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

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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. Micro-structural 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.

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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 isoinionic 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
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 a mixed system. In most previous studies of milk protein gelation, 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 gelation 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 into 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 (Tc) and melting (Tm) 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):

\[ η^* = \sqrt{G'^2 + G''^2/ω} \]  

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 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\( η^*/dT \)) 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 (~1 mm³) 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 CO2 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 (\( p < 0.05 \)).
3. Results and discussion

3.1. Rheology results

Fig. 1 shows representative rheological results for a 2.5% gelatin gel at pH 6.6 through the three stages (cooling, annealing and heating). G' increased rapidly and surpassed G'' during the cooling stage, which indicated that the system gelled (Fig. 1A). When the temperature was held at 10°C for 2.5 h during annealing, both G' and G'' showed an increase (Fig. 1B), with G' increasing more than G''. After around 40 min, the slope of the curve decreased and gelation became slow and stable, but gelation was not complete in the 2.5 h. Similar observations were made by Joly-Duhamel, Hellio, Ajdari, and Djabourov (2002). From optical rotation measurements, these authors found that the helix concentration of gelatin increased with the annealing time and did not reach 100% within the “normal time scale” of observation. During the heating stage, G' decreased quickly and became lower than G'' (Fig. 1C), which indicated melting of the gel. Similar trends for G' and G'' were observed for samples at different concentrations and pH, and on addition of the milk proteins. However, samples with 1 and 2.5% gelatin at pH 3.0 did not gel during the cooling stage.

3.1.1. Gelling temperature

As described above, the gelling temperatures (T_G) of the samples were measured in the cooling stage, and Table 1 summarises the ANOVA output on the dependence of the gelling temperature on type of proteins, concentration and pH. Only the main effects (concentration and protein) were significant, and the trends are shown in Fig. 2A and B.

At any pH, the higher the concentration of gelatin, the higher the T_G of the gels (Fig. 2A). The 5.0% gelatin solution with or without milk proteins gelled at temperatures in the range 20–22°C and the 2.5% solution in the range 15–18°C (except at pH 3.0, explained in the next paragraph); samples with 1.0% gelatin did not gel during the cooling stage. Similar results were reported by Michon, Cuvelier, and Launay (1993). In their study, T_G of the gelatin gel ranged from 26.4°C at 1.1% to 32.6°C at 20% concentration. Joly-Duhamel et al. (2002) also reported that T_G increased with concentration for both mammalian and fish gelatin. The gelling temperatures of gelatin gels can also be different at the same concentration because of different thermal histories. The gelling point in a polymerizing system is considered to be the point at which a three-dimensional network, infinite in extent, first appears (Eldridge & Ferry, 1954). For a gelatin gel, a threshold level of proline II helixes is needed to form the three-dimensional network, which could be achieved within a relatively short time and high temperature at high gelatin concentration.

pH from 4.6 to 8.0 had no significant effect on the T_G of gelatin gels with or without milk proteins at the concentrations studied (data not shown). The gelling properties of all samples were substantially affected by pH 3.0. At pH 3.0, the T_G of samples with 5.0% gelatin was much lower than that at other pH, and samples with 1.0 or 2.5% gelatin did not gel at all during cooling stage. Thus, it could be concluded that gelation of gelatin was inhibited at pH 3.0. This is attributable to protonation of amino acids of gelatin at low pH, which prevents formation of hydrogen bonds. Hydrogen bonds are very important in forming the gelatin gel framework (Bello et al., 1962). In a circular dichroism study of a 0.2% gelatin solution, Wustneck, Wetzel, Buder, and Hermel (1988) observed that when the temperature of the gelatin solution was decreased from 25 to 10°C, an increase in peak area for triple helical structures occurred at pH 4.9, 7.0, and 10, but not at pH 2.0. They attributed this to partial destruction of the gelatin molecules at very low pH.

In the pH range studied, addition of MPC and SMP significantly increased the T_G of gelatin gels at all gelatin concentrations both at pH 6.6 and 8.0, while addition of WPI did not affect T_G significantly (Fig. 2B). These results indicated that interactions occurred between gelatin and MPC/SMP, but not between gelatin and WPI. From these observations it can be inferred that, under the experimental conditions of this study, gelatin was able to interact with caseins but not with whey proteins. The reasons for these differences are discussed in Section 3.1.3.

