Effect of Tetrasodium Pyrophosphate on the Physicochemical Properties of Yogurt Gels

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ABSTRACT

The effect of tetrasodium pyrophosphate (TSPP) on the properties of yogurt gels was investigated. Various concentrations (0.05 to 0.2%) of TSPP were added to preheated (85°C for 30 min) reconstituted skim milk, which was readjusted to pH 6.50. Milk was inoculated with 2% starter culture and incubated at 42°C until the pH reached 4.6. Acid-base buffering profiles of milk and total and soluble calcium levels were measured. Turbidity measurements were used to indicate changes in casein dispersion. Storage modulus (G′) and loss tangent (LT) values of yogurts were monitored during fermentation using dynamic oscillatory rheology. Large deformation properties of gels were also measured. Microstructural properties of yogurt were observed using fluorescence microscopy. The addition of TSPP resulted in the disappearance of the buffering peak during acid titration at pH ~5.1 that is due to the solubilization of colloidal calcium phosphate (CCP), and a new peak was observed at lower pH values (pH 4.0–4.5). The buffering peak at pH 6.0 during base titration virtually disappeared with addition of TSPP and a new peak appeared at pH ~4.8. The addition of TSPP reduced the soluble Ca content of milk and increased casein-bound Ca values. The addition of up to 0.125% TSPP resulted in a reduction in turbidity because of micelle dispersion but at 0.15%, turbidity increased and these samples exhibited a time-dependent increase in turbidity because of aggregation of casein particles. Gels made with 0.20% TSPP were very weak and had a very high gelation pH (6.35), probably due to complete dispersion of the micelle structure in this sample. The LT value of gels at pH 5.1 decreased with an increase in TSPP concentration, probably due to the loss of CCP with the addition of TSPP. The G′ values at pH 4.6 of gels made with ≤0.10% TSPP were not significantly different but the addition of ≥0.125% TSPP significantly decreased G′ values. The addition of 0.05 to 0.125% TSPP to milk resulted in a reduction in the yield stress values of yogurt compared with yogurt made without TSPP. Greater TSPP levels (>0.125%) markedly reduced the yield stress values of yogurt. Lowest whey separation levels were observed in yogurts made with 0.10% TSPP. High TSPP levels (>0.10%) greatly increased the apparent pore size of gels. Addition of very low levels of TSPP to milk for yogurt manufacture may be useful in reducing the whey separation defect, but at TSPP concentrations ≥0.125% very weak gels were formed.
Key words: yogurt, tetrasodium pyrophosphate, rheology, microstructure

INTRODUCTION

Yogurt is one of the most popular fermented milk products in the world. The texture of yogurt is an important consumer attribute influencing its acceptability. Manufacturers often modify yogurt texture by altering process conditions (e.g., milk heat treatment) or by the addition of ingredients (e.g., milk solids or stabilizers; Horne, 1999; Tamime and Robinson, 1999). Another approach is to modify the casein micelle structure through the addition of materials that chelate calcium, such as citrates (Ozcan-Yilsay et al., 2007). We recently observed that the addition of low concentrations of trisodium citrate (**TSC**) to milk improved gel stiffness and reduced whey separation of yogurts. Calcium chelating agents disrupt casein micelles because colloidal calcium phosphate (**CCP**) is a key structural or cross-linking unit in micelles (Horne, 1998). Ozcan-Yilsay et al. (2007) suggested that low levels of CCP removal facilitated greater rearrangement and molecular mobility of the micelle structure, which may have helped to increase the formation of cross-links between strands in yogurt gel networks. In contrast, when high levels of CCP were dissolved, complete micelle disruption occurred and this caused the formation of very weak yogurt gels. Both citrates and phosphate are commonly used in the dairy industry to sequester calcium;

for example, in the manufacture of process cheese where they are called melting or emulsifying salts (Berger et al., 1998). It is well known that the various types of phosphates do not have identical influences on the texture of process cheese, although they all can bind calcium. It was recently reported (Mizuno and Lucey, 2007) that the addition of low concentrations (between 0.1 and 0.2%) of tetrasodium pyrophosphate (**TSPP**) to milk could induce slow protein gelation even at room temperature. The gelation mechanism proposed by these authors included an initial partial dispersion of caseins and slow association of caseins that involved calcium pyrophosphate complexes. It appeared that pyrophosphate-induced gelation depended on a critical balance between electrostatic repulsion and attraction because gelation was only observed for a narrow TSPP concentration range and other types of phosphates were not as good at forming gels; TSC addition did not result in gelation. The present study was performed to investigate the effect of low concentrations of TSPP on yogurt gelation. We expected that very weak gels would be formed when the concentration of TSPP was sufficiently high to completely disperse the casein micelles so we worked in the range ≤0.2% TSPP. To our knowledge, there is no published information on the effect of TSPP addition to milk on the gelation properties of yogurt.

MATERIALS AND METHODS

Materials

Low-heat skim milk powder with 7.34 mg/g (wt/wt) of undenatured whey protein nitrogen (Bradley et al., 1992) was supplied by Dairy Farmers of America (Fresno, CA). Commercial yogurt starter culture (YC-087) consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* was obtained from Chr. Hansen Inc. (Milwaukee, WI). Tetrasodium pyrophosphate was supplied by Astaris (St. Louis, MO).

