Microalgae proteins: From bulk to techno-functional proteins with nutritional and sustainable benefits

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Microalgae gain increasing importance as alternative protein source for food production and biotechnological systems. Their protein-content of up to 70% bears potential for multiple functional applications in the food, nutraceutical and cosmetics industries. This potential coupled with microalgae's ability to be grown on non-arable land and to fixate CO₂ when cultivated photoautotrophically, motivates the foreseen impact on major challenges of the sustainable development goals. Economic constraints and a low technical readiness level impede large-scale applications of their bulk proteins, so far.

We extruded whole microalgae biomass, showed the stabilizing effect of aqueous extracts on fluid-fluid interfaces and investigated in continuous high-temperature short-time (HTST) processing the thermal stability and functionality decrease of phycocyanin, a high value low volume protein-pigment complex. Thereby, we could showcase microalgae's potential in various food processing categories and relevant functionalities of its proteins ranging from bulk to specific techno-functional fractions.

High moisture extrusion with incorporated microalgae

Plant-based meat analogues produced by high moisture extrusion cooking can provide relevant alternatives to animal protein products. Microalgae combined to soy concentrates were used to create fibrillary textured extrudates. The incorporation of spray-dried microalgae biomass in up to 50% affected the formation of fibers, which could be balanced by reducing moisture levels. The elevated fat content of microalgae biomass led to lubrication effects, while probably undisrupted microalgae cells acted as passive fillers and limited the access of intracellular proteins. Both effects may have reduced texturing but increased tenderness in comparison to pure soy based extrudates. By using heterotrophically cultivated *Auxenochlorella protothecoides* with a light-yellow coloration, adverse effects on visual appearance could be eliminated. Microalgae integration improved the extrudate's nutritional profile by incorporating vitamins B and E, where over 95% was retained in the final product.

Stabilizing multiphase food systems with aqueous microalgae extracts

Spray-dried *Arthrospira platensis*, commonly named *Spirulina*, was disrupted by highpressure homogenization to yield an aqueous extract by centrifuging cell biomass from soluble components. Proteins were precipitated from the supernatant at the isoelectric point. The resoluble fraction was further purified by diafiltration. Foams and emulsions were formed with different aqueous extracts and compared to model structures based on whey protein isolate. Foam height was recorded and bubble size distribution was determined by image processing microscopic pictures. Creaming height was tracked and droplet size distribution measured with a laser diffraction particle size analyser. Thus, stability of the multiphase systems could be shown and quantified. To identify the nature of the stabilising mechanism, decrease in surface tension was recorded by bubble pressure tensiometry and interfacial viscoelastic properties of the fluid-fluid interface were investigated by interfacial oscillatory shear rheology. The adsorption behaviour and viscoelastic profile of the differently purified aqueous extracts against dodecane stressed the vital impact of proteins in stabilizing the fluid-fluid interface.

Disentangling phyocyanin's thermal degradation by HTST processing

Phycocyanin is a protein-pigment complex derived from *A. platensis*. This complex has proven functionality as only natural blue coloring food, fluorescent marker and therapeutic agent. The structure-function relationship is heat sensitive, making thermal processing in its production and its subsequent application a crucial aspect. By differential scanning calorimetry, it was shown that purified phycocyanin (mixture of allophycocyanin and c-phycocyanin) disassembled and denatured between 50 and 70°C. Combined with UV-Vis absorbance and fluorescence spectra of samples treated in continuous HTST processing, three characteristic transition temperatures were allocated to specific quaternary aggregates. In contrast to sequential chemical denaturation, changes in phycocyanin's chromophores coincided with alterations in the secondary protein structures in continuous HTST. The insights gained help to define processing windows and enable targeted process control for preserving maximum color shades, fluorescence properties and biological functionalities of phycocyanin for multiple applications in research and industry.

This research highlights the potential of microalgae proteins for the food and cosmetics industry emphasizing its capabilities as nutritious and sustainable alternative in high moisture extrusion cooking for plant-based textures, in stabilizing

fluid interfaces for multiphase systems, and in providing blue color for multiple product categories.