![Fig. 1. Changes in G' (•) and G'' (×) of pure gelatin gels at concentration 2.5%, pH 6.6. Cooling step from 40 to 10°C (A); annealing step at 10°C (B); heating step from 10 to 40°C (C).](image-url)
3.1.2. Melting temperature

The melting temperatures ($T_M$) of the samples were obtained from the heating stage, but 1.0% gelatin did not gel at pH 3.0, and no melting temperature was obtained. $T_M$ of all samples was significantly affected by gelatin concentration (Table 1), and the higher the concentration, the higher was $T_M$ (Fig. 3A and B). From pH 4.6 to 8.0, gels with 5.0, 2.5, and 1.0% gelatin melted at temperatures in the range 29–33 °C, 27–32 °C and 25–27 °C, respectively. These results are in agreement with previous studies (Bello et al., 1962; Boedtker & Doty, 1954). Although MPC also contains some minerals, the amount is less than that in SMP because of their removal during manufacture (Hemar, Hall, Munro, & Singh, 2002). It was also found that gels containing SMP showed significantly higher melting temperatures at all concentrations and pH studied than other gels (Fig. 3B). Results contradictory to this study have been reported by Salvador and Fiszman (1998) who found that the melting temperature of a gelatin gel was not affected by the addition of milk components. However a different type of gelatin was used in that study.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration</th>
<th>pH</th>
<th>Protein</th>
<th>Concentration × pH</th>
<th>Concentration × protein</th>
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<td>$G_{0.5}$</td>
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a Deductions are for pure gelatin and gelatin–whey protein mixtures or for pure gelatin and gelatin–milk proteins mixtures [in brackets [ ]]; $P < 0.05$, *; $P < 0.01$, **; $P < 0.0001$, ***.

b $T_c$ – gelling temperature, $T_m$ – melting temperature, $G_0'$ – initial $G'$ of time sweep, $G_{0.5}$ – $G'$ at infinite time in time sweep, $K$ – rate of gelation during annealing.

3.1.3. Annealing stage

In order to fully understand the gelation process during the annealing stage, the rheograms were described using a modified first-order kinetic model (Eq. (2)) as it was thought that the time-sweep outputs described a first order kinetic. The storage modulus was focussed on because $G''$ was effectively zero for the gels at all the conditions studied. The modified first-order kinetic model has been extensively used to describe time-course starch digestion, and Sorba and Sopade (2013) used it, written with viscosity parameters, to describe time-course viscosity changes in starch during digestion. Moreover, these authors opined that the model is identical to some creep models, even though the parameters might indicate different concepts.
1500 Pa for 5.0% gels. The effect of pH on the casein has been studied in 488 of the annealing stage. At all pH, indicating that the system had not gelled at the beginning gelatin gels, while MPC- and SMP-containing gels showed significant differences among these three milk protein preparations, it is apparent that the casein component may be the cause of the higher charge of the whole molecule. Other authors reported that the viscoelastic properties of gelatin gels were greatly improved by higher amounts of triple helical structure, instead of the aggregates of gelatin molecules (Boedtker & Doty, 1954; Sarabia, Gomez-Guillen, & Montero, 2000). Large aggregates could be formed around the IP (Boedtker & Doty, 1954; Lin et al., 2002), while no significant difference of G’ of gelatin gels was observed among the pH close to IP (4.6 and 5.3) and far away from IP (8.0) in our study, except pH 3.0.

Addition of WPI significantly decreased the value of G’ at gelatin concentrations 2.5 and 5.0%, but not at 1.0%. MPC and SMP caused a significant increase in G’ at a gelatin concentration of 5.0%, but not at 2.5 and 1.0% (Fig. 4C and D), which indicated the gelatin concentration dependence of the milk protein effect. Thus the effect of milk proteins on gelatin gels was more significant at higher gelatin concentrations, which is in agreement with a study on casein micelles/κ-carrageenan interactions. It was found that the chance for these two polymers to interact increased with increased concentration of κ-carrageenan. At very low carrageenan concentrations, there were fewer carrageenan chains so that the junction zones connected by casein micelles may not be statistically significant (Ji, Corredig, & Goff, 2008; Langendorff et al., 1997). Also increasing the gelling agent concentration could lead to an increase in the amount of milk protein aggregates (Martin, Goff, Smith, & Dalgleish, 2006), which would affect the rheological properties of the gels.

