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Enhancement of transglutaminase-induced protein cross-linking by preheat treatment of cows' milk: A statistical approach

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Abstract

Optimization of heat treated milk towards protein cross-linking induced by transglutaminase was carried out. Capillary electrophoresis was employed to study the extent of cross-linking under different preheating temperatures (70–90 °C) and times (15–60 min). The experiments were arranged according to a central composite statistical design (3^2 + centre points). Response surface methodology was used to assess factor interactions and empirical models regarding relative peak area (%) of individual protein (α_{s2}-casein, α_{s1}-casein, α_{s0}-casein, κ-casein, β-casein A^1, β-casein A^2, α-lactalbumin and β-lactoglobulin) and total α_s-caseins, total β-caseins and whey proteins (sum of α-lactalbumin and β-lactoglobulin). Multi-response optimization was also performed on the total α_s-caseins, total β-caseins, κ-casein and whey proteins data set of the factorial design. The desirability function was the statistical tool employed in this multi-optimization step. The optimum preheating conditions that maximized the cross-linking reactions catalyzed by transglutaminase were achieved within 60 min at 84.5 °C.

Keywords: Protein cross-linking; Transglutaminase; Heat treatment; Response surface methodology; Multi-response optimization

1. Introduction

The use of enzymes to modify the functional properties of foods is an area that has attracted considerable interest, since consumers perceive enzymes to be more 'natural' than chemicals (Singh, 1991). In the last few years, enzymatic cross-linking of proteins has gained increasing importance in food technology (Rasiah, Sutton, Low, Lin, & Gerrard, 2005; Gujral & Rosell, 2004a; Gerrard, 2002; Thalmann & Lotzbeyer, 2002; Motoki & Seguro, 1998; Dickinson, 1997) and, in particular, an enzyme that has recently received much attention for its ability to crosslink proteins is transglutaminase (Kolodziejska, Kaczorowski, Piotrowska, & Sadowska, 2004; Uresti, Téllez-Luis, Ramírez, & Vázquez, 2004; Gujral & Rosell, 2004b; Ramírez-Suárez & Xiong, 2003; Walsh, Cleary, McCarthy, Murphy, & FitzGerald, 2003; Schorsch, Carrie, Clark, & Norton, 2000).

Transglutaminase (protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) catalyzes an acyl transfer reaction between the γ-carboxyamide group of a peptide-bound glutamine residue (acyl donors) and a variety of primary amines (acyl acceptors), including the amino group of lysine (Yokoyama & Kikuchi, 2004). When the ε-amino group of a peptide-bound lysyl residue is the substrate, peptide chains are covalently connected through ε-(γ-glutamyl)lysine (G–L) bonds (Lauber, Noack, Klostermeyer, & Henle, 2001).

Transglutaminase has been used to catalyze the crosslinking of a number of proteins, such as soy proteins, wheat glutens, egg yolk, myosin and actomyosin (Zhu, Rinzema, Tramper, & Bol, 1995; Yokoyama & Kikuchi, 2004) and, among the milk proteins, both casein and whey α-lactalbumin and β-lactoglobulin are excellent acyl donor and/or acceptor substrates for transglutaminase (Cozzolino et al., 2003). Caseins

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especially appear to be readily cross-linking because of their flexible, random-coil structures and the absence of any disulphide bonds in the α_{s1}- and β-casein. Due to their compact globular structures, whey proteins tend to cross-link less efficiently (Sharma, Zakora, & Qvist, 2001b).

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In order to optimize the performance of transglutaminase in dairy products, the aim of this work includes statistical optimization of variables that have been reported to play a very significant role in enhancing the enzymatic cross-linking of milk proteins. The classical method of determining these optima is by varying one parameter while keeping the other at a specified constant level or carrying out experiments with every possible factorial combination of the test variables. These laborious and time-consuming approaches often do not guarantee determination of optimal conditions (Box, Hunter, & Hunter, 1978; Wernimont, 1985; Haaland, 1989). In this regard, the effects of the milk preheating temperature and time on the transglutaminase-induced cross-linking were evaluated using response surface methodology.

