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Topic III – Gentle and smart food processing

Simple purification method for recombinant milk protein expressed from *Pichia pastoris*

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Background: Recombinant proteins are gaining attention as promising alternative protein sources for food. Recombinant proteins are produced by micro-organisms, such as bacteria or yeasts, that are modified to produce a specific (foreign) target protein. In that way, the recombinant protein closely resembles the amino acid composition and possibly folding and functionality of the natural target protein. This is a major advantage over other alternative proteins, such as plants and insects, which have different nutritional value and functionality. Recombinant production of proteins is already widely used for the production of vaccines, therapeutics, diagnostic reagents and enzymes (Tripathi & Shrivastava, 2019). Now it is also an emerging technology in the food industry, for example, to produce milk proteins without cows (Keppler et al., 2021). However, conventional downstream processing involves expensive chromatographic methods for pharmaceutical grade purification. To produce recombinant food protein in a cost and environmentally sustainable manner, simpler and cheaper purification methods are required.

Methods: For food applications, alternative purification processes were investigated to purify recombinant protein expressed from *pichia pastoris*. The major whey protein β-lactoglobulin (BLG) was used as a model protein. We compared purification with anion exchange chromatography to a specific salt precipitation method. The recombinant BLG was extensively characterized before and after purification, using bovine BLG as a control.

Results and Discussion: The recombinant expression of BLG in the yeast *P. pastoris* resulted in a material with a crude protein content of ~35%, while the major fraction contained carbohydrate impurities. Preliminary results showed that separation of proteins and carbohydrates based on size and polarity was inefficient, while separation based on charge significant increased the protein purity. A simple purification method using salt precipitation was established that resulted in a similar protein purity (77%) and yield (26%) to anion exchange chromatography (protein purity and yield of 74% and 23%, respectively). The salt purification process had no major impact on structural properties of both recombinant and bovine BLG. The denaturation temperature was also similar, while stability changed depending on the variant. Minor sequential differences were found between the bovine and recombinant BLG, but this had no major impact on the structural properties. Functionality experiments will be executed, but the current characterization results already demonstrate the potential use of this method for the purification of recombinant food protein. Finally, we are envisioning ways to recover the salts and water used in this process, as well as to increase the protein recovery, to reduce the environmental footprint of the purification process presented.

References

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