Heat-induced aggregation of faba bean proteins: influence of heating temperature and protein concentration on induced molecular interactions

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Faba bean (*Vicia faba* L.) proteins are promising sustainable alternatives to animal proteins in food applications. During a typical wet isolation process to generate faba bean protein ingredients, a heat treatment is applied to *i.a.* reduce the microbial load. However, the impact of such heating step on the protein dispersibility remains poorly understood, particularly in terms of the underlying aggregation mechanisms that are induced during isolation. A key, yet often overlooked, factor in this regard is the protein concentration during heating, which in lab scale processes is often low, but much higher (>10%) in industrial processes. How protein concentration modulates the balance between different molecular interactions governing heat-induced aggregation has not yet been systematically investigated. Therefore, this study aimed to gain mechanistic insight into the nature of heat-induced aggregation of faba bean proteins by systematically investigating the influence of heating temperature (60, 80, 100 °C for 15 min) and protein concentration (2% and 12% w_p /v) on heat-induced aggregation of faba bean proteins during their isolation via aqueous extraction (pH 7.0) and isoelectric precipitation (pH 5.0). The heat treatment was performed on the protein pellet following isoelectric precipitation, after redispersion in water and pH adjustment to 7.0. After subsequent freeze drying, the characteristics of the different obtained protein isolates were further studied.

At 2% w_p /v, heating did not reduce protein dispersibility of the isolate, but dispersible aggregates were formed. In contrast, at 12% w_p /v, protein dispersibility of the isolate decreased strongly with increasing temperature (from 76.7 ± 0.1% for unheated samples to 26.9 ± 10.7% after heating at 100 °C). Confocal microscopy showed that protein aggregates were larger after heat treatment at 12% w_p /v than at 2% w_p /v. Size-exclusion HPLC after extraction in media containing different bond-breaking chemicals confirmed that aggregation via disulfide bonds always occurred, regardless of the heating temperature and concentration. Surface hydrophobicity and free SH-group measurements showed that, at high protein concentration, hydrophobic interactions became more dominant, leading to the formation of large, non-dispersible protein aggregates. These findings illustrate that at higher protein concentrations, non-covalent aggregation is the main driver of the reduced protein dispersibility during heat treatment as part of faba bean protein isolation.

Taken together, the results show that while disulfide bonds always form during heating, contributing to the formation of dispersible aggregates, protein concentration governs the extent of non-covalent aggregation, and consequently the loss of dispersibility in faba bean protein isolates. These results provide strategies for minimizing dispersibility losses during protein isolation, for instance by adjusting the protein concentration and/or by preventing the formation of non-covalent interactions.

Keywords:
Faba bean, protein isolation, heating, aggregation, molecular interactions