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**DEPARTMENT OF FOOD TECHNOLOGY
MASSEY UNIVERSITY
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***AGGREGATION AND GELATION OF
BOVINE β -LACTOGLOBULIN, α -LACTALBUMIN AND
SERUM ALBUMIN***

**A THESIS
PRESENTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF TECHNOLOGY IN FOOD TECHNOLOGY
AT MASSEY UNIVERSITY**

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1995

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DEDICATION

**To my family, Luke, Rose, Joyce, Peter, Zvaitika,
Luke (Jnr) and Chido.**

ABSTRACT

Gelation is one of the most important functional properties of whey proteins in food systems. The properties of whey protein gels are affected by the chemical and physical properties of its protein components, β -lactoglobulin AB (β -Lg), α -lactalbumin (α -La) and bovine serum albumin (BSA).

Heat-induced aggregation and gelation of individual whey proteins, β -Lg, α -La and BSA and in mixture was studied by dynamic rheology and electrophoresis analysis. The proteins were dispersed in an ionic buffer containing 0.009 M CaCl_2 , 0.012 M NaCl, 0.012 M K_2HPO_4 and 0.007 M $\text{Na}_3\text{citrate}$ (pH 6.8) which was comparable to the ionic composition of 12% whey protein concentrate solution. Rheological properties of the protein solutions were measured using a Bohlin VOR rheometer after heating to 70, 75 and 80°C, holding at these temperatures for 60 min and after cooling to 25°C. Gel electrophoresis under non-dissociating (Native-PAGE in the absence of dissociating and reducing agents) and dissociating but non-reducing conditions (SDS-PAGE) was used to determine the extents of aggregation in some of the heated protein samples.

Gelation temperatures of 10%, w/v, protein solutions were found to be in the range 82.5 - 84°C for β -Lg and 68 - 70°C for BSA while α -La did not gel even at 90°C. Gelation temperatures of protein mixtures containing β -Lg and BSA were dependent on the relative proportion of the two proteins in the mixture. In contrast, the protein mixtures containing β -Lg and α -La gelled at temperatures (\sim 83°C) comparable to that of β -Lg alone.

Rheological measurements on pure β -Lg and BSA showed that BSA solutions formed self-supporting gels at lower protein concentrations and lower temperatures. Increasing the heating temperature or protein concentration of either β -Lg or BSA resulted in higher values of the storage

modulus (G').

It was apparent from the electrophoretic data that protein aggregates were formed as an intermediate prior to the formation of gel net-work. These aggregates appeared to be non-covalently linked initially and became increasingly disulphide-linked during heating.

Analysis of mixtures containing β -Lg and BSA during heat treatment showed that at both 70 and 75°C the gelation time decreased with the increasing proportion of BSA. Similarly, the values of G' after 60 min of heating were greater for the gels containing more BSA. G' values of these mixtures were dependent on the heating temperature and the relative proportion of the two proteins.

Gel electrophoresis data for a mixture of 5% β -Lg and 5% BSA heated at 70°C showed that prior to gelation most of the BSA had been transformed into aggregates while most of the β -Lg was essentially in the native form. Aggregates of both β -Lg and BSA were formed during heating at 75°C. At both temperatures, gelation commenced after most of the BSA had become covalently cross-linked but before all the β -Lg had become cross-linked. This effect was also apparent for other mixtures. Initially the aggregates appeared to be non-covalently linked and became increasingly disulphide linked with heating. From these results it is apparent that during heating at 70°C, BSA is the main protein forming the gel net-work and some β -Lg aggregates are probably attached to the net-work strand through either hydrophobic interactions or disulphide linkages. During heating at 75°C, two gel net-works are presumed to be formed independently, again with some interactions between the strands of the two net-works.

The rheological properties of protein mixtures containing β -Lg and α -La showed that β -Lg was the dominant gelling protein. G' values decreased with increasing relative proportion of α -La in the mixture at both 75 and

80°C. Gelling times increased with increasing proportion of α -La in the mixture at both 75 and 80°C.

No aggregate formation was observed during heating of α -La at 75 or 80°C. However, in the presence of β -Lg, α -La aggregated rapidly during heating. This aggregation appears to involve sulphydryl disulphide interchange reactions particularly when the mixtures were heated at 80°C. Almost all the proteins had aggregated through disulphide linkages before any significant increase in G' . It is suggested that during heating and prior to gelation co-polymers of both β -Lg and α -La were formed and this resulted in heterogeneous net-work strands being formed.

The results presented in this study suggest that slight differences in the protein composition of WPC are unlikely to affect the gelation properties of WPC. Further studies into the effects of immunoglobulins (Igs) are needed in order to gain further understanding of the contributions of these proteins to rheological properties of WPC gels.

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