Milk Reconstitution and Milk Samples

Reconstituted skim milk (10.7% wt/vol) was preheated at 85°C for 30 min in a thermostatically controlled water bath and cooled rapidly to ~4°C in ice water. Some milk samples were used for chemical analysis and 0.02% (wt/wt) NaN_3 was added to these samples to prevent bacterial growth. Milk samples were stored overnight at 4 to 6°C. Milks were warmed to 25°C and various concentrations (0.05, 0.10, 0.125, 0.150, and 0.20% wt/wt) of TSPP were added with continuous stirring. The pH was then adjusted with 1 *N* HCl to a final pH of 6.50 ± 0.02 at 25°C. Milks were stirred

for 1 h to allow for equilibrium. Starter cultures were prepared as described by Lee and Lucey (2004a, b). Milk was warmed to 42°C before inoculation with 2% (wt/wt) working culture and incubated until the pH reached pH 4.6. pH measurements were recorded during fermentation as described by Ozcan-Yilsay et al. (2007).

Turbidity Measurements

Turbidity measurements were made at 700 nm on a Beckman DU 520 UV/Vis Spectrophotometer (Beckman Coulter, Fullerton, CA) using a cell with a 1-mm light path. Turbidity was measured 1 h after TSPP addition and pH was adjusted to pH 6.50 at 25°C.

Particle Size of Casein Micelles

Estimates of (changes in) micelle size were derived from the slope of the double-log plot of dispersion turbidity as a function of wavelength, as described by Holt et al. (1975) and Holt (1975), measured on a Shimadzu UV/Vis Spectrophotometer (model 160 1 PC, Shimadzu, Columbia, MD) equipped with a cell with a 1-cm light path. Analyses were performed 1 h after addition of TSPP and pH adjustment. The TSPP-treated milk samples were diluted by a factor of 23 in UF permeate and were clarified by filtration. The sensitivity of the procedure was improved by addition of 10% EDTA for complete dissociation of casein micelles to permit correction of turbidity readings for nonmicellar contributions to the scattered light. A double log plot of τ versus λ was found to be linear over the wavelength range 400 to 700 nm. Holt et al. (1975) showed that this slope could be written as

$$d (\log \tau)/d (\log \lambda) = -4 + \beta + \gamma, \quad [1]$$

where τ is turbidity, λ is the wavelength of light, and β and γ are, respectively, defined by

$$\beta = d (\log Q)/d (\log \lambda) \quad [2]$$

$$\text{and } \gamma = 2 \, d \, [\log n(dn/dc)]/d (\log \lambda) = 0.3. \quad [3]$$

The factor, Q, results from internal interference of light scattered at all angles, θ, by the particle and is the integral, therefore, of the particle form factor over those angles. The wavelength dependence of Q, expressed through the slope parameter β, therefore conveys entirely the same information as the particle form factor and can be used to determine size information in the same manner as the use of the particle form factor as

a function of scattering angle. Differing only by known constants, this information is conveyed also by the raw slope, d (log τ)/d (log λ) (Eq. 1). As Holt et al. (1975) demonstrated, deriving an average particle size from this parameter requires foreknowledge of the micellar size distribution function. Here, however, we employ our data only to indicate shifts in average particle size and their direction, a more elaborate analysis not being justified.

Acid-Base Buffering Properties

The acid-base buffering method described by Lucey et al. (1993) was used. Samples were titrated using a Mettler Toledo DL50 Autotitrator (Mettler Toledo, Greifensee, Switzerland). Milk samples were titrated from the initial pH of ∼6.5 to pH 3.0 with 0.5 N HCl and back-titrated to pH 9.0 with 0.5 N NaOH. The change in pH (dpH), caused by the incremental addition of acid or base, and the volume of titrant used in the titration were recorded by the titrator software and used to calculate buffering indices (dB/dpH), which were plotted as a function of pH.

Ca Analysis

A Prep/Scale-TFF membrane (Millipore, Billerica, MA), which was made from regenerated cellulose, was used to obtain UF permeates (Mizuno and Lucey, 2005). The molecular mass cut-off of this membrane was 10 kDa. Experiments were performed at 25°C. The Ca contents of milk and UF permeate (diluted with 0.2 N nitric acid) were determined using inductively coupled plasma optical emission spectrometry (Vista-MPX Simultaneous ICP-OES, Varian Inc., Palo Alto, CA). Wavelength of plasma emission used to measure the Ca was 317.9 nm (Park, 2000). Casein-bound Ca was calculated according to the equation of White and Davies (1958):

$$\text{Casein-bound Ca} = \text{Total Ca} - \text{Ca in UF permeate.} \quad [4]$$

Casein-bound calcium includes CCP and any other calcium associated with caseins (sometimes called caseinate calcium).

