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Mineral Salt Effects on Whey Protein Gelation†

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The effects of salts on the rheological properties (shear stress and shear strain at failure) of heat-induced whey protein isolate (WPI) gels were examined. WPI gels formed with low levels of added NaCl (25–30 mM) were translucent and gelatin-like with low shear stress and high shear strain values, whereas WPI gels formed with low levels of added $CaCl_2$ (7.5 mM) were opaque and curd-like with low shear stress and low shear strain values. Increasing levels of either salt caused a sharp increase in the shear stress of the gels to a maximum at 50–75 mM NaCl or 20 mM $CaCl_2$. However, while increases in $CaCl_2$ also caused an increase in gel shear strain to a maximum at 100 mM $CaCl_2$, increases in NaCl caused a decrease in shear strain to a minimum with 150 mM NaCl. The differing effect of NaCl and $CaCl_2$ on shear strain was shown to be general monovalent (Na, Li, K, Rb, Cs) or divalent (Ca, Mg, Ba) cation effects. In mixed systems ($KCl/CaCl_2$), the divalent cation determined shear strain.

INTRODUCTION

An important functional property of whey proteins is their ability, under appropriate conditions, to form heat-induced gel structures capable of immobilizing large quantities of water and other food components (Hermansson and Akesson, 1975; McDonough et al., 1974; Sternberg et al., 1976). In recent years, considerable effort has focused on understanding and improving the functionality of whey protein products to enhance utilization of whey by the food industry. The variability and complexity of whey protein concentrate (WPC) and whey protein isolate (WPI) products have, however, hampered more rapid advancement in our understanding of the roles that compositional and physicochemical factors play in gelation. Further quantitative research is needed to increase our understanding of the relationship between the basic physicochemical properties and the functionality of whey proteins. Improved control of functional consistency together with more effective manipulation of gel texture for specific functional applications would greatly enhance the suitability of WPC and WPI for incorporation into processed and formulated food products, including many meat, bakery, and dairy items.

Schmidt et al. (1978) determined that the mineral components have a significant influence on the gel characteristics of WPC. Subsequently, a number of researchers have investigated the effects of varying the salt content and/or type of salt on the structural failure properties of heat-induced whey protein gels (Schmidt et al., 1979; Johns and Ennis, 1981; Dunkerley and Zadow, 1984; Kohnhorst and Mangino, 1985; Mulvihill and Kinsella, 1988; Zirbel and Kinsella, 1988).

Schmidt et al. (1978) found that dialyzed whey protein concentrate formed stronger, more cohesive, less springy gels which were more sensitive to salt addition than nondialyzed WPC. Maximum gel hardness values of dialyzed WPC are attained when protein suspensions contain 200 mM NaCl or 11.1 mM $CaCl_2$, and hardness decreases with higher salt concentrations (Schmidt et al., 1978, 1979). Mulvihill and Kinsella (1988) supported these observations with their findings that hardness values of β-lactoglobulin gels maximized at 200 mM NaCl or 10 mM $CaCl_2$. They further noted that $CaCl_2$ appeared to be far more effective than NaCl in increasing gel strength since lower concentrations of $CaCl_2$ were required to produce similar increases in hardness. Zirbel and Kinsella (1988) reported maximum hardness values of WPI gels with 20 mM $CaCl_2$ and noted that WPI suspensions, without the addition of salt, only readily formed gels at protein concentrations in excess of 14% (pH 7.0, heated 15 min at 90 °C).

Previously we found that dialysis markedly increased the shear stress at failure (shear stress) values of WPC

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and WPI gels (Kuhn and Foegeding, 1990). Moreover, dialysis caused a significant increase in the shear strain at failure (shear strain) values of the WPC gels which resulted in a normalization of all WPC and WPI gel strain values (Kuhn and Foegeding, 1990). The rheological property of shear strain at failure has not been investigated in previous studies by other investigators. Salts may, therefore, prove to be a factor that can be used to alter the strength (shear stress) and deformability (shear strain) of whey protein gels.

