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Changes in physicochemical properties and gelation behaviour of caseinomacropeptide isolate by treatment with transglutaminase

Meral Kilic-Akyilmaz*, Esra Kocaman, Zehra Gulsunoglu, Ceren Sagdic-Oztan, Solmaz Mohammadipour Mavazekhan

Istanbul Technical University, Department of Food Engineering, 34469 Istanbul, Turkey

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ABSTRACT

The gelation behaviour of caseinomacropeptide isolate (CMPI) treated with transglutaminase at levels of 1 and 25 U g^{-1} protein was investigated at different pH and temperatures. Cross-linking of CMPI protein fractions by transglutaminase was confirmed using tricine-sodium dodecylsulphate-polyacrylamide gel electrophoresis. Cross-linking reduced the isoelectric point and hydrophobicity of CMPI. The gelation temperature of CMPI at pH 3 was reduced from 54 to 42 °C; a gel point (G'>1 Pa) was not observed at pH 4.5 after enzyme treatment during temperature sweep measurements. Cross-linked CMPI formed a gel with lower stiffness and fracture stress at 90 °C at pH 3.0 or 4.5 compared with gels of untreated CMPI. However, stiffness and fracture stress of CMPI gels formed at 70 °C at pH 3.0 increased by three- and four-fold, respectively, by cross-linking with 25 U g^{-1} protein of enzyme. Transglutaminase affected gelation of CMPI by cross-linking of both CMP and residual whey proteins.

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1. Introduction

Caseinomacropeptide (CMP) is a polypeptide released into whey by rennet action during cheesemaking. The rennet enzyme hydrolyses the Phe_{105}–Met_{106} peptide linkage of the κ-casein, forming para-κ-casein and CMP (Mollé & Léonil, 1995). Para-κ-casein is processed into the cheese while the water-soluble CMP passes into the whey.

CMP is the third most abundant protein fraction of whey, after β-lactoglobulin (β-lg) and α-lactalbumin (α-la). It has two structural forms, including glycosylated gCMP with sugar residues and non-glycosylated aCMP. These two forms are present at the same ratio in native CMP from bovine milk (Thomä-Worringer, Sorensen, & Lopez-Fandino, 2006). aCMP exists in two forms, A and B, both with a molar mass of around 6.8 kDa, while the molar mass of gCMP was reported as 9–11 kDa depending on the sugar moiety and degree of glycosylation (Kreuß, Strixner, & Kulozik, 2009b; Minkiewicz, Slangen, Dziuba, Visser, & Mioduszewska, 2000; Mollé & Léonil, 1995).

CMP provides structural functionality including gel, emulsion and foam formation (Farías, Martinez, & Pilosof, 2010; Kreuß, Krause, & Kulozik, 2009a; Kreuß et al., 2009b; Martinez, Farías, & Pilosof, 2011; Martín-Diana, Gomez-Guillén, Montero, & Fontecha, 2006; Thomä-Worringer et al., 2006). This functionality is related to its amphiphilic structure comprising a hydrophilic sugar and a hydrophobic and charged peptide chain (Kreuß et al., 2009a,b). CMP is also a bioactive peptide with reported prebiotic, antibacterial, antiviral, immunomodulating, antithrombotic and antihypertensive activities, which makes it a valuable ingredient for food products (Abd El-Salam, El-Shibiny, & Buchheim, 1996; Neelima, Rajput, & Mann, 2013). The lack of aromatic amino acids also renders it a suitable ingredient for diets of patients with certain amino acid metabolism disorders.