Rheological Properties

A Universal Dynamic Spectrometer (Paar Physica UDS 200 controlled stress rheometer, Physica Messtechnik GmbH, Stuttgart, Germany) was used to measure the rheological properties of yogurt. The small-strain
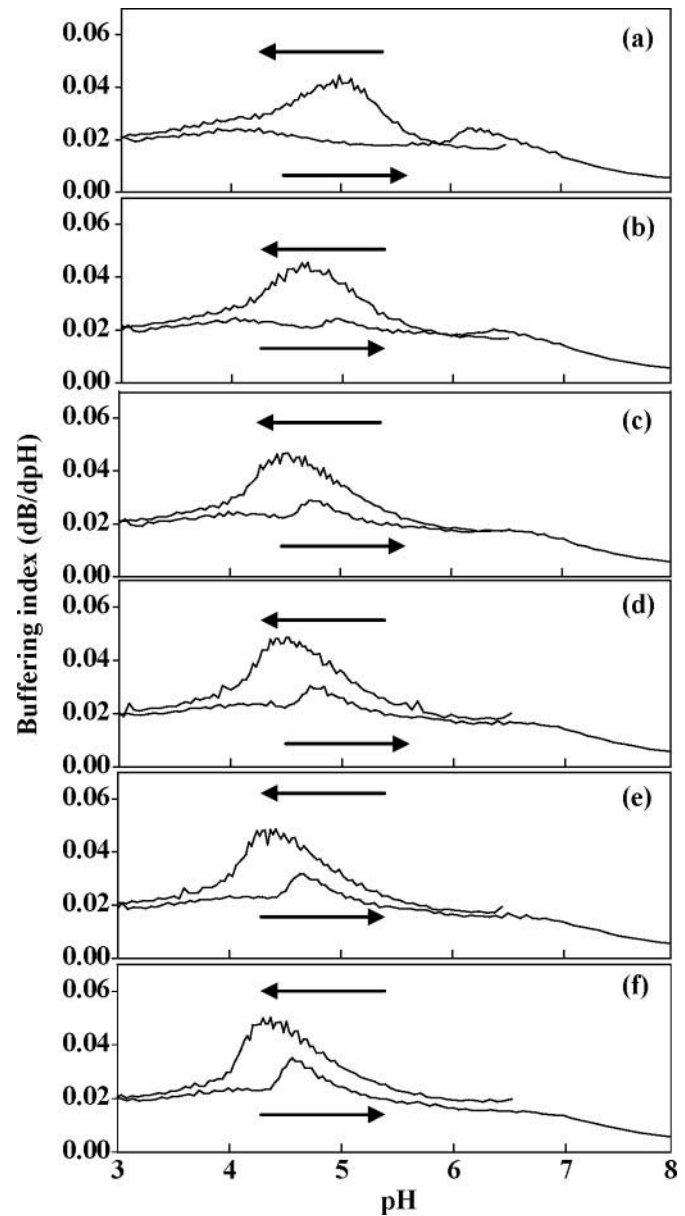

Figure 1. Acid-base buffering curves of milks containing different levels of tetrasodium pyrophosphate (TSPP): a) 0, b) 0.05, c) 0.10, d) 0.125, e) 0.150, and f) 0.2% TSPP (arrows indicate direction of titration).

rheological parameters storage modulus (**G′**) and loss tangent (**LT**) were recorded every 5 min until pH 4.6. A profiled cup-and-bob measuring geometry with co-axial cylinders (inner diameter 25 mm; outer diameter 27.5 mm) was used. The applied strain was 1% and the oscillatory frequency was 0.1 Hz. The milk sample was warmed to 42°C for 30 min in a waterbath, and 2% (wt/wt) starter culture was added to the milk, and mixed thoroughly for 2 min before 14 mL of the mixture was immediately placed in the cup of the rheometer. A few

drops of vegetable oil were added to the surface of the milk to prevent evaporation. Gelation was arbitrarily defined as the point at which the G′ of gels was greater than 1 Pa. The large deformation properties of yogurt gels formed in situ were determined by applying a single, constant shear rate (0.01 s^{-1}) up to the yielding of the gel. The yield stress (σ_{yield}) and shear deformation at yielding were defined as the point when the shear stress started to decrease. Yield strain (γ_{strain}) was the strain value at the yield point (Lucey et al., 1997).

Whey Separation

The (spontaneous) whey separation was assessed using the flask method of Lucey et al. (1998b). The degree of whey separation was calculated as a percentage of the total weight of milk. At least 8 flasks were used for each treatment.

Fluorescence Microscopy

The microstructure of yogurt gels was examined at pH 4.6 using the fluorescence microscopy method of Choi et al. (2007). Heat-treated skim milks were mixed with TSPP and adjusted to pH 6.5 with 1 N HCl. Fifty milliliters of skim milk were warmed to 42°C, inoculated with 2% (wt/wt) working starter culture, and mixed with 350 μL of acridine orange (0.2% wt/wt; Sigma Chemical Co., St Louis, MO), a fluorescent dye that binds to protein. After stirring, a few drops of the mixture were transferred to slides with a cavity, and a coverslip was placed over the sample. The slide was placed in a temperature-controlled incubator (model 650F, Fisher Scientific, Hanover, IL) and incubated at 42°C until the pH was ~4.6. Microstructure of yogurt samples was viewed with a fluorescence microscope (Axioskop 2 plus, Carl Zeiss, Eching, Germany). Deconvolution was used to remove out-of-focus material (Choi et al., 2007). Triplicate slides were prepared at each time point, and randomly selected fields that were typical for each slide were reported.

Statistical Analysis

The GLM procedure of SAS (version 9.01, SAS Institute Inc., Cary, NC) was used to analyze the experimental data. Analysis of least squares means was performed to determine differences among means. Level of significance was indicated by $P < 0.05$.