The objective of this study was to systematically investigate the influence of salts of monovalent and divalent cations on the rheological properties of heat-induced whey protein isolate gels.

MATERIALS AND METHODS

Whey Protein Isolate Suspensions. By use of proximate analysis data for protein determination (N × 6.38, macro-Kjeldahl) (AOAC, 1984), 10% protein suspensions of commercial whey protein isolate (Le Sueur Isolates, Le Sueur, MN) were prepared in specified salt solutions and adjusted to pH 7.0. Each salt solution was prepared in deionized water.

Three studies were undertaken in triplicate. The first and third studies were performed on two different lots of WPI, while the second study was performed on three different lots of WPI. In the first study, suspensions were prepared with a range of added $CaCl_2$ (5–500 mM) or NaCl (20–500 mM) concentrations. The second study was designed to determine if there was a difference between the effects of monovalent and divalent cations at equal ionic strength. WPI suspensions were prepared in a series of 90 mM solutions of monovalent cations (LiCl, NaCl, KCl, RbCl, CsCl) and a series of 30 mM solutions of divalent cations ($MgCl_2$, $CaCl_2$, $BaCl_2$). The last study was a competition study. WPI suspensions were prepared with two sets of $CaCl_2$ and KCl mixtures. Either the $CaCl_2$ concentration was held constant at 40 mM while the KCl concentration was varied (50, 100, 200 mM) or the KCl concentration was held constant at 120 mM (equivalent ionic strength to 40 mM $CaCl_2$) while the $CaCl_2$ concentration was varied (5, 10, 20 mM).

Once hydrated (11% w/v protein) in the salt solutions, the suspensions were degassed in a vacuum chamber connected to an aspirator for 2 h to remove all visible air bubbles, readjusted to pH 7.0 with 0.5 N NaOH or 0.5 N HCl, and diluted to 10% protein. Fractions of each suspension were poured into polycarbonate tubes, 19 mm in diameter, which were precoated with Haynes sanitary lubricating spray (The Haynes Manufacturing Co., Cleveland, OH) and closed at one end with rubber stoppers. Gels were formed by heating in an 80 °C water bath for 30 min. After cooling for 30 min at 23 ± 1 °C, the gels were removed from the tubes and stored overnight at 4 °C. The following day they were equilibrated to room temperature, i.e., 23 ± 1 °C, for rheological testing.

Determination of Rheological Properties at Failure. Twisting of gel samples to failure on a torsion apparatus was used to measure the rheological properties of true shear stress at failure and true shear strain at failure (Diehl et al., 1979). The samples were cut into 28.7 mm long cylinders, and plastic disks were glued with cyanoacrylate glue (Krazy Glue, B. Jadow and Sons, Inc., New York, NY) to each end of the gel cylinders. Once ground into dumbbell shapes with a center diameter of 10 mm as described by Montejano et al. (1983), they were vertically mounted and twisted to failure on a torsion apparatus attached to a Brookfield digital viscometer (Model 5XHBTD). The values of true shear stress at failure and true shear strain at failure were calculated from the torque and angular displacements as described by Diehl et al. (1979).

Mineral Analysis. Calcium content was determined by using a Perkin-Elmer Plasma II emission spectrophotometer; sodium was determined with an Instrumentation Laboratory Model 443 flame photometer.

Statistical Analysis. Data were analyzed by analysis of variance using the general linear model procedure (SAS, 1982). Differences among the failure shear stress and failure shear strain value means of gels were determined by using the Waller–Duncan K-ratio t-test.

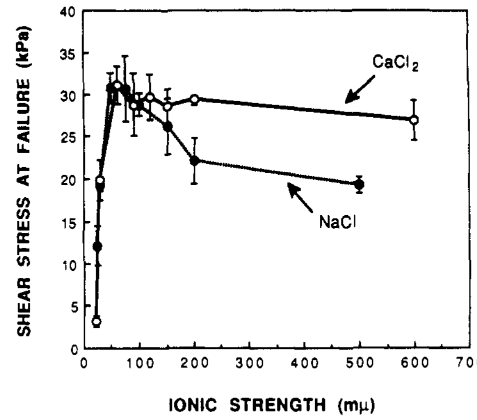

Figure 1. Effect of increasing concentrations of NaCl vs $CaCl_2$ on the shear stress at failure of 10% protein WPI gels.

