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**A comprehensive study on the relative
importance of disulphide and non-covalent
interactions between proteins on the heat-
induced aggregation and functional
property of acid milk gels**

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Abstract

Understanding the interactions between the milk proteins during heat treatment of milk can be employed to manipulate the functional properties of dairy products. The ability to control the functional properties can be beneficial to the dairy industry. When being heated, milk proteins interact via two main types of bonding: disulphide bonds and non-covalent interactions. They are both considered to be important in the properties of heated milks and the resulting milk products. This research aimed to investigate the relative importance of each interaction type on the heat-induced aggregation between the proteins in milk and the functional properties of a milk product in a model food system.

Experiments involved adding low concentrations of a disulphide-bond reducing agent or a thiol blocking reagent to milk systems to either enhance or inhibit the thiol-disulphide exchange reactions between the proteins. The reagent was added to unheated milks, heated milks and unheated milks followed by heating. The effect of modifying the extent of thiol-disulphide exchange reactions between the proteins on the level of proteins participating in intermolecular disulphide bonds, on the degree of interactions between the casein micelles/casein proteins and the whey proteins were investigated. The treated milks were acidified to form acid milk gels of which the rheological properties and the microstructure were examined.

Results demonstrated that the proportion of proteins participating in intermolecular disulphide bonds can be controlled by systematically modifying the thiol-disulphide exchange reactions between the milk proteins. It was shown that the initial interactions between the proteins in milk upon heating were non-covalent and disulphide bonds were subsequently formed to strengthen the bonding between the proteins in the heat-induced aggregates. When the milks were made to acid gels, both types of protein interactions in the milk were equally important in influencing the storage modulus (G') values of the resulting gels with the higher the degree of connections, the higher the G' values. On the other hand, disulphide bonds played a more important role than non-covalent interactions in determining the yield properties of the acid gels. The yield stress values can be increased by increasing the proportion of disulphide bonds in the milk system before acidification or by enhancing the formation of disulphide bonds between the particles during the formation of acid gels.

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List of Symbols

G'	Storage modulus
G''	Loss modulus
pI	Isoelectric point
pKa	The negative base-10 logarithm of the acid dissociation constant (K_a) of a solution. The lower the pKa values, the stronger the acid.
tan δ	Loss tangent, ratio of G''/G'

List of Abbreviations

APS	Ammonium persulphate
CCP	Colloidal calcium phosphate
Cys	Cysteine residue
DSC	Differential scanning calorimetry
DTT	Dithiothreitol
GDL	Glucono- δ -lactone
MF	Micro-fluidic
NEM	N-ethylmaleimide
PAGE	Polyacrylamide gel electrophoresis
SDS	Sodium dodecyl sulphate
SH	Thiol group
TEM	Transmission electron microscopy
TEMED	Tetramethylethylenediamine
Tris-base	Tris (hydroxymethyl) methylamine
WPE	Whey protein enriched
WPF	Whey protein free
WPI	Whey protein isolate
UV	Ultraviolet