RESULTS AND DISCUSSION

Acid-Base Titration and Ca Analysis

The acid-base titration curves of milks with different levels of TSPP added are shown in Figure 1. Changes in the CCP content of milk can be inferred from the acid-base buffering profiles (Lucey et al., 1993). The buffering peak at pH ~5.1 during acid titration of milk is caused by the solubilization of CCP, and the peak at pH ~6 during base titration is due to precipitation of Ca phosphate (Lucey et al., 1993; Figure 1a). The addition of TSPP resulted in a decrease in the pH value of the buffering peak observed during acid titration (Figure 1b–f). Addition of 0.05% TSPP resulted in a reduction in the buffering peak observed at pH ~6 during titration with base and a small peak was also observed at pH 5 during titration with base (Figure 1b). Greater levels of TSPP (>0.05%) resulted in a shift in the acid-titration buffering peak observed in milk at pH 5.1 (Figure 1a) to a lower pH (between pH 4.0 and 4.5; Figure 1c–f). Greater levels of TSPP (>0.05%) also resulted in a shift in the peak observed during back titration with NaOH to pH values between 4 and 5 (Figure 1c–f), instead of at pH ~6.0 in control milk (Figure 1a). These new peaks are probably due to the formation of Ca pyrophosphate (Mizuno and Lucey, 2005). The disappearance of buffering peak at pH 6.0 during base titration could be related to conversion of CCP into a new type of Ca phosphate salt involving pyrophosphate. These results are in agreement with Mizuno and Lucey (2005) who reported on the effect of TSPP on the buffering curves of 5% milk protein concentrate solutions that had been preacidified to pH 5.8.

When even very low levels of TSPP (e.g., 0.05%) were added to the milks, a significant ($P < 0.05$) decrease in soluble Ca content and increase in amount of casein-bound Ca was observed (Table 1). The increase in the casein-bound Ca was probably due to the association of TSPP with casein molecules, resulting in the formation of a new type of Ca phosphate complex (i.e., that had different acid-base buffering properties than CCP). Similar findings for soluble Ca levels were observed with the addition of TSPP to milk protein solutions (Morr, 1967; Vujicic et al., 1968). Mizuno and Lucey (2005) reported that there was an increase in both casein-bound Ca and P when TSPP was added to milk protein concentrate solution.

Light-Scattering Properties

The influence of TSPP addition on turbidity of milk and particle size of micelles, as evidenced by the slope of the double-log plot of solution turbidity versus wavelength, is shown in Figure 2. With the addition of TSPP the turbidity of milk decreased until 0.125% TSPP was added; a time-dependent increase in turbidity was observed at greater levels. The decrease in turbidity argues for a partial disintegration of the micelles
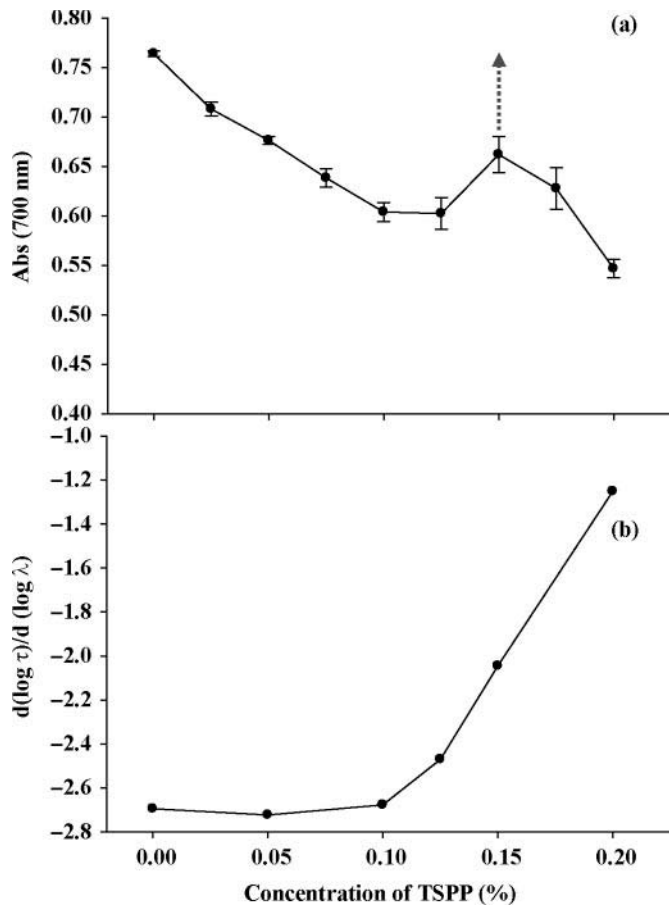
Figure 2. Effect of different levels of tetrasodium pyrophosphate (TSPP) on a) turbidity (absorbance at 700 nm) and b) particle size (where τ is turbidity, λ is the wavelength of light) of casein micelles. Means of triplicates; error bars indicate S; the dotted line in panel (a) indicates that an increase in turbidity was observed with time.

as a result of a sequestration of calcium by the TSPP, behavior that does not produce significant changes in apparent particle size as the unchanging slope parameter in this range of TSPP concentrations indicates (Figure 2b). The decrease in absolute value of slope

parameter combined with the time-dependent changes in turbidity indicate that larger particles or aggregates were formed at TSPP concentrations >0.1% (Figure 2). The subsequent drop in turbidity at the highest TSPP concentration employed is possibly due to sedimentation of the largest aggregates. Mizuno and Lucey (2007) reported that gelation occurred when 0.075 to 0.275% TSPP was added to 5% milk protein concentrate (pH 5.8) and the samples were held at 25°C for 24 h. Mizuno and Lucey (2007) suggested that TSPP-induced gelation occurs when the added TSPP acts together with calcium to behave as a cross-linking agent between dispersed caseins and the balance between (a reduced) electrostatic repulsion and (enhanced) attractive (hydrophobic) interactions becomes suitable for aggregation and eventual gelation of casein molecules. Thus, this gelation reaction is highly dependent on pH and TSPP concentration. Panouillé et al. (2004) reported that caseins could undergo heat-induced aggregation and gelation in the presence of polyphosphates.