Increase in G’ value of gelatin gel by SMP has been reported previously (Salvador & Fiszman, 1998). It was attributed to stabilization of the network by SMP through changes in hydrogen bonding, which is the basis of the formation of the gelatin network. The disturbance of the gelatin network by added WPC has also been reported (Walkenstrom & Hermansson, 1996). Considering the differences among these three milk protein preparations, it is apparent that the casein component may be the cause of the higher G’ of the mixed gels than that of pure gelatin gels. However these results are different from those reported previously. Based on size distribution and turbidimetric titration results, Lefebvre and Antonov (2001) reported that no interaction occurred between gelatin type B and caseins at pH 6.7, at gelatin: casein ratios from 0.03 to 13. A rheological study by Koh et al. (2002) indicated that addition of gelatin B had little influence on G’ of sodium caseinate gels at pH 4.6. They reported that in the pH range 4.5—5.0, both casein and gelatin B carry a very low net charge, so they would not have a strong interaction. Our study of gelatin/MPC and gelatin/
SMP gels was conducted at pH 6.6 and 8.0 (both gelatin and the milk proteins carried some negative charge at those pH) which is different from the study of Koh et al. (2002). Moreover it has been suggested that a “positive patch” exists between residues 97 and 112 of $\kappa$-casein, which is on the surface of casein micelles, and that this positive patch even exists at pH above PI (Snoeren, 1975). Therefore, it is possible that interactions occurred between gelatin and the positive patch on $\kappa$-casein under the experimental conditions of this study.

Similar to those obtained in this study, effects of addition of milk proteins have been reported on gelation of $\kappa$-carrageenan gels. It was reported that $\kappa$-carrageenan gel with SMP showed a lower loss tangent with increase of pH from 6.0 to 8.0, indicating higher elasticity. However, no explanations were given for this result (Xu, Stanley, Goff, Davidson, & Lemaguer, 1992). Hemar et al. (2002) reported that addition of SMP and MPC showed a greater effect on the viscoelastic properties of $\kappa$-carrageenan gels than addition of WPI, and the results obtained for MPC- and SMP-containing gels were similar. They attributed this effect to the phase separation caused by addition of MPC and SMP. The casein particle network formed due to depletion flocculation contributed to the increase in viscosity and elasticity of the $\kappa$-carrageenan gel, while for WPI-containing gels, no significant phase separation occurred that would cause improvement in the rheological properties of the gels. Those authors reported in another study that the depletion flocculation mechanism depended on the biopolymer size; particles in SMP and MPC solutions have an average diameter of 0.2 $\mu$m, which is mainly due to casein micelles, while particles in WPI solutions are of nanometer size (Hemar, Tamehana, Munro, & Singh, 2001). This hypothesis is also reasonable for explaining the results of our study on gelatin. Phase separation of different mixtures could be seen in our microscopy study (Fig. 6).

Fig. 4E and F show the results of $K'$ during the annealing stage, which indicated the gelation rate. The $K'$ of all the gels increased with increasing concentration of gelatin at all pH values. At pH 3.0 a significant decrease in $K'$ was observed for pure gelatin gels at 2.5 and 5.0% gelatin concentrations, but no significant difference could be seen for WPI-containing gels. Interestingly, it was observed that the $K'$ of WPI-containing gels was higher than for pure gelatin gels, except at 1.0% gelatin concentration (Fig. 3E). However, WPI-containing gels showed the lowest values for other parameters. A significant increase in $K'$ due to addition of MPC and SMP could only be seen at 1.0% gelatin concentration, and not at higher concentrations like the other parameters. The gelation rate not only determines the final gel strength, but also is crucial to the structure of the gels. High gelation rates tend to lead to a coarse network
Further study needs to be done to understand the mechanism of rate of increase of $K'$.

