PREVENTION OF PLASMIN-INDUCED HYDROLYSIS OF CASEINS

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Abstract

Bovine plasmin is a proteolytic enzyme that is naturally present in milk. Plasmin can have a detrimental impact on product quality including proteolysis, age-gelation and bitterness. The activity of plasmin is difficult to control as its precursor, plasminogen, and its activators can survive severe heat treatments such as ultra-high-temperature processing.

The aim of this work was to understand and control the plasmin-induced hydrolysis of caseins in milk systems. A sequential approach was used. In the first stage, the effect of substrate modification on plasmin-induced hydrolysis in a pure β-casein model system was studied; this allowed us to propose a control mechanism to limit the availability of the substrate by protein modification. In the second stage, different protein modifications were applied to a real milk system. In the analysis of this system, the casein micelle structure, whey protein denaturation and whey protein association with the casein micelle were considered. The final stage investigated plasmin-induced dissociation of casein micelles in real milk systems to understand the effect of plasmin activity on gelation and sedimentation in heat-treated milks.

Modification of lysine residues on the protein decreased plasmin-induced hydrolysis. Lactosylation had a greater effect than succinylation and transglutamination at the same level of lysine modification. A mechanism for this phenomenon was proposed. Lactosylation involves the attachment of lactose and, in advanced stages, cross-linking, thus modifying lysine and making it unrecognisable to plasmin; in addition, the cross-linking may affect the release of plasmin-generated peptides. Transglutamination also modifies lysine by cross-linking and has a similar effect to lactosylation, but to a lesser extent. In contrast, succinylation modifies the charge associated with lysine, making it unrecognisable to plasmin. Collectively, this knowledge can be used to make protein resistant to plasmin activity.

The combined effect of micellar structure and protein modification on plasmin activity was also studied. Calcium chelation and dissociation of the casein micelle increased plasmin activity because of reduced steric hindrance, which made the protein more readily available to plasmin. In contrast, succinylation decreased plasmin activity, which could be attributed to the formation of succinyl-lysine rendering β-casein unrecognisable to the substrate-binding pocket of plasmin, resulting in a decrease in hydrolysis with an increase in modification. These results indicated the importance of the casein micelle structure as a tool for controlling the activity of plasmin on milk proteins in food systems.

The effect of high heat treatment on plasmin-induced hydrolysis was also investigated. A high-heat-treated skim milk (120°C/15 min) was found to have greater resistance to plasmin activity than non-heated skim milk. Both whey protein association with the casein micelles and lactosylation decreased the availability of protein to plasmin. Whey-protein-free milk was the most plasmin resistant, followed by skim milk and lactose-free milk. Collectively, these results suggest that lactosylation plays a more significant role than whey protein association with the casein micelles in making protein resistant to plasmin activity.

The plasmin-induced dissociation of the casein micelle was explored by identifying peptide release from the micelle. Upon plasmin-induced hydrolysis of the casein micelle, hydrophilic peptides, i.e. proteose peptones, were the first to dissociate from the casein micelle, followed by hydrophobic peptides, which had dissociation patterns that were identical to those of κ-casein. This suggests that the release of κ-casein from the micelle is too slow to cause gelation. Extensive plasmin-induced hydrolysis of the casein micelle leads to sedimentation in heat-treated milk because of the formation of β-lactoglobulin–κ-casein complexes and their aggregation with hydrolysed hydrophobic peptides.

Overall, the results of the present study showed that casein modification can be useful in controlling plasmin activity and has developed our understanding of the plasmin-induced dissociation of casein micelles. Further research work is needed to understand the mechanism of plasmin's selective hydrolysis pattern and the

structural aspects of the substrate-binding pocket of plasmin. Studies on casein micelle dissociation separately and in conjunction with physicochemical changes during storage could be useful in further understanding the phenomenon of age gelation.

To my wife Namrata and son Sharav

For your love and support

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Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature: _____

Date: _____

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List of Conference Paper/Poster Presentations and Publications

Bhatt, H., Cucheval, A., Coker, C., Patel, H., Carr, A., & Bennett, R. (2012). *Mechanism of plasmin resistance through protein lactosylation.* Poster presented at the Gordon Research Conference: Proteases and their Inhibitors, 17–22 June 2012, Barga, Italy.