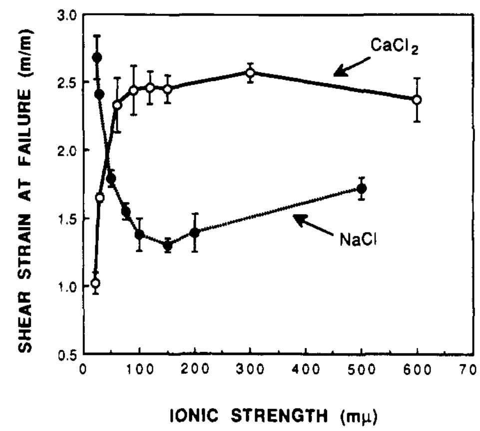

Figure 2. Effect of increasing concentrations of NaCl vs $CaCl_2$ on the shear strain at failure of 10% protein WPI gels.

RESULTS AND DISCUSSION

The whey protein isolate samples used in the study contained 0.17–0.23% calcium and 0.43–0.45% sodium on a dry weight basis. The 10% protein suspensions contained approximately 4.0 mM calcium and 7.0 mM sodium. These suspensions did not, however, form heat-induced gel structures under the experimental conditions used (10% protein, pH 7.0, and 80 °C for 30 min) unless additional salts were added. The minimum added salt to form a self-supporting gel strong enough for rheological testing was 25 mM NaCl or 7.5 mM $CaCl_2$ (concentrations of similar ionic strength). Both gels had low shear stress at failure (shear stress) values but differed greatly in shear strain at failure (shear strain) values and appearance. Gels formed with protein solutions containing 25–30 mM NaCl had very high shear strain values and were translucent and gelatin-like in appearance, whereas the gels formed in the presence of 7.5 mM $CaCl_2$ had very low shear strain values and were opaque and curd-like, resembling coagulums rather than gels (Figures 1 and 2). Increasing the concentration of either salt caused a sharp increase in shear stress values to a maximum with 50–75 mM NaCl or 20 mM (60 mμ) $CaCl_2$ (Figure 1). Such increases in shear stress values (gel strength) with low-level increases in salt concentration support previous results (Schmidt et al. 1978, 1979; Mulvihill and Kinsella, 1988; Zirbel and Kinsella, 1988). However, due to differences in the systems examined, our results differ regarding exact salt concentrations required to maximize gel strength. Schmidt et al. (1978, 1979) reported maximum hardness of 10%

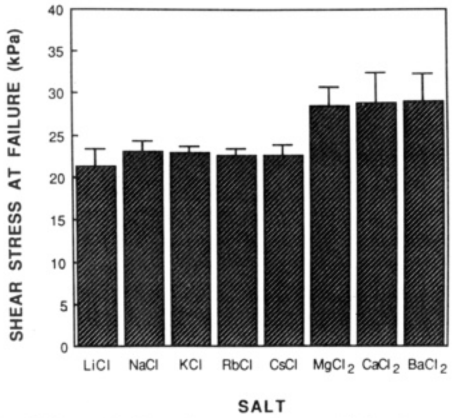

Figure 3. Effect of salts of monovalent and divalent cations at equal ionic strength (90 mμ) on the shear stress at failure of 10% protein WPI gels.

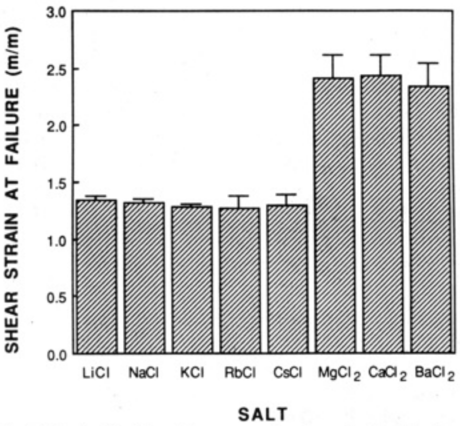

Figure 4. Effect of salts of monovalent and divalent cations at equal ionic strength (90 mμ) on the shear strain at failure of 10% protein WPI gels.