CMP was reported to form a gel at room temperature at concentrations of 3–10% at 25 °C and pH below 4.5 (Farías et al., 2010). Martinez, Farías, and Pilosof (2010) showed that heating to 50 °C can shorten gelation time, with no change in gelation time above 55 °C. Gelation of CMP takes place in two stages (Farías et al., 2010; Martinez et al., 2010). In the first stage, CMP molecules form pH-irreversible dimers by hydrophobic interactions at pH below 6.5. Dimers aggregate at pH below 4.5 by electrostatic interactions forming a network that is reversible by pH. Electrostatic interactions occur depending on charges of different CMP forms that are determined by their pI: 3.15 for gCMP and 4.15 for aCMP (Kreuß et al., 2009b). Electrostatic interactions continue up to pH 2.2, which is the pK of sialic acid, but the gels are weaker compared with those formed at pH 3.5 (Martinez et al., 2010).

Transglutaminase is an enzyme that can alter protein structure by covalent cross-linking between glutamine and lysine residues in

* Corresponding author. Tel.: +90 212 2856016.
E-mail address: meral.kilic@itu.edu.tr (M. Kilic-Akyilmaz).

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proteins. The degree of crosslinking by transglutaminase depends on pH, temperature and time. It can form both intramolecular and intermolecular cross-links depending on enzyme and protein concentration (Eissa & Khan, 2005). Transglutaminase can influence gelation behaviour of proteins. It has been used for strengthening and stabilisation of structure of milk and meat products (Bönisch, Heidebach, & Kulozik, 2008; Eissa & Khan, 2005; Jaros, Partschefeld, Henle, & Rohm, 2006; Lauber, Henle, & Klostermeyer, 2000). Transglutaminase was applied to crosslink CMP for the purpose of its isolation from whey protein concentrate (Tolkach & Kulozik, 2005). CMP was found to be selectively and almost completely cross-linked by transglutaminase, which allowed its separation from other whey proteins (Tolkach & Kulozik, 2005).

The application of transglutaminase and its effect on the gelation of CMP have not been reported in the literature. Cross-linking of CMP could improve its gel properties since it is a smaller molecule compared with proteins. In this study, gelation and gel properties of transglutaminase-crosslinked CMP were investigated and compared with those of untreated CMP.

2. Materials and methods

2.1. Materials

A commercial caseinomacropeptide isolate (CMPI) (Lacprodan® CGMP-10) with 80% protein (80% of protein being CMP), 2% lactose, 0.5% fat and 6.5% minerals was supplied by Arla Food Ingredients (Viby J, Denmark). Native WPI (Prolacta® 95LL Instant) containing 89% protein, 0.2% lactose, 1.9% fat and 3.0% minerals was supplied from Lactalis Ingredients (Bourgbarré, France). Transglutaminase (Activa® MP; Ajinomoto Foods Europe, Mesnil-Saint-Nicaise, France) with an activity of 100 U g^{-1} was used. Deionised water was used for preparation of the solutions.

2.2. Cross-linking of CMPI with transglutaminase

An aqueous solution of CMPI was prepared at a concentration of 20% (w/v), which included approximately 12.8% CMP according to the manufacturer's specification. The solution was first stirred for 15 min using a magnetic stirrer, then placed for 15 min in an ultrasonic bath and stored at 4 °C overnight for hydration. The transglutaminase was added at activities of 1 and 25 U g^{-1} protein. The mixture was incubated with stirring at 40 °C for 1 h. After incubation, the samples were cooled and kept in ice-water bath until analyses. The level of enzyme, temperature and time were chosen according to the literature and preliminary experiments. Tolkach and Kulozik (2005) reported almost complete cross-linking of CMP in whey protein concentrate by transglutaminase at a level of 3 U g^{-1} protein at 40 °C for 1 h. The enzyme reaction was carried out in an aqueous solution of CMPI as phosphate buffer was found to be inhibitory to the enzyme.

Additional experiments with WPI, with and without transglutaminase, were carried out to elucidate the effect of whey proteins on gelation of CMPI. WPI solutions were prepared at a protein concentration of 3.2% (w/v) so as to have an amount of whey protein similar to that present in the CMPI solution. Transglutaminase was added at the same level on protein (25 U g^{-1} protein) and also solution basis (4 U mL^{-1} solution = 125 U g^{-1} protein) as used for the 20% CMP solution. WPI samples were prepared with and without transglutaminase following the same procedures as outlined above for the CMPI.

