STUDIES ON HEAT-INDUCED INTERACTIONS AND GELATION OF WHEY PROTEIN

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BY

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TO ‘ESETA, my mother

“By wisdom the Lord laid the earth’s foundation, by understanding he set the heavens in place; by his knowledge the deeps were divided, and the clouds let drop the dew.”

Proverbs 3:19-20 (The Holy Bible)

ABSTRACT

The purpose of this study was to gain greater understanding of the interactions of whey proteins during heat-induced gelation of whey protein concentrate (WPC) solutions. Attention was focused on gaining better knowledge of the relationship between composition of WPC and its ability to form heat-induced gels, and to explore the mechanisms of protein aggregation and gelation in WPC solutions.

Interactions of whey proteins (β-lactalbumin, α-lactalbumin and BSA) were studied in three types of commercial WPC (rennet, cheese and acid) solutions, as well as in pure protein model systems, using one-dimensional (1D-) and two-dimensional (2D-) polyacrylamide gel electrophoresis (PAGE), size exclusion chromatography, light scattering and ultracentrifugation. The formation and structure of aggregates and gels were determined by oscillatory rheometry, confocal scanning laser microscopy (CSLM) and transmission electron microscopy (TEM) techniques.

Examination of heated (75 °C) rennet WPC solutions at a range of concentrations (10-120 g/kg, pH 6.8) revealed that the extent of protein aggregation and the formation of the intermediate molecular weight products were concentration-dependent. The rates of loss of β-lactoglobulin, α-lactalbumin and BSA during heating increased as the WPC concentration was increased from 10 to 120 g/kg. 2D-PAGE showed that some disulphide-linked β-lactoglobulin dimers were present in heated 10 g/kg solution, but very little was present in heated 120 g/kg solution. SDS was able to dissociate monomeric protein from high molecular weight aggregates in heated 120 g/kg WPC solution but not in 10 g/kg WPC solution. This suggested that in addition to disulphide-linked aggregates, hydrophobic aggregates involving β-lactoglobulin, α-lactalbumin and BSA were formed in heated WPC solutions at high protein concentrations.

Examination of the heated acid WPC and cheese WPC solutions (120 g/kg), using 1D-PAGE and size exclusion chromatography, revealed that the loss of β-lactoglobulin, α-lactalbumin and BSA from the cheese WPC solution was faster than the loss of the same proteins from the acid WPC solutions. It was also found that a considerable proportion of aggregates formed in heated cheese WPC solution was linked by hydrophobic association, whereas the aggregates formed in heated acid WPC solutions were linked predominantly by disulphide bonds. TEM and CLSM showed that the aggregates formed in cheese WPC solution were relatively large and "particulated," whereas the aggregates formed in acid WPC solution were small and "fine stranded." The gels formed from the heated cheese WPC solutions had low gel strength and high syneresis, whereas the gels obtained from the acid WPC had high gel strength and good water holding capacity.

Results of the dialysis experiments revealed that the differences between the properties of the acid WPC and the cheese WPC gels could be explained largely by their different mineral compositions. Relatively higher concentrations of divalent cations, Ca and Mg, in the cheese WPC was considered to be responsible for high rates of loss of native-like proteins, and the formation of large, hydrophobically-associated and "particulated" aggregates. High concentrations of monovalent cation in the acid WPC solutions probably resulted in slower loss of native-like proteins and formation of small and "fine-stranded" aggregates.

Attempt was made to characterise the nature of "insoluble" material in the unheated acid and cheese WPC solutions. Although, both the acid and the cheese WPC solutions contained considerable amounts of "insoluble" material, the amounts in the cheese WPC were greater. This material contained disproportionately higher levels of aggregated BSA and the minor whey proteins; in the cheese WPC it also contained considerable amounts of aggregated β-lactoglobulin and α-lactalbumin as well as phospholipids. The "insoluble" material in acid WPC, had higher casein content. The presence of this material did not appear to affect the gelation characteristics of the cheese WPCs, but had a positive effect on acid WPC gelation.

Studies on model systems of pure proteins showed that β-lactoglobulin, α-lactalbumin and BSA interacted to form homogeneous aggregates of each other as well as heterogeneous aggregates. 2D-PAGE clearly showed that when a mixture of these proteins was heated, initially BSA formed aggregates with itself and β-lactoglobulin and α-lactalbumin formed co-aggregates at a later stage of heating. Based on these results, the structure of WPC gel was suggested to be a heterogeneous network formed largely by co-polymers of β-lactoglobulin and α-lactalbumin embedded with "clusters" or "strands" of BSA aggregates.

Based on the results of this study, recommendations are made on how this information can be used in the development of new or improved whey products.

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