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Rheology, texture and microstructure of gelatin gels with and without milk proteins

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ABSTRACT

The effects of gelatin concentration, pH and addition of milk proteins on the physical and microstructural properties of type B gelatin gels were studied by small deformation rheology, texture analysis and scanning electron microscopy. Whey protein isolate (WPI), milk protein concentrate (MPC) and skim milk powder (SMP) were used as sources of milk proteins. The elasticity of gelatin gels was significantly affected by the concentration of gelatin. Higher gelatin concentrations led to a stronger gel, and higher gelling and melting temperatures. However, all the gelatin gels at concentrations from 1.0 to 5.0% melted below human body temperature. Rheological properties of gelatin gels were independent of pH in the range pH 4.6–8.0. At pH 3.0 gelation of gelatin was significantly inhibited. Addition of SMP and MPC significantly enhanced the rheological properties of gelatin gels, while addition of WPI had a negative effect on them. However, the effect of addition of milk proteins was dependent on the gelatin concentration. Textural results showed that addition of all milk powders increased the hardness of gelatin gels at high gelatin concentration (5.0%). The fracturability of the gels was greatly influenced by pH. Addition of milk proteins and high gelatin concentration (5.0%) both caused loss of gel fracturability. Microstructural results showed that gelatin concentration and pH had a marked influence on the gel structure, and the addition of MPC and SMP changed the structure of the gelatin gels; a structure similar to pure gelatin gel was observed after addition of WPI.

1. Introduction

Gelatin is an animal protein produced from collagen (Boran, Mulvaney, & Regenstein, 2010). It has high flexibility of the polypeptide chains and a non-random occurrence of imino acids (i.e., proline or hydroxyproline) in its sequence which is unusual among the gel-forming agents (Karim & Bhat, 2009). The intermolecular contacts in gelatin gels are hydrogen bonds, which make the gels thermally reversible. Specifically, a gelatin gel melts below human body temperature, which gives it the well-known "melt-in-mouth" property (Djabourov, 1988). These unique properties make gelatin an important and widely used biopolymer in the food industry. However the properties of gelatin gels are affected by factors such as pH and concentration. Gelation of a gelatin solution and subsequent changes in the gel network arise through the partial return of disordered gelatin molecules (coil) to the collagen-like structure (polyproline II helix) (Djabourov, Lechaire, & Gaill, 1993). It has been reported that at low gelatin concentration, three regions of the helix may be derived from one chain to give an intramolecular collagen-like structure which makes no contribution to the gel network. At higher gelatin concentrations, the three regions of the helix can come from two or three different chains, so that useful junction zones that induce gelation can be formed (Djabourov, 1988). Although gelatin provides stable gels over a very wide range of pH values, pH should still be considered in gelatin gelation. pH can greatly affect the viscosity of gelatin solutions, which is minimum at its isoionic point (IP) because of the maximum molecular folding at that pH (Petrie and Becker, 1970). It was reported that aggregation of gelatin type A (IP 9.0) increased and the gelatin gel turned from transparent to opaque as the pH was increased from 5.4 to 7.5 (Walkenstrom & Hermansson, 1997). Gelation of both gelatin type A and B is inhibited greatly and the gel strength is low outside the pH range 4.0–10. This is attributed to strong electrostatic forces that inhibit the ability of chains to form junction zones (Bello, Vinograd, & Bello, 1962).

Milk protein–gelatin mixtures are widely used in food products, as they play an essential role in the texture and stability of many

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food systems. Gelatin is also used widely for modifying the texture and shelf-life of dairy-based foams, gels, dispersions and emulsions (Hemar, Liu, Meunier, & Woonton, 2010; Koh, Merino, & Dickinson, 2002). The interaction between milk proteins and food hydrocolloids has been reviewed extensively (Dickinson, 1998; Lal, O'Connor, & Eyres, 2006; Syrbe, Bauer, & Klostermeyer, 1998). Hydrocolloids can be classified as non-ionic and anionic, which determines the behaviour of protein–hydrocolloid solutions (Syrbe et al., 1998). Gelatin is expected to interact with milk proteins at pH values where the two polymers carry opposite charges. Gelatin A (IP 9.0) interacts with the oppositely charged micellar casein at pH 6.7, while gelatin B (IP 5.0) does not (Lefebvre & Antonov, 2001). However, in the gelatin A–whey proteins system, no interaction has been observed at pH 4.6 or 5.4 (Walkenstrom & Hermansson, 1994). Therefore, the milk protein type plays an important role in mixed system. In most previous studies of milk protein–gelatin system, milk proteins have been denatured either by heating or acidification, which leads to gelation of the milk proteins (Fiszman & Salvador, 1999; Koh et al., 2002; Walkenstrom & Hermansson, 1996).

The aim of this study was to investigate the effects of pH, concentration and addition of milk proteins on the gelling behaviours of type B gelatin. Small deformation rheology, texture analysis and microscopy were used to investigate the properties of the gels. In this study, gelatin was the only gelling agent in the systems used to investigate the effect of milk proteins on gelation properties of gelatin.