2. Materials and methods

2.1. Materials

Sodium hydroxide and phosphoric acid were of analytical grade. Hydroxylpropyl cellulose (HPMC), dithiothreitol (DTT) and urea were obtained from Sigma (St. Louis, MO, USA). All solutions were prepared with purified water (Milli-Q system, Millipore Corp, Bedford, MA, USA). Microbial Transglutaminase from *Stretoverticillium mobaraense* (Activa® TI) was a gift from Ajinomoto Co., Ltd. Japan (100 U g^{-1}). The enzyme preparation was used without any further purification. Standards of α_s-casein (C-6780), β-casein (C-6905), κ-casein (C-0406), α-lactalbumin (L5385) and β-lactoglobulin (L3908) from bovine milk were all supplied by Sigma (St. Louis, MO, USA).

2.2. Cross-linking conditions of total proteins in milk

Preliminary studies of the effect of treating milk with transglutaminase were carried out with raw cows' milk preheated at 85 °C for 15 min and untreated milk (Sharma, Zakora et al., 2001b). Before heating, the milk was skimmed by low-speed centrifugation (3000 *g* for 20 min) (Correding & Dalglesish, 1999). A mixture of milk and enzyme (final enzyme/substrate ratio of 0.2 or 0.02 (w/w)) was incubated at 40 °C for 30, 60, 90 and 180 min. The required quantity of transglutaminase was added directly to the milk.

The effect of the preheat treatment of milk on cross-linking behaviour was studied with milk heated at different temperature conditions (70–90 °C) for different lengths of time (15–60 min) before incubation with microbial transglutaminase at 40 °C, and at an enzyme to substrate ratio of 0.02% (w/w). Samples were heated in test tubes in a temperature controlled water bath, and after heat treatment the samples were rapidly cooled at 4 °C. The enzymatic reaction was terminated by heating the mixture at 80 °C for 2 min (Sharma, Zakora et al., 2001b). Control samples were prepared following the procedure described above, except that enzyme was omitted.

2.3. Capillary electrophoresis

Capillary electrophoresis was carried out using a Beckman P/ACE^{TM} system MDQ, equipped with a UV detector, a temperature-controlled capillary compartment and an autosampler. Separations were performed using a fused-silica capillary column eCapTM (Beckman Instruments, Fullerton, CA, USA) of 60 cm × 50 μm ID (50 cm to the detector window). Sample solutions were injected for 5 s at 0.5 psi. The separations were conducted at 20 kV and the separation temperature was kept constant at 30 °C. UV-detection was performed at 214 nm. The running buffer (50 mM) was prepared by mixing 14.7 M H_3PO_4 (847 μL) and 0.05% HPMC with 6 M of urea solution (250 mL). The pH was adjusted at 3.0 with 2 M NaOH. Sample buffer (pH 8) consisted of 10 mM H_3PO_4, 8 M urea, 10 mM DTT and 1 mM Lys-Try-Lys. Before each injection, the capillary was washed with 0.1 M NaOH (5 min), deionized water (5 min), and 1 M HCl (5 min) and equilibrated with the running buffer (5 min) (Rodriguez-Nogales, Revilla, & Vivar-Quintana, 2005).

Standard proteins were dissolved in a sample buffer at 10 mg mL^{-1} for α-casein and β-casein and at 5 mg mL^{-1} for κ-casein, α-lactalbumin and β-lactoglobulin. Sample solutions of milk treated with transglutaminase were prepared dissolving 150 μL of reaction mixture in 1 mL of sample buffer (Miralles et al., 2001). The samples were filtered through 0.45 μm filters (Millex-GV_{13}, Millipore, Molsheim, France) before analysis by capillary electrophoresis.

2.4. Factorial design and the desirability function

A central composite design with two variables (3^2 + centre points) was applied to find the optimum conditions and analyze how sensitive the responses were to variations in the settings of the experimental