A Study of Factors Contributing to Gel Formation and to Syneresis of Gels with Particular Reference to Rennet Casein Systems.

A Thesis

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"EXHILARATION IS THAT FEELING YOU GET JUST AFTER A GREAT IDEA HITS YOU AND JUST BEFORE YOU REALISE WHAT'S WRONG WITH IT"

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ABSTRACT

An "artificial milk" system, prepared under controlled conditions, was developed. This was used for the study of the initial phase of rennet action on casein which results in gel formation. It was also used for a study of factors contributing to syneresis of rennet-casein gels.

"Artificial milks" containing calcium alone (60 - 125 mg% added calcium)* were shown to contain micelles very similar in gross structure and physical organisation to those in normal skim milk. It was demonstrated by electron microscopy that the presence of phosphate ion in the "artificial milks ", whether as Na_2HPO_4 or as H_3PO_4, sharply reduced the size of casein micelles which were then quite unlike those in skim milk.

Rennet action at 0^oC on both sodium caseinate and calcium caseinate "milks" was found to significantly reduce the viscosity and hydration constant 'K' of the caseinate systems. An ultracentrifugal technique using haemoglobin as an indicator, showed that rennet action on casein micelles in calcium caseinate "milks" released, at or about gel point, a quantity of "immobilised" water which amounted to about 70% of the weight of the "wet" protein.

Electron Micrographs of calcium-caseinate "milks", calcium caseinate - calcium phosphate "milks" and skim milk indicated that gel formation was essentially a micelle-surface phenomenon. No indication was found for the distortion of the micelles due to rennet action, nor did rennet action appear to reduce the size of the micelles.

Whole casein was found to contain both cystine and cysteine. However, sulphydryl - disulphide or disulphide exchange reactions did not appear to play a significant role in gel formation or in the syneresis

* added as $CaCl_2 \cdot 2H_2O$

of rennet-casein systems. The studies indicated that the presence of para-kappa casein in its entirety, probably on the micelle surface, was necessary for the development of a 3-dimensional gel network. Modification of para-kappa casein through scission of the disulphide bond was found to give a precipitate rather than a gel network after rennet action.

Calcium was shown to be important for both gel formation and syneresis. In its absence, neither phenomena occurred in rennet-casein systems. Despite this observed fact, evidence has been presented to show that syneresis was not a continuation of the gelation phenomena. Factors which greatly affected syneresis were found not to affect gel formation. Both rennet modification of casein and the presence of calcium was found to be necessary for syneresis to occur. In the absence of either, no syneresis was observed even when the pH was lowered below the isoelectric point.

The level of calcium in the "milks" was found to greatly influence both the rate and the extent of syneresis. A sharp drop in syneresis was observed in rennet-casein systems containing between 125 mg% and 175 mg% added calcium. It was postulated that the addition of such a level of calcium neutralised by interaction, the available negative charges on the caseinate and that this caused the gel network to acquire a net positive charge, thus giving rise to electrostatic forces promoting swelling and opposing syneresis.

Dinitrophenylation studies and studies on heated "artificial milk" systems containing reducing sugars showed that β casein played a major role in the interactions causing syneresis. α_s and kappa casein did not appear to play a significant role in this phenomenon. The studies emphasised the important and possibly direct role played by the free

amino groups of β-casein, probably the epsilon amino groups of lysine, in syneresis. The attractive forces causing shrinkage of the system after gel formation has occurred initially appear to be primarily electrostatic in character.

It appears possible that free amino groups are exposed by rennet action on kappa casein and these are involved with calcium in the gelation phenomenon.

It is postulated that both gelation and subsequently syneresis result from the formation of co-ordinate covalent linkages between calcium and the separately involved free amino groups of the kappa casein for gelation and β casein for syneresis.

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