Bhatt, H., Cucheval, A., Coker, C., Patel, H., Carr, A., & Bennett, R. (2013a). *Effect of substrate modification on plasmin-induced hydrolysis: a comparative study.* Paper presented at the NIZO Conference, 2013, Papendal, Netherlands.

Bhatt, H., Cucheval, A., Coker, C., Patel, H., Carr, A., & Bennett, R. (2013b). *Effect of succinylation of skim milk on its plasmin-induced hydrolysis*. Paper #563 presented at the ADSA–ASAS Joint Annual Meeting, 08–12 July 2013, Indianapolis, IN, USA.

Bhatt, H., Cucheval, A., Coker, C., Patel, H., Carr, A., & Bennett, R. (2013c). *Effect of transglutamination on plasmin-induced hydrolysis*. Paper presented at the NZIFST Conference 2013, Hawkes Bay, New Zealand.

Bhatt, H., Cucheval, A., Coker, C., Patel, H., Carr, A., & Bennett, R. (2013d). *Effect of whey proteins on plasmin-induced hydrolysis in high heat-treated milk.* Paper presented at the NZIFST Conference 2013, Hawkes Bay, New Zealand.

Bhatt, H., Cucheval, A., Coker, C., Patel, H., Carr, A., & Bennett, R. (2013e). *The mechanism of resistance to plasmin activity through protein succinylation: a model study using* β*-casein*. Poster #T217 presented at the ADSA–ASAS Joint Annual Meeting, 08–12 July 2013, Indianapolis, IN, USA.

Bhatt, H., Cucheval, A., Coker, C., Patel, H., Carr, A., & Bennett, R. (2013f). *Plasmin resistance of high-heat-treated skim milk: a sequential study.* Poster presented at the 8th NIZO Conference, 2013, Papendal, Netherlands.

Bhatt, H., Cucheval, A., Coker, C., Patel, H., Carr, A., & Bennett, R. (2013g). *Plasmin resistance of the lactosylated protein.* Paper and poster presented at the IFT Conference 2013, Chicago, IL, USA. – **Won second prize for the best oral presentation.**

Bhatt, H., Cucheval, A., Coker, C., Patel, H., Carr, A., & Bennett, R. (2014a). *Cross-linking of milk proteins can reduce its susceptibility to plasmin-induced hydrolysis*. Poster presented at the ADSA–ASAS Joint Annual Meeting, 2014, Kansas City, MO, USA.

Bhatt, H., Cucheval, A., Coker, C., Patel, H., Carr, A., & Bennett, R. (2014b). Effect of lactosylation on plasmin-induced hydrolysis of β-casein. *International Dairy Journal, 38*(2), 213–218. doi: 10.1016/j.idairyj.2014.01.017.

Bhatt, H., Cucheval, A., Coker, C., Patel, H., Carr, A., & Bennett, R. (2014c). *Effect of micellar structure of casein and their modification on plasmin-induced hydrolysis.* Paper and poster presented at the IFT Conference 2014, New Orleans, LA, USA. – **Won second prize for the best oral presentation.**

Bhatt, H., Cucheval, A., Coker, C., Patel, H., Carr, A., & Bennett, R. (2014d). *Elucidating the role of plasmin in sedimentation and age gelation in a heat-treated milk system.* Poster presented at the IFT Conference 2014, New Orleans, LA, USA.

Bhatt, H., Cucheval, A., Coker, C., Patel, H., Carr, A., & Bennett, R. (2014e). *Interactions of whey protein with casein play a major role in preventing plasmin-induced hydrolysis in heated milk system*. Poster presented at the 7th International Whey Conference, 2014, Rotterdam, Netherlands.

Bhatt, H., Cucheval, A., Coker, C., Patel, H., Carr, A., & Bennett, R. (2014f). *Plasmin-induced dissociation of the casein micelle: effect of casein micelle*

modification on plasmin-induced hydrolysis of skim milk. Paper presented at the ADSA–ASAS Joint Annual Meeting, 2014, Kansas City, MO, USA.