3.2. Texture analysis

In the texture analysis, only two gelatin concentrations (2.5 and 5.0%) were studied. Since a 1.0% gelatin gel was too soft, no penetration force peak could be obtained. Fig. 5A and B show representative penetrometry profiles of the samples. Gel firmness was calculated as initial slope from the penetrometry curves and the results are shown in Fig. 5C and D.

Fig. 5A and B show how the penetration curve varied among different gels. All gels showed a rapid increase in the force over a short time as the probe moved into the samples, although the initial slope was different for different gels. The gel firmness was much higher at 5.0% concentration than at 2.5% for all gels (Fig. 5C and D), similar to the concentration effect on rheological results. These results are also in agreement with previous studies (Ferry, 1948; Fiszman & Salvador, 1999; Salvador & Fiszman, 1998).

The gel firmness at pH 3.0 was significantly lower than that at other pH ($p < 0.05$) for all gels (Fig. 5C), which corresponded to the rheological results. The low initial slope indicated that the gels at pH 3.0 deformed easily and tended to flow more than break. The effect of pH on the firmness of gelatin gels is probably due to changes in the electrostatic interactions in the system (Fiszman & Salvador, 1999). The firmness of a high concentration (27%) gelatin gel was studied in the pH range 2.0–12 by Cumper and Alexander (1952). They found that at the extreme pH, the number of basic or acidic amino acid residues available for bond formation decreased rapidly and consequently a sharp decrease in firmness occurred. This could explain the low firmness of the gelatin gel at pH 3.0 in our study. Our results were also consistent with the study of Choi and Regenstein (2000), in which different kinds of gelatin were studied. They observed a marked decrease in gel strength of gelatin gels below pH 4.0.

Gel firmness was independent of pH in the range 4.6–8.0 for all gels except gels containing SMP. Gels with SMP showed significantly higher firmness at pH 8.0 than at pH 6.6 at both 2.5 and 5.0% gelatin concentration. This could be due to the minerals in the SMP as discussed before for gel melting temperature. Addition of milk proteins significantly increased the gel firmness at 5.0% gelatin concentration, while at 2.5% the values of the mixed gels were not significantly different from those of the pure gelatin gels. These results again indicated the dependence on the gelatin concentration of the effect of the milk proteins on gelatin gels.

In addition to gel firmness, another important characteristic that could be observed from penetrometry curves was the breaking point, which is a measure of the fracturability of the gel. As can be seen from Fig. 5B, only some of the pure gelatin gels at 2.5% gelatin concentration showed a breaking point. At pH 5.3 and 6.6, the pure gelatin gels were not easily deformed and showed a clear breaking point during penetration (between 8 and 9 s) (Fig. 5B). No breaking point was observed for pure gelatin gels at pH 3.0 and the penetration force kept increasing until the compression ended at 10 s, indicating that the gel had no fracturability. The force recorded at the end of compression (10 s) is not indicative of any physical characteristic (Fiszman & Salvador, 1999) and hence was not recorded. At pH 4.6, the profile of the pure gelatin gel showed a shoulder at about 8.5 s, which indicated it had an initial resistance to penetration; however this was considered as a questionable breaking point because the penetration force did not show an apparent decrease after this point. The small inflection also indicated a structural change which was not strong enough to break the gel and needs to be confirmed by a microscopy study. At pH 8.0, no apparent breaking point was observed either. The fracturability of gels could be related to IP. Molecular aggregation could be caused

\[ K' = f(pH) \times \text{concentration} \times \text{milk protein} \]

**Fig. 5.** Representative penetrometry profiles for gels and the effect of protein × concentration × pH interaction on gel firmness. Effect of gelatin concentration and addition of milk protein on the texture profile of gelatin gels at pH 6.6 (... 5% pure gelatin gel, — 2.5% pure gelatin gel, - - - 2.5% gelatin gel with MPC) (A); effect of pH on the texture profile of 2.5% pure gelatin gels (... pH 3.0, — pH 4.6, - - - 5.3, - - - pH 8.0) (B); firmness of pure gelatin gel and gelatin gel with WPI (C) and firmness of pure gelatin gel and gelatin gels with three milk proteins (D).
by the strong attraction of oppositely charged groups on gelatin chains around the IP (Boedtker & Doty, 1954), leading to fragile gels. However a gel at pH 4.6, which is also very close to the IP of gelatin type B, did not show apparent fracturability. The difference between the gels formed at pH 4.6 and at pH 5.3 and 6.6 could also be seen in microscopy results (Fig. 6).

