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**HEAT-INDUCED WHEY PROTEIN
REACTIONS IN MILK**

*KINETICS OF DENATURATION AND AGGREGATION AS
RELATED TO MILK POWDER MANUFACTURE*



**A THESIS PRESENTED IN PARTIAL
FULFILMENT OF THE REQUIREMENTS
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ABSTRACT

The objective of this study was to gain a better understanding of the heat-induced whey protein reactions that occur during the manufacture of milk powders. Attention was focused on the preheating step, because most of the whey protein reactions that affect powder properties occur during this step.

Skim milk was heated at a range of temperatures (70 to 130°C) and times (5 s to 1800 s), normally used in powder manufacture, using a pilot-scale UHT plant equipped with direct steam injection. The temperature and time conditions were characterized by residence time distribution analysis. After heating the milk samples were analyzed by quantitative polyacrylamide gel electrophoresis under non-dissociating and dissociating conditions.

Using reaction progress data (reactant concentration versus time) determined over a range of temperatures, apparent reaction orders, reaction rates and Arrhenius parameters were determined by non-linear regression. This one-step approach gave Arrhenius parameters of considerably higher precision than the commonly used alternative of first determining the rate constants and then the Arrhenius parameters from the temperature dependence of those constants. Kinetic parameters were calculated for β -lactoglobulin A, β -lactoglobulin B, α -lactalbumin, immunoglobulin G and bovine serum albumin. Reaction orders for β -lactoglobulin varied from 1.0 to 1.6, while values for α -lactalbumin were in the range 0.9 to 1.1. The denaturation of Immunoglobulin G could be described by a 2nd order reaction, whereas bovine serum albumin followed a reaction order of 2.8. There was a marked change in activation energy for β -lactoglobulin at 90°C (51.18 to 301.73 kJ mol⁻¹) and α -lactalbumin at 80°C (52.87 to 203.26 kJ mol⁻¹). No such change was observed for Immunoglobulin G and bovine serum albumin over the temperature range 70-90°C. At temperatures <80°C the rates for β -lactoglobulin and α -lactalbumin denaturation were similar, but at higher temperatures α -lactalbumin denatured at a slower rate than β -lactoglobulin.

The aggregation of β -lactoglobulin mainly involved the formation of disulphide-linkages, whereas α -lactalbumin aggregates were formed through both hydrophobic interactions and disulphide-linkages. The kinetics of β -lactoglobulin aggregate formation followed an Arrhenius relationship similar to β -lactoglobulin denaturation, with comparable values of reaction orders, activation energies ^{and} reaction rates.

The rates of β -lactoglobulin and α -lactalbumin association with the casein micelles were slower than the corresponding rates for denaturation and aggregation. At temperatures $>80^{\circ}\text{C}$ β -lactoglobulin associated at a faster rate than α -lactalbumin, but $<80^{\circ}\text{C}$ the rates of association were similar. Under all heating conditions only a portion ($\approx 55\%$) of the denatured β -lactoglobulin and α -lactalbumin associated with the casein micelles; the rest remained in the serum as aggregates.

Based on the interrelationships between denaturation, aggregation and association behaviour of β -lactoglobulin and α -lactalbumin a novel mechanism was proposed and a mathematical model was developed. This model could accurately predict the formation of β -lactoglobulin aggregates and their subsequent association with the casein micelles.

The extent of whey protein denaturation, aggregation and association in milk was affected by compositional factors, such as pH, whey protein concentration and total solids content. Increasing the pH of milk from 6.48 to 6.83 prior to heating had little effect on β -lactoglobulin and α -lactalbumin denaturation and aggregation, but greatly decreased their association with the casein micelles. When the whey protein concentration in milk was increased from 0.52 to 1.24 g/100 g there was a marked increase in the extent of denaturation, aggregation and association of α -lactalbumin with the casein micelles, the effect being less marked on β -lactoglobulin. As the total solids content increased from 6% to 13% the extent of β -lactoglobulin and α -lactalbumin reactions increased. Examination of whey protein reactions in milks obtained during the New Zealand dairying season, showed that the extent of denaturation, aggregation and association was greater in late season milk. This increase was possibly caused by the increased whey protein and κ -casein concentrations.

Preliminary studies were carried out on the evaporation and spray drying processing steps. Little further denaturation and aggregation of whey proteins occurred during the evaporation and spray drying steps, while the association of whey proteins with the casein micelles increased slightly during evaporation. However both these processing steps caused considerable changes in soluble minerals and calcium ion activities.

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