Polymerisation of CMPI and WPI with transglutaminase was confirmed using tricine-sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Solutions of CMPI and WPI were diluted to 0.5% (w/v) with deionised water and mixed with a reducing buffer comprising tricine sample buffer and 0.2 M DTT. Heat treatment at 95 °C was applied for 15 min before loading into the gel. Ready-made Tris-tricine gels (Mini-PROTEAN® Tris/Tricine Precast Gel, Bio-Rad, Hercules, CA, USA) were used in a mini electrophoresis unit (Mini-Protean® Tetra Cell, Bio-Rad), applying a voltage of 100 V. The gel was fixed with methanol: acetic acid: water (40:10:50) for 30 min, stained with 0.25% (w/v) Coomassie brilliant blue G-250 in fixing solution and destained in fixing solution.

The surface hydrophobicity of CMPI solutions was measured by fluorescent 8-anilino-1-naphthalenesulfonic acid (ANS) probe according to the method reported by Alizadeh-Pasdar and Li-Chan (2000) with some modifications. Samples were diluted to concentrations in the range of 0.01–0.05% (w/v). ANS solution was prepared at a concentration of 3 mM in phosphate buffer, pH 7. Ten millilitres of sample was mixed with 50 μL ANS solution and the mixture was held at room temperature in the dark for 15 min. A blank was prepared by using water instead of sample. The fluorescence intensities of samples with and without ANS were measured at 480 nm after excitation at 390 nm with a slit width of 5 nm (LS55 fluorescence spectrometer; Perkin Elmer, Waltham, MA, USA). The net fluorescence intensity of the sample was determined by subtracting fluorescence intensities of the sample without ANS and blank from that of the sample with ANS. Readings were standardised using 10 mL methanol and ANS for correction of instrumental variation. Surface hydrophobicity (S_0) of the sample was determined as the slope from a plot of net fluorescence of the sample versus CMPI concentration.

The zeta potential of CMPI solutions were measured after dilution to a concentration of 0.2% (w/v); the pH of the solutions was adjusted to values between 2.5 and 6.7 using 1 M HCl. The zeta potential of the samples were measured at 1 Hz frequency and 15 mA cm^{-1} intensity (Zetasizer Nano ZS, Malvern, Worcestershire, UK). pI of CMPI was determined from a polynomial equation fitted to data.

2.3. Determination of gelation properties of CMPI

The pH of CMPI solution at a concentration of 20% was 6.5. Its pH was reduced to 3.0 and 4.5 using 4 M HCl for studying the effect of pH on gelation properties of CMPI. The gelation properties of the samples were determined using a rheometer (Rheostress 1; Thermo Electron GmbH, Karlsruhe, Germany). Measurements were made using a cone-plate sensor with a diameter of 35 mm (C35/2° Ti, Thermo Electron GmbH) in the linear viscoelastic region. The open edges of the samples between cone and plate were covered with paraffin oil to prevent water loss. The gelation temperature of the samples was determined by temperature sweep at a shear strain of 0.01% at 0.1 Hz frequency in the temperature range of 25–90 °C at a heating rate of 4.3 °C min^{-1}. Gelation temperature was determined as the temperature at which the elastic modulus (G') became greater than 1 Pa.

In a separate set of experiments to determine the strength of CMPI gels, samples were gelled in a water bath at 70 or 90 °C for 30 min and then cooled to room temperature in ice-water bath. Samples were taken from gels and stress sweeps were carried out on them by applying shear stress between 0 and 1000 Pa at 0.1 Hz at 25 °C. Maximum elastic modulus value was used to express gel stiffness and shear stress value, at which elastic and viscous moduli crossed was taken as fracture stress. Gelation experiments were repeated with WPI solutions under the same conditions.

2.4. Statistical analysis

The experiments and measurements were repeated at least two times. One-way analysis of variance (ANOVA) was applied to evaluate effect of treatments on the measured variables. The differences

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