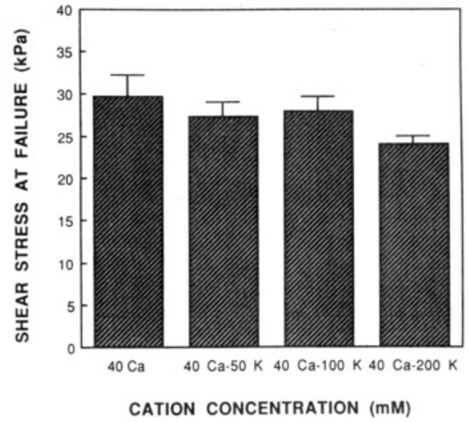

Figure 5. Effect of combining a range of different concentrations of KCl with a constant concentration of 40 mM $CaCl_2$ on the shear stress at failure of 10% protein WPI gels.

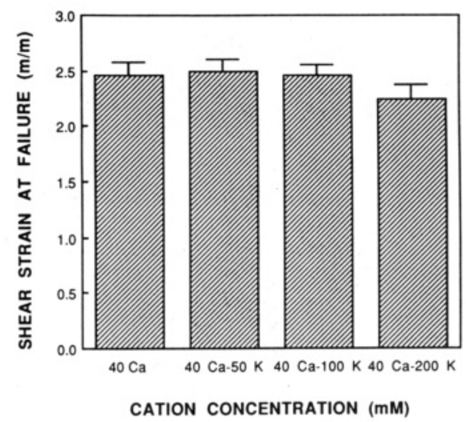

Figure 6. Effect of combining a range of different concentrations of KCl with a constant concentration of 40 mM $CaCl_2$ on the shear strain at failure of 10% protein WPI gels.

protein WPC gels with 200 mM NaCl or 11.1 mM $CaCl_2$. Mulvihill and Kinsella (1988) reported maximum hardness of 10% protein β-lactoglobulin gels with 200 mM NaCl or 10 mM $CaCl_2$, and Zirbel and Kinsella (1988) reported maximum hardness of 20% protein WPI gels with 20 mM $CaCl_2$.

Increasing NaCl concentration above that determined to maximize gel strength caused a sharp decrease in shear stress values followed by a gradual leveling off (Figure 1). Shear stress values, however, remained elevated when $CaCl_2$ concentration was increased (30–300 mM). The effect of increasing salt concentration on shear strain values was found to differ greatly between NaCl and $CaCl_2$ (Figure 2). Gels had very high shear strain values with low levels of added NaCl (25–30 mM) but very low shear strain values with low levels of added $CaCl_2$ (7.5 mM). Increasing NaCl concentration caused a sharp decrease in shear strain values to a minimium at 150 mM NaCl followed by a gradual increase with higher levels. Increasing levels of added $CaCl_2$, however, caused a sharp increase in shear strain values to a maximum at 100 mM $CaCl_2$, followed by a gradual decline with higher concentrations. The significant difference in the effect of NaCl and $CaCl_2$ on the shear strain suggested that gel strain was greatly dependent upon the charge of the cation of the added salt.

At equal concentrations (90 mM), salts of monovalent cations (LiCl, NaCl, KCl, RbCl, CsCl) had similar effects on the shear stress and shear strain values of heat-induced gels (Figures 3 and 4). Addition of salts of divalent cations ($MgCl_2$, $CaCl_2$, $BaCl_2$) at 30 mM had similar effects to each other on gelation; however, the gels formed had significantly higher shear stress and shear strain values than those formed with monovalent cations at equivalent ionic strength (Figures 3 and 4). Shear stress values ranged from 21.3 to 23.0 kPa with monovalent cations and from 28.5 to 29.0 kPa with divalent cations, while shear strain values ranged from 1.27 to 1.34 with monovalent cations and from 2.34 to 2.44 with divalent cations. Thus, there was a great similarity in the effect of cations with the same charge on the shear stress and shear strain values of WPI gels, but a significant difference between the effect of monovalent cations and divalent cations at equal ionic strength, especially on the shear strain values of the gels.