2. Materials and methods

2.1. Materials

The gelatin used in this study was supplied by Gelita (Beaudesert, Australia). It was a light coloured edible bovine skin (type B) gelatin powder with bloom 200 and mesh 20, which is a commercial product commonly used in the food industry. The milk powders, whey protein isolate (WPI), milk protein concentrate (MPC) and skim milk powder (SMP) were obtained from Murray Goulburn Co-Operative Ltd (Melbourne, Australia). The protein contents of WPI, MPC and SMP were 90.2, 85.0 and 33.3%, respectively (information provided by supplier).

2.2. Methods

2.2.1. Sample preparation

Solutions with three concentrations (1.0, 2.5 and 5.0%, w/v) of gelatin were prepared by allowing the gelatin to swell in distilled water overnight (about 15 h) followed by heating at 45 °C for 30 min to dissolve it. Then 1 M NaOH or HCl was used to adjust the pH to 3.0, 4.6, 5.3, 6.6 or 8.0. In mixed gels, the milk protein concentration used was 4.5% (w/w), which was obtained by adding the appropriate amounts of WPI, SMP or MPC. Milk powders and gelatin were dissolved together in distilled water overnight followed by heating at 45 °C for 30 min. The pH was then adjusted to 6.6 and 8.0 for gels containing MPC and SMP, since MPC and SMP easily form aggregates at pH < 5.3, and to 3.0 to 8.0 for WPI-containing gels.

2.2.2. Small deformation rheological measurement

Dynamic oscillatory measurements were performed on a stress-controlled rheometer (Model AR-G2, TA Instruments, Elstree, UK). Test samples were poured at about 45 °C onto the bottom plate of the rheometer, and a cone (4 cm, diameter; 2° angle) and plate geometry was used. A strain sweep test revealed that 0.5% strain at 1 Hz frequency was within the linear viscoelastic region (LVR) for the samples. The measurements were carried out in a three-stage process (Salvador & Fiszman, 1998):

a. Cooling: equilibration at 40 °C and a temperature sweep to 10 °C at a cooling rate of 1 °C/min to promote gelatin gel formation.
b. Annealing: a time sweep at 10 °C for 2.5 h to observe the maturation of the gelling samples.
c. Heating: a temperature sweep from 10 to 40 °C at a heating rate of 1 °C/min to observe melting of gelatin gels.

The gelling (T_G) and melting (T_M) temperatures were calculated when there were appreciable increases and decreases, respectively, in complex viscosity (η^*), and two values were obtained for each temperature to calculate the average gelling and melting temperatures. The complex viscosity, η^* was defined as in Eq. (1):

$$\eta^* = \sqrt{G'^2 + G''^2/\omega} \tag{1}$$

where, G' = storage modulus, G'' = loss modulus and ω = frequency. Following the procedures in Sopade, Halley, and Junming (2004):

i. The cross-over temperature was obtained when G'' equals G' or loss tangent, which is the ratio of G'' to G', equals to 1.
ii. Temperature of maximum or minimum change in complex viscosity per unit change in temperature. This was defined as the point of inflection. It was obtained by differentiating the complex viscosity with respect to temperature (first derivative, $d\eta^*/dT$) and finding the temperature when the derivative was zero (=0).

All rheological measurements were performed in duplicate and the samples were randomised for the analysis.

2.2.3. Texture analysis

Texture measurements were performed using a TA–XT2 Texture Analyser (Godalming, Surrey, UK). Samples were transferred to an incubator at 10 °C after the pH was adjusted, and kept for 2.5 h before measurement. All measurements were carried out at 10 °C in triplicate. The probe used was cylindrical with a flat base of 12.7 mm diameter, operating at a speed of 1 mm/s. The sample height was 30 mm in a cylindrical container of about 40 mm. The probe penetrated the gel during a total displacement of 10 mm. Two parameters were obtained from the force–time curves: (a) fracturability (N/mm), defined as the force at the first significant break in the curve; (b) firmness (N/mm), defined as the initial slope of the penetration curve within the first 2 s (Fiszman & Salvador, 1999).

2.2.4. Microstructure

Gels were formed in the same way as for texture analysis. Gels were cut into small pieces ($\sim$1 mm^3) and fixed with 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 6.8), dehydrated in ethanol with a serial concentration of 50, 70, 90 and 100% (v/v) and dried with a CO_2 critical point dryer (Tousimis Automatic, Rockville, USA) prior to mounting on aluminium stubs and sputter-coated with a Baltek platinum coater. The microstructure of the gels was examined using a scanning electron microscope (JEOL 6610, Tokyo, Japan) at an acceleration voltage of 10 kV.

2.2.5. Statistical analysis

Minitab ver. 16 software (Minitab Inc., USA) was used for analysis of variance (ANOVA), test of significance ($p < 0.05$).

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