Rheological Properties

The effects of TSPP on the rheological and physical properties of yogurt are summarized in Table 1. The gelation pH decreased with the addition of ≤0.125% TSPP and, as a result, gelation time also increased (Table 1). Gels made with 0.15% TSPP had similar gelation time and pH as the control sample. The addition of >0.15% TSPP resulted in a large increase in the gelation pH (6.3) and a very short gelation time. The initial reduction in gelation pH with low concentrations of TSPP could be due to the partial disruption of the casein particles. The high gelation pH (~5.4) of high-heat-treated milk (the control milk) is due to the presence of denatured whey proteins on the surface of casein micelles (Lucey et al., 1998a). It is believed that the greater isoelectric point of the main whey protein, β-lactoglobulin (~5.3), is responsible for increasing the gelation pH of yogurt made from high-heat-treated

Table 1. Effect of tetrasodium pyrophosphate (TSPP) on the Ca equilibrium in milk and the rheological and physical properties of yogurt made from TSPP-treated milks[1]

TSPP (%)	Gelation time (min)	pH at gelation	G′ at pH 4.6 (Pa)	LT value at pH 5.1	σ_{yield} (Pa)	γ_{yield}	Soluble Ca (mg/100 g of milk)	Casein-bound Ca (mg/100 g of milk)
0	120[c]	5.42[b]	180[a]	0.52[a]	71[a]	0.52[a]	25.8[a]	99.5[f]
0.050	134[b]	5.28[c]	156[b]	0.47[c]	45[b]	0.44[a]	22.7[b]	102.6[e]
0.100	141[ab]	5.21[cd]	178[a]	0.44[d]	44[b]	0.48[a]	18.7[c]	106.5[d]
0.125	146[a]	5.17[d]	143[b]	0.43[d]	48[b]	0.49[a]	16.0[d]	109.2[c]
0.150	115[c]	5.47[b]	8[c]	0.50[ab]	3[c]	0.47[a]	14.6[e]	110.7[b]
0.200	40[d]	6.35[a]	8[c]	0.48[cb]	1[c]	0.48[a]	12.4[f]	112.5[a]

[a–f]Means of quadruplicate values with different letters in the same column significantly different ($P < 0.05$).

[1]Gelation time was defined as the point when gels had a storage modulus ≥1 Pa; G′ = storage modulus; LT = loss tangent; σ_{yield} = yield stress; γ_{strain} = strain value at yielding (no unit).

milk compared with the low gelation pH (4.9–5.0) for yogurt made from unheated milk. Low levels of TSPP partly disrupted casein micelles, as indicated by the reduction in turbidity (Figure 2a). When these (disrupted) particles aggregated during acidification, the surface of these altered casein particles probably had less denatured whey proteins and so the gelation pH tended toward the gelation pH of unheated caseins. A similar trend of a reduction in gelation pH and increase in gelation time was observed when TSC was added to milk before yogurt manufacture (Ozcan-Yilsay et al., 2007).

The increase in gelation pH with greater levels of TSPP ($\geq$0.15%) is probably due to aggregation of TSPP-treated caseins because it has previously been reported that, at similar concentrations of TSPP, caseins may undergo aggregation and gelation (Mizuno and

Lucey, 2007). Supporting evidence for the aggregation of TSPP-treated micelles ($\geq$0.15%) is also provided by the time-dependent increase in turbidity (Figure 2a) and increased particle size (Figure 2b) observed with this level of TSPP addition.