In the competition study, the effects of combining KCl with $CaCl_2$ in a number of different ratios were examined. The shear stress values of gels formed with salt mixtures fell within the ranges found with similar concentrations of the individual salts (Figures 5 and 7). The shear strain values, however, were found to be greatly dependent upon the concentration of the divalent cation (Figures 6 and 8). The shear strain values of gels formed in suspensions including 40 mM $CaCl_2$ and <200 mM KCl did not significantly differ from those formed with only 40 mM $CaCl_2$ ($p < 0.01$) (Figure 7). However, combining 5–20 mM $CaCl_2$ with 120 mM KCl caused a sharp increase in shear strain (Figure 8). Thus, $CaCl_2$ had a dominant effect over KCl on gel rheology, even when sufficient KCl (120 mM) was present to form a strong, self-supporting gel structure. Alteration of the $CaCl_2$ concentration thus appears to be an effective way of modifying WPI gel shear strain, the measurable parameter determined by Montejano et al. (1985) to correlate most closely and frequently with sensory parameters used in food texture evaluation.

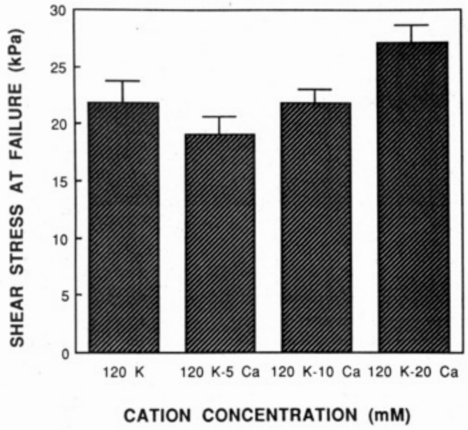

Figure 7. Effect of combining a range of different concentrations of $CaCl_2$ with a constant concentration of 120 mM KCl on the shear stress at failure of 10% protein WPI gels.

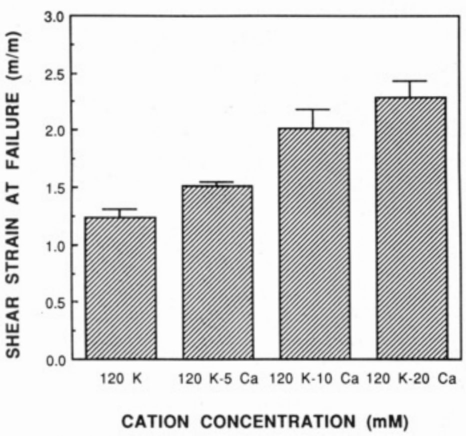

Figure 8. Effect of combining a range of different concentrations of $CaCl_2$ with a constant concentration of 120 mM KCl on the shear strain at failure of 10% protein WPI gels.

CONCLUSIONS

Although the effects of low levels of added NaCl (<75 mM) and $CaCl_2$ (<20 mM) were very similar on the shear stress values of WPI gels, they differed greatly on the shear strain values, indicating that shear strain is greatly dependent on the charge of the cation of the added salt. Gels formed with salts of cations with the same charge all had similar shear stress and shear strain values. Gels formed with monovalent cations, however, differed significantly from gels formed with divalent cations, indicating that there is a monovalent vs divalent cation effect on the heat-induced whey protein gel structure. Competition studies with salt mixes revealed that $CaCl_2$ had a dominant effect over KCl on gel structure. Alteration of the $CaCl_2$ concentration was found to be an effective way of modifying the rheological properties, especially shear strain, of a strong gelling WPI.

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