STUDIES ON HEAT- AND PRESSURE-INDUCED INTERACTIONS OF MILK PROTEINS

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ABSTRACT

The present study was aimed at understanding the high pressure (HP) processing-induced interactions of milk proteins in whey protein concentrate (WPC) solutions, in skim milk and in pure protein systems. The changes in milk proteins induced by heat treatments in the same systems under selected conditions were also evaluated.

The main approach taken was to elucidate changes in the whey proteins in heat- and pressure-treated samples from common aliquots, under identical conditions, using various one-dimensional (1D) and two-dimensional (2D) polyacrylamide gel electrophoresis (PAGE) techniques in the absence or presence of a disulphide bond reducing agent. In some instances, the samples were also analysed using small deformation rheology, size exclusion chromatography (SEC) and transmission electron microscopy (TEM).

The results of the present study indicated that, in general terms, heat treatment and HP treatment had common effects, i.e. denaturation and subsequent aggregation of whey proteins. Both heat treatment and HP treatment generated disulphide-bonded and hydrophobically bonded aggregates of whey proteins. However, the sensitivities of each of the whey proteins to heat treatment [immunoglobulin (Ig) > lactoferrin (LF) > bovine serum albumin (BSA) > β-lactoglobulin B (β-LG B) > β-LG A > α-lactalbumin (α-LA)] and pressure treatment (β-LG B > β-LG A > IgG > LF > BSA > α-LA) were considerably different. Also, HP treatment generated a comparatively greater proportion of smaller aggregates than did heat treatment.

The effects of protein concentration, intensity of pressure treatment, holding time and pressurising temperature on whey protein aggregation in WPC solutions were investigated. The rate of aggregation of whey proteins increased with an increase in the concentration of protein in the WPC solution and the pressurising temperature. The combination of low protein concentration, mild pressure treatment (200 MPa) and low pressurising temperature (20°C) led to minimal loss of native-like and SDS-monomeric β-LG, whereas the combination of high protein concentration, severe pressure treatment (600 MPa) and higher pressuring temperature (40°C and higher) led to significant loss of both native-like and SDS-monomeric β-LG. The sensitivity of pressure-resistant

whey proteins, such as α-LA and BSA, to the aggregation was significantly increased at pressurising temperatures of 40°C and higher. Self-supporting gels were formed when 8 or 12% (w/v) WPC solutions were pressure treated at 600–800 MPa, 20°C. Detailed analysis of the behaviour of the proteins during the formation of these gels revealed a novel pathway, suggesting that intermolecular disulphide bond formation occurred at high pressure but that hydrophobic association became important after the HP treatment.

In the later part of the study, heat- and HP-induced interactions of caseins and whey proteins were studied in a more complex system, i.e. skim milk. With the application of modified PAGE techniques, it was possible to show that the high molecular weight disulphide-bonded aggregates that were formed by HP treatment of skim milk contained disulphide-linked complexes consisting of α_{s2}-casein (α_{s2}-CN) as well as κ-CN, β-LG and other whey proteins. The results showed that the effects of heat treatment and HP on the interactions of the caseins and whey proteins in milk were significantly different. The accessibility of α_{s2}-CN and the formation of complexes involving α_{s2}-CN, κ-CN and whey proteins in the HP-treated milk, as demonstrated using the modified 2D PAGE technique, and as explained by possible proposed reactions of the caseins and whey proteins in pressure-treated milk, was an important finding of the present study.

Finally, a study on the effects of HP treatment in model systems using pure proteins in solution, both singly or in binary and ternary combinations, generated very useful information and clarified the role of each protein in pressure-induced aggregation and interactions of milk proteins in complex systems such as WPC and milk. It was found that the reactions of β-LG were not significantly affected by other proteins such as α-LA or BSA, but that the presence of β-LG in the system catalysed the reactions of other proteins such as α-LA or BSA.

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