The G´ values at pH 4.6 of gels made with 0.1% TSPP were not significantly different from the control, although the addition of 0.05% TSPP produced slightly weaker gels (Table 1). The addition of $\geq$0.125% TSPP significantly ($P < 0.05$) decreased the G´ values at pH 4.6 of yogurt gels. The decrease in the G´ values at pH 4.6 of yogurt gels with $\geq$0.125% TSPP could be due to the reduction in CCP cross-linking (as indicated by the reduction in CCP buffering peak at pH 4.8; Figure 1) and casein dispersion. The G´ values at pH 4.6 are a reflection of the number and strength of bonds between casein particles and the distribution of casein
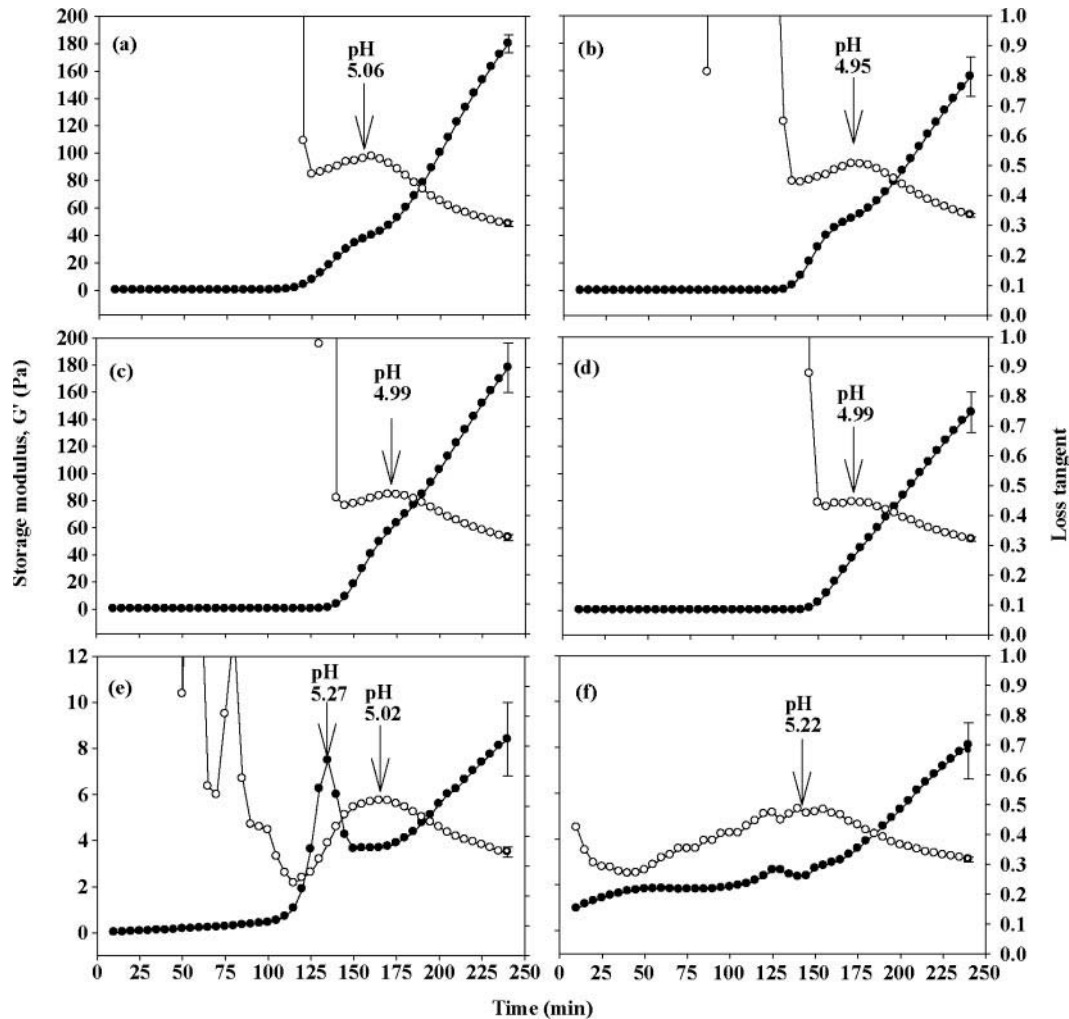

Figure 3. Storage modulus (G´, ●) and loss tangent (LT, ○) as a function of time for yogurts made from milk treated with tetrasodium pyrophosphate (TSPP): a) 0, b) 0.05, c) 0.10, d) 0.125, e) 0.150, and f) 0.2% TSPP. Note the different scale for the G´ axis in Figures 3e and f. Means of triplicates; error bars indicate SD.
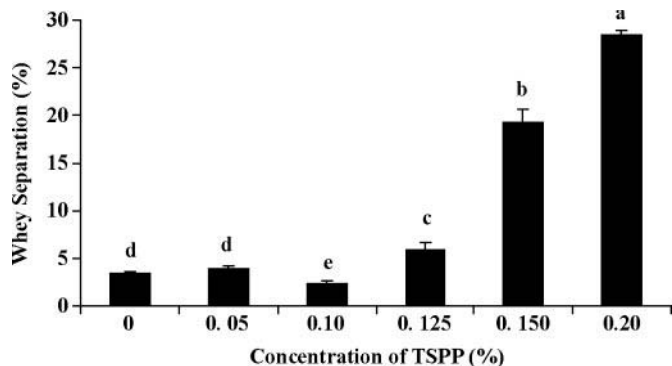
Figure 4. Whey separation of yogurts made from milk with different levels of tetrasodium pyrophosphate (TSPP). Whey separation was measured when gels reached at pH 4.6. Means of triplicates; error bars indicate SD. [a–e]Bars with different letters are significantly different ($P < 0.05$).

strands in the network (Zoon et al., 1988). High levels of TSPP addition also increased the gelation pH, which indicated that another aggregation reaction may be occurring that initiated gelation sooner than just milk acidification alone.

