EFFECT OF TRANSGLUTAMINASE ON MILK PROTEINS

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ABSTRACT

In this study transglutaminase was used to achieve ε-γ-(glutamyl) lysine cross-linking of milk proteins, in Trim™ and Full Fat milks, the same milks with a variety of added protein concentrates, and finally in yogurt and Petite Suisse acid gel systems. The effects of a preheat treatment, enzyme incubation temperature, enzyme inactivation after the enzyme incubation period and homogenization on the cross-linking of the three major casein and two whey proteins were also studied. The degree of cross-linking was established by the use of SDS PAGE gel electrophoresis.

The results indicated that cross-linking of the major casein and whey proteins was maximized if the milk was preheated for 10 minutes at 90°C and then cooled before addition of the transglutaminase. However, the preheat treatment was not always advantageous in Trim™ milk systems, but was essential for Full Fat milk systems. Maximal cross-linking of milk proteins occurred if the enzyme/milk system was incubated at 37°C for two hours rather than at 55°C for the same period. The extent of cross-linking increased in an almost linear fashion with increasing transglutainase concentration in most milk systems, with maximal cross-linking occurring when the enzyme concentration was 100 U/mL. Studies on one milk system showed that whey loss and gel strength deteriorated if more than 100 U/mL of enzyme was used.

The study demonstrated that homogenization was an essential step for protein cross-linking if the system contained any fat. Casein and whey protein transglutaminase mediated cross-linking was maximized in Full Fat milk systems if the milk was homogenized before transglutaminase was added. Maximal cross-linking, particularly of whey proteins, occurred in Full Fat milk systems if the milk was preheated for 10 minutes at 90°C, cooled to 60°C and then homogenized at 50/150, cooled further to 37°C and then incubated with 100 U/mL of enzyme for two hours.

Addition of sodium caseinate or milk protein isolate to Trim™ and Full Fat milk systems was shown to significantly improve protein cross-linkage by up to 50% for β-casein and whey protein respectively. Transglutaminase addition to milk systems containing the previously mentioned protein concentrates further enhanced cross-linking compared to the non-enzyme controls, particularly when the enzyme concentration was 100 U/mL

Addition of transglutaminase to acid milk gels dramatically improved the whey holding and gel properties of the products, particularly when the enzyme concentration was 100 U/mL. The reduction in whey loss was proportional to transglutaminase concentration up to 100 U/mL. A 100% reduction in whey syneresis and a 10g F improvement in gel strength improvement were obtained when 0.5 % sodium caseinate and 100 U/mL of transglutaminase were added to a gel milk system compared to a control sample with no enzyme. The physical properties of the milk acid gels were further improved if the transglutaminase in the acid gel systems was not inactivated prior to the addition of the enzyme.

The addition of milk protein concentrates such as sodium caseinate and total milk proteinate were shown to have dramatic effects on the whey holding and gel properties of acid gels. Moreover, the properties showed little reduction over a two week storage period compared to yogurt with no added protein. The addition of transglutaminase at a concentration of 100 U/mL further enhanced the above physical characteristics of the acid milk gels. Variations in cross-linking within systems containing either sodium caseinate, milk protein concentrate and milk protein isolate were observed. These variations need to be examined in further work. The addition of NaCNTMP further enhanced the gel and whey-holding properties compared to systems containing either sodium caseinate or total milk proteinate.
The final study was conducted on Petite Suisse, a high fat acid milk gel, and here the addition of transglutaminase at 100 U/mL dramatically improved the gel strength of the system by 500% compared to the control.

Finally, this research confirmed that transglutaminase effectively cross-linked milk proteins, and in particular β- and κ-casein and β-lactoglobulin.

Transglutaminase addition to milk and acid milk systems clearly improved some of the physical properties of the systems. However, much work is needed before it could be recommended for use by industry. The effect of adding transglutaminase to acid milk gels and milk systems should be evaluated by consumer panels to ensure that the sensory properties of these systems have in no way been compromised. Furthermore the economic costs of adding transglutaminase should be determined to ensure that the process would not

be uneconomic. If the above evaluations prove to be beneficial then the process could be investigated and further studies carried out to see whether improvements could result by addition of transglutaminase to such milk products as yogurts, desserts, cheese etc, and to create new products with different textural and water holding characteristics.

Further work is needed on a scientific front to assess the effects of transglutainase and added proteins on the structure of milk gels and the precise mechanism of filament formation in these gels. Some questions were also raised concerning the exact mechanism that was responsible for removal of monomeric forms of whey protein in the various milk systems evaluated in this study, and these should be determined by further research work.

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1.0 INTRODUCTION

Proteins are important components of food for human nutrition and are widely used as functional ingredients for improving the texture or viscoelastic properties of foods (Sakamoto et al., 1994).

The functional properties, e.g. viscosity, water-holding capacity, gelation, mouthfeel, and emulsifying and foaming properties, are closely related to a protein's molecular structure and interaction; it is therefore of great importance to increase our knowledge of the relationship between protein structure and functionality (Faergemand, 1998).

Protein gelation plays a major role in the preparation and acceptability of dairy products (Boye et al., 1995). Gels made from milk protein are traditionally formed by thermal denaturation of the whey proteins or by treating caseins with acid or a proteolytic enzyme (chymosin). In milk protein gels, the resulting network structure is typically held together by non-molecular physical cross-links, electrostatic interactions, hydrogen bonding and hydrophobic bonds (Dickinson and Yamamoto, 1996).

An alternative way of making a milk protein gel could be by enzymatically cross-linking the protein molecules to produce a network of covalent linkages. This new protein network might have different rheological properties from a conventional milk protein gel (Dickinson and Yamamoto, 1996).

The enzyme transglutaminase can catalyze an acyl transfer reaction between γ-carboxymide groups of glutamine residues and ε-amino groups of the lysine residues of peptide chains, giving as a result a covalent ε-(γ-glutamyl) lysine bond between protein molecules. Thus transglutaminase could have great potential for improving the physical properties of many foods without affecting the sensory properties of the product such as flavor and odor.

The aim of our study was to investigate the interaction between transglutaminase, at different concentrations and thermal conditions, and milk proteins and protein concentrates.

Once the optimal conditions were defined, transglutaminase was applied to yogurt manufacture with the objective of improving the texture and reducing syneresis.