efficiency can be increased by the use of enzymes. In summary, the use of acids, bases, or enzymes can hydrolyze the protein material during the isolation process, thereby reducing the molecular weight. This has to be taken into account for applications in which the molecular weight of the protein is important as the isolation process has an effect on the chemical and physical properties of proteins. Applications of proteins can range from food(additive), feed(additive), and as ingredient in pharmaceuticals or in technical applications.

In this study the extraction of proteins from *S. latissima* was being evaluated. Protein extractions from seaweed biomass but also from a residue streams (coming from the alginate extraction) were described. Finally, a protocol for the isolation of protein from *S. latissima* was written, both from a liquid phase (soluble proteins) and from a solid phase (membrane bound proteins).

Protein extractions of seaweed under alkaline conditions (pH 12) resulted in partial solubilization of small protein fragments in the liquid phase. Due to the buffering capacity of seaweed, high amounts of NaOH were needed to reach pH 12, making this procedure less interesting.

The solid residue sample after Na-alginate solubilization appeared to be an interesting source of protein as the protein content of this fraction was around 20 wt% DM (40 wt% DM of the initial protein content in seaweed). This residue fraction was high in ash content due to extraction of alginate with soda. A quick win was obtained by washing this sample with water, thereby removing a large part of the salts and increasing the protein content to 25 wt% DM. This process can be further optimized, leading to a protein-enriched fraction as a valuable residue stream from the production of alginate. More research needs to be done to determine the properties of this protein fraction.

Also the liquid fraction, obtained after screw pressing of fresh seaweed, might be an interesting source for (native) seaweed proteins. SDS-page showed the presence of high-molecular weight proteins. Isolating proteins from this screw press liquid fraction might also be an interesting option for the production of a protein-enriched fraction from *S. latissima*.

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