The rheological properties of yogurts made with TSPP are shown in Figure 3. The G′ profiles for yogurts made with 0 or 0.05% TSPP exhibited a shoulder at pH ~5.1 to 5.0, which coincided with the occurrence of the maximum in the LT (Figure 3a,b). However, no clear shoulder was observed in the G′ profiles for yogurts with 0.1 or 0.125% TSPP and no distinct maximum in the LT was observed in these gels (Figure 3c,d). The G′ profiles for yogurts made with 0.15% TSPP were unusual as they exhibited a local maximum in G′ at around pH 5.27, and then G′ values decreased before increasing again (Figure 3e). A possible explanation of the local maximum in G′ could be the formation of a weak TSPP-induced casein gel that weakened with the ongoing decrease in pH. The Ca pyrophosphate complexes involved in the TSPP-induced gelation (Mizuno and Lucey, 2007) presumably start to solubilize at pH values around 5 (Lyster, 1979) because this pH range coincides with the start of the buffering peak observed during acid titration of this sample (Figure 1e). The G′ values for yogurt made with 0.15% TSPP were very low (<10 Pa). The "gel" made with 0.2% TSPP appeared to aggregate shortly after starter culture addition but did not exhibit a local maximum in G′ and only formed a very weak gel at pH values <5.0 (Figure 3f). The very high gelation pH (6.3) indicates that TSPP-induced aggregation probably occurred in this sample but that the ongoing decrease in pH during fermentation did not facilitate the formation of a strong gel network.

The LT profiles as a function of time for yogurts gels are shown in Figure 3. Gels with low TSPP levels

(≤0.1%) exhibited maximum in the LT, which coincided with the appearance of a shoulder in the G′ profile. The maximum in LT indicates a loosening of the intermolecular forces in casein particles (van Vliet et al., 1991) that are the structural components of the network resulting from the solubilization of CCP cross-links (Lucey, 2001). Yogurt gels made from heat-treated milks exhibit a maximum in LT (e.g., Lee and Lucey, 2004a,b). Gels made with 0.125% TSPP did not exhibit a distinct maximum in LT after gelation, only a relatively constant LT value initially and then a slow decline as the pH decreased toward 4.6 (Figure 3d). Gels made with 0.05 to 0.125% TSPP had significantly lower LT values at pH ~5.1 compared with the control yogurt (Table 1). A very large increase in LT after gelation was observed in gels made with 0.15% TSPP, which indicated increased bond mobility and the likelihood of greater particle rearrangement. These factors could have contributed to the local maximum observed in the G′ profile of this sample (Figure 3e). The LT profile for the yogurt sample made with 0.2% TSPP exhibited a broad LT peak that started from pH ~6.2 and had a maximum at around 5.2.

The large deformation rheological properties for yogurts are listed in Table 1. The addition of TSPP had a significant affect ($P < 0.05$) on σ_{yield} values of yogurt. The σ_{yield} significantly decreased with the addition of TSPP but γ_{yield} was not significantly affected (Table 1). Yogurts with 0.05 to 0.125% TSPP had similar σ_{yield} values albeit significantly lower than those in samples made with 0% TSPP. Presumably, the reduction in the number of CCP cross-links in these samples contributed to the reduction in their σ_{yield} values. The addition of ≥0.150% TSPP to milk resulted in yogurt gels with very low σ_{yield} values (<3 Pa), probably because of dispersion of casein particles and the effect of TSPP-induced casein aggregation.

Whey Separation

The levels of whey separation in yogurts made with different concentrations of TSPP are shown in Figure 4. Gels made with 0 and 0.05% TSPP had similar whey separation levels. High TSPP levels (>0.1%) resulted in significantly ($P < 0.05$) increased whey separation. High TSPP levels (>0.1%) produced very weak gels. Lee and Lucey (2004a,b) and Ozcan Yilsay et al. (2007) also reported that weak yogurt gels (low G′ and low σ_{yield} values) exhibited high levels of whey separation.

