Heat-induced co-aggregation of rapeseed cruciferin with pea legumin and soy glycinin: Understanding protein interactions for clean-label applications

Jasper H. Seibt [1,2], Anna V. Klinnert [2], Christoph S. Hundschell [1,2], Anja M. Wagemans [1,2]

[1] Chair of Food Engineering, Institute of Natural Material Technology, Technische Universität Dresden , Bergstr. 120, 01069 Dresden, Germany

[2] Department of Food Biosciences, Institute of Food Technology and Food Chemistry, Technische Universität Berlin , Straße des 17. Juni 135, 10623 Berlin, Germany

jasper.seibt@tu-dresden.de

Plant protein aggregates are key structural elements of novel food gels formed via heat- or acid-induced protein gelation. However, protein gels based on pea or soy aggregates often lack sufficient gel stiffness, commonly addressed by additives [1]. Clean-label strategies therefore focus on protein combinations to achieve tailored functional properties while increasing consumer acceptance. Rapeseed proteins are a promising candidate for protein combination due to their high aggregation and gelation potential and wide availability as oilseed by-products [2]. However, their co-aggregation behaviour with pea or soy proteins is not yet fully understood. Since these proteins contain structurally distinct protein fractions, their individual roles must be examined to understand the mechanisms of co-aggregate formation. Therefore, this study aimed to elucidate the relationships between denaturation and aggregation of isolated rapeseed cruciferin in combination with pea legumin and soy glycinin during heat-induced co-aggregation.

For this purpose, native cruciferin, glycinin, and legumin fractions were isolated from low-processed protein sources. The denaturation characteristics of the individual protein fractions and their mixtures were assessed by micro-DSC measurements. Heat-induced aggregation and the molecular interactions driving aggregate formation were examined by viscometric temperature sweeps from 25 to 90 °C at pH 7. The composition of insoluble and soluble co-aggregates and the specific protein subunits involved were further analysed by SDS-PAGE.

During heat treatment, cruciferin formed predominantly insoluble aggregates, revealing a strong tendency for intermolecular bonding and self-association. When combined with legumin, aggregate solubility increased by 60% compared to cruciferin alone. SDS-PAGE analysis showed an even distribution of cruciferin subunits between soluble and insoluble aggregates, suggesting that legumin terminates cruciferin's self-association tendency and promotes the formation of soluble co-aggregates. Mixing cruciferin and glycinin resulted in the formation of cruciferin-glycinin complexes likely due to attractive electrostatic interactions. The associated conformational rearrangement lowered the denaturation temperature of the complexes from about 98 °C to 91 °C, compared to glycinin alone. This reduced thermal stability led to more extensive protein unfolding during heat treatment up to 90 °C, exposing additional interaction sites that accelerated co-aggregation, as reflected by a steeper increase in relative viscosity with increasing temperature. SDS-PAGE analysis further indicated additional electrostatic interactions between the glycinin basic subunit with cruciferin subunits. Consequently, a complex co-aggregation mechanism between cruciferin and glycinin is proposed, primarily driven by hydrophobic and specific electrostatic interactions.

Overall, these findings highlight cruciferin's potential as a clean-label modulator of plant protein aggregation. The mechanistic insights gained from this study can be employed to modulate the properties of cruciferin-legumin/glycinin aggregates, enabling the formation of clean label plant protein gels with improved functionality and consumer appeal.

Keywords:
plant proteins, soy, pea, rapeseed, protein aggregation, co-aggregation, denaturation, fractionation, protein interactions, clean-label

References:

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