Additionally, no apparent breaking point was observed for pure gelatin gels at 5.0% concentration at all pH conditions studied. At high concentrations, gelatin gels have higher elasticity, which makes them harder to break (Hansen, Blennow, Pedersen, Norgaard, & Engelsen, 2008). The absence of a breaking point in 5.0% gelatin gels indicated that the changes in electrostatic interactions due to pH only influenced the aggregation of the mobile chains in weak gels like the 2.5% gelatin gels. The results were in agreement with a study by Salvador and Fiszman, (1998), although different texture profiles were obtained because of a different gelatin type used and different experimental conditions. They found that at 1.5% gelatin concentration, the breaking force of gelatin gels decreased as the pH moved further away from its IP, while no significant differences were found at 5.0% concentration (Fiszman & Salvador, 1999). It can also be observed that all the gels containing milk protein showed no obvious breaking point, suggesting that the presence of milk proteins changes the texture of gelatin gel in such a way that prevents fracturability. This could be because milk proteins filled in the pores of gelatin gel, thereby making the gel less fracturable.

3.3. Microstructure

The microstructure of gels with high gelatin concentration (2.5 and 5.0%) was too dense and no clear gelatin strands could be seen. Hence, the effect of pH and addition of milk protein on the microstructure of gelatin gel was studied at 1.0% gelatin concentration. The structural changes in pure gelatin gels were studied in the pH range 3.0–8.0. The effect of addition of milk proteins on the microstructure of gelatin gels was studied at pH 6.6. High milk solids content tended to cover the gelatin structure, so in this study only 2% milk solids were added. The microstructure of

![Fig. 6. Scanning electronic micrographs of gels: 2.5% pure gelatin gels at pH 6.6 (A); 1% pure gelatin gel at pH 3.0 (B), pH 4.6 (C), pH 5.3 (D), pH 6.6 (E) and pH 8.0 (F); 1% gelatin with WPI (G), MPC (H) and SMP (I) at pH 6.6. Scale bars in the images are 1 µm.](image-url)
gelatin gels with and without milk proteins were examined by SEM (Fig. 6).

As can be seen in the micrographs, the microstructure of gelatin gels differed greatly with concentration. A 2.5% gelatin gel at pH 6.6 formed a very dense structure with small voids (Fig. 6A). No strands could be seen in the gel structure while for all the gels with 1.0% gelatin, the structure was much looser and the strands could be seen clearly (Fig. 6B–F). Some particles were observed in pure gelatin gels (Fig. 6A), which could have been an impurity in the gelatin product, such as unhydrolyzed collagen. These results were in agreement with the rheology and texture study, which showed that stronger and firmer gels were formed at higher gelatin concentrations.

The microstructure of pure gelatin gels was also significantly affected by pH (Fig. 6B–F). The microstructure of 1.0% gelatin gel formed at pH 3.0 was much looser with larger pores than those at higher pH (Fig. 6B). The microstructure at higher pH was much denser, suggesting that the gels were more organized at these pH values. Pure gelatin gels at pH 4.6 appeared to form more strands than those at pH 3.0 (Fig. 6C). From pH 5.3, the network became more three dimensional, and no significant differences were observed between the microstructures of pure gelatin gels at pH 5.3 and 6.6 (Fig. 6D and E). The microstructure of the pure gelatin gel at pH 5.0 was denser with some large pores, but still individual strands could be observed (Fig. 6F). These results could further explain the results obtained during the textural measurements. At pH 3.0, the gel was too soft to be fractured. pH 4.6 seemed to be the transition pH for the gels to change from flat to “three-dimensional”, which could have induced a questionable breaking point in the penetration curve and a relatively low melting temperature. At pH 5.3 and 6.6, the gels formed well-organized structures, which could have led to enough gel strength to have a clear breaking point. At pH 8.0, the gel structure was very well organized and it could not be broken within the penetration distance used in this study.

The gelatin gel with WPI at pH 6.6 had a similar microstructure as the pure gelatin gel (Fig. 6G). The strands’ organization did not change, and the fine and homogeneous gel structure could still be observed. The only difference was that the gel appeared denser and more protein aggregates were seen in the WPI-containing gel, which could be attributed to the presence of protein aggregates in WPI (Hemar et al., 2001). No apparent phase separation between the gelatin and whey protein was observed. This was in agreement with a previous study by Walkenström and Hermansson (1994), in which light microscopy was used to study the microstructure of WPI/gelatin mixed gels. In the gelatin gel with MPC, no individual casein micelles could be seen; they appeared as large aggregates linked by gelatin strands (Fig. 6H). The gel structure had changed significantly from that of the pure gelatin gel. The regularity of the pure gelatin gel seemed disturbed. It was not as homogenous as the pure gelatin gel and large voids were observed. It seems there were two separate phases, containing dense milk protein aggregates and gelatin strands, which could be caused by depletion flocculation of milk proteins in the aqueous phase (Hemar et al., 2002). These microstructure results suggest interactions between the gelatin and milk proteins, which is in agreement with the observation of the rheology study. Similar results have been obtained by confocal microscopy of MPC and κ-carrageenan gels (Hemar et al., 2002). In the gelatin gel with SMP (Fig. 6I), a compact and interconnected network was formed and more branched structures were observed than in the pure gelatin gel. Phase separation could again be observed, although the milk proteins filled the voids and formed clusters and chains connected by gelatin strands. Different from the gel with MPC, the SMP-containing gel showed smaller protein aggregates and smaller voids, which was in agreement with the confocal microscopy study of Hemar et al. 2002. The SMP-containing gel also appeared to be more branched, which could be attributed to the ions in SMP affecting the structure of the gelatin gel and the interaction between gelatin and casein by influencing the hydrogen bonding (Salvador & Fiszman, 1998). SMP/carrageenan gel was also studied by Martin et al. (2006), who found that the SMP aggregates were linked by carrageenan. It was attributed to attractive electrostatic interactions between carrageenan and casein. Similar electrostatic interactions could be responsible for the changes in the microstructure of gelatin gels containing MPC and SMP in this study. The interaction could be between the “positive patch” on κ-casein and gelatin, as discussed before. There are no reports in the literature indicating such positive patches on whey protein molecules. Hence, no interactions between gelatin and WPI are expected, since both carry a small negative charge at pH 6.6.

4. Conclusions

This study provides an insight into the effect of concentration, pH and addition of milk proteins on the gelation behaviour of gelatin. According to our results, mixed gels of gelatin and milk proteins with different properties could be obtained by manipulating these three factors. Stronger and firmer gels could be formed with high gelatin concentrations and addition of casein, while whey protein has different effects on the rheology and texture of gelatin gels. Gelatin behaviour is constant in pH range from 4.6 to 8.0 in terms of rheology and texture, which makes it suitable for a wide range of applications. However the difference of fracturability caused by pH should be noted. The differences caused by addition of different milk proteins on gelatin gel can be understood in two ways. Firstly, κ-casein has a “positive patch” on the surface of its molecule, which can interact with negatively charged gelatin at pH 6.6 and 8.0, while whey proteins do not have such a “positive patch”. Secondly, casein micelles in MPC and SMP can more easily cause depletion flocculation, which induces phase separation, than the much smaller particles present in WPI. This phase separation may lead to higher elasticity of the gels. A further study with purified milk proteins, such as κ-casein, β-casein, α-lactalbumin and β-lactoglobulin, could be undertaken to better understand the mechanism of interaction between gelatin and milk proteins. SEM seems to be a useful technique to explain the physical properties of gels at the microstructure level. In this study, modelling the annealing stage was very useful to obtain a comprehensive understanding of the gel properties. The parameters modelled in this study revealed some useful information which was not apparent from just measuring the traditional rheological parameters such as G’. The dependence of gelatin concentration on the effect of milk proteins was only observed from G* and the slightly weaker gel formed at pH 4.6 could only be seen from parameter G’. Further study needs to be done to elucidate the mechanism of increase in rate of G’ during the annealing stage.

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References