The lowest whey separation level was observed for gels made with 0.1% TSPP (Figure 4). Ozcan Yilsay et al. (2007) reported that low levels of TSC (5 to 20 mM) addition to milk reduced whey separation in yogurt. Ozcan Yilsay et al. (2007) suggested that partial removal of CCP cross-links may have facilitated greater
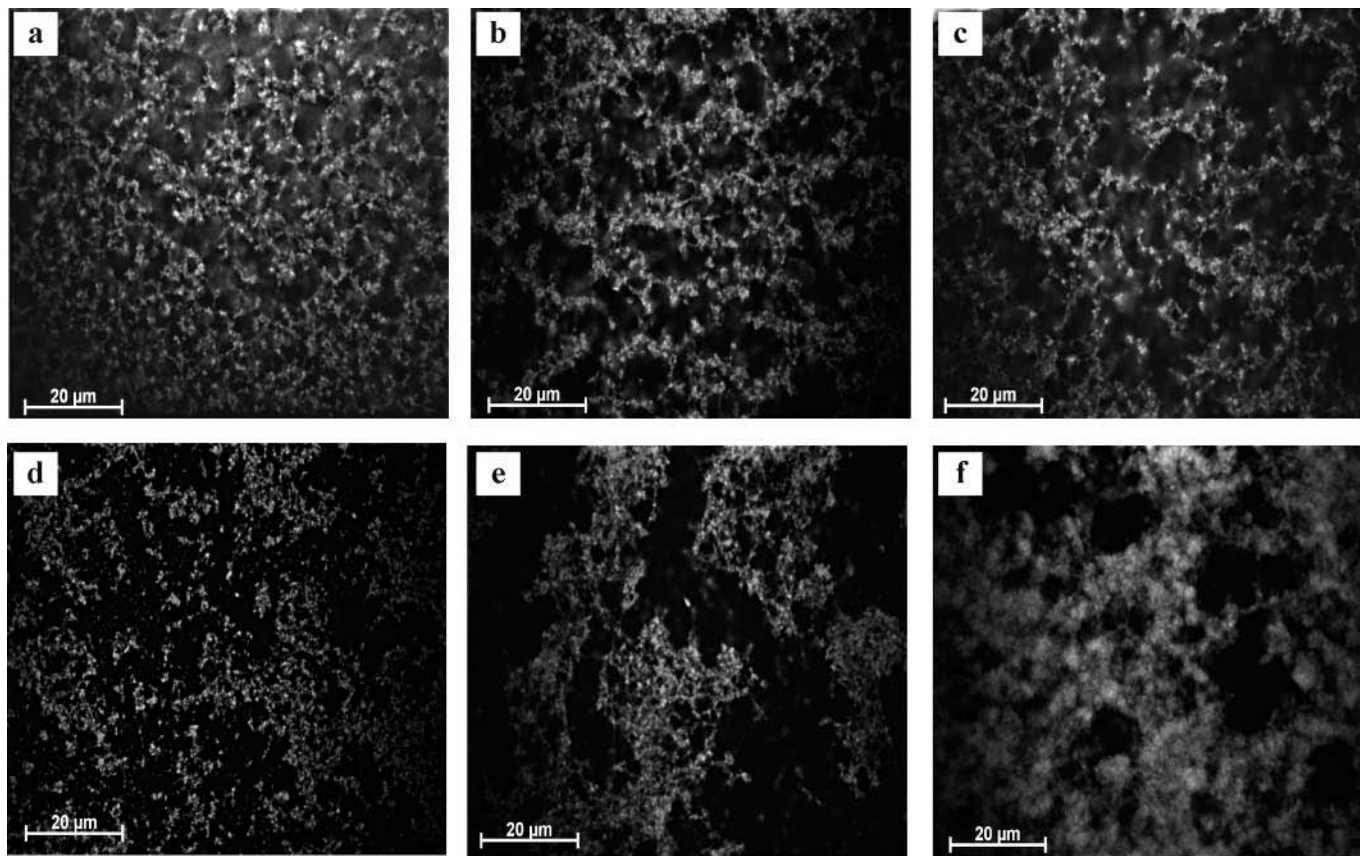
Figure 5. Microstructure of yogurt gels made from milk treated with a) 0, b) 0.05, c) 0.10, d) 0.125, e) 0.150, or f) 0.2% tetrasodium pyrophosphate (TSPP). Yogurt gels were formed at 42°C and examined at pH 4.6. The protein matrix is white and pores are dark. Scale bar = 20 μm.

rearrangement and molecular mobility of the micelle structure, which may have helped to increase G′ and LT values of TSC-treated gels by increasing the formation of cross-links between strands. A different mechanism may be occurring in the 0.1% TSPP-treated milks because the G′ values did not increase and LT values actually decreased compared with gels made from 0% TSPP. The addition of 0.1% TSPP caused a reduction in the gelation pH and probably more CCP was already solubilized before gelation; greater solubilization of CCP from casein particles after gelation has been related to increased whey separation (Lucey, 2001). At low concentrations of TSPP there was partial disruption of micelles (reduced turbidity) but an increase in the casein-bound Ca (Table 1) presumably because of the formation of Ca pyrophosphate complexes (Mizuno and Lucey, 2007). It may be that partial disruption of the micelle in conjunction with greater cross-linking of caseins by Ca pyrophosphate altered the microstructure such that there was a reduction in pore size or permeability. The addition of ≥0.125% TSPP caused a measurable aggregation of casein particles (Figure 2) but these TSPP-induced aggregates or the resultant

weak gel network appeared to be disrupted during subsequent acidification (Figure 3e).

Microstructure

The microstructure of yogurt gels made from milk with various levels of added TSPP is shown in Figure 5. Yogurt gels made with 0, 0.05, and 0.1% TSPP (Figure 5a–c) had small pores and a lot of interconnections between strands. In gels made with 0.125% TSPP there appeared to be a change or transition in the type of gel structure with fewer interconnections between clusters (Figure 5d). Gels made with 0.150 and 0.2% TSPP had very large pores and very little interconnectivity (Figure 5e,f) in agreement with the high whey separation and low G′ value at pH 4.6 (Table 1). These trends are in agreement with low whey separation in gels made with ≤0.1% TSPP and very weak gels with considerable wheying-off at greater TSPP concentrations.

CONCLUSIONS

The addition of TSPP had a significant effect on the properties of yogurt gels. At very low levels of TSPP

($\leq$0.1%), the G´ values at pH 4.6 were not significantly different from yogurt made without TSPP, and there was a decrease in the LT values at pH 5.1 and the σ_{yield} values. There was an increase in casein-bound Ca due to the association of Ca pyrophosphate with caseins. The partial disruption of casein micelles, the increased association of Ca with caseins (cross-linking), and the reduced bond mobility (as indicated by the reduced LT) probably helped to reduce whey separation in gels made with 0.1% TSPP. When greater concentrations of TSPP were added to milk, TSPP-induced aggregates were formed, resulting in an increase in the gelation pH; the addition of 0.2% TSPP resulted in a gelation pH of >6. This TSPP-induced weak gel network weakened during acidification of milk as these types of Ca phosphates were solubilized with acidification. At pH values <5 milk is unsaturated with respect to all types of Ca phosphates (Lyster, 1979). Large clusters and pores were visible in the micrographs of yogurts made with high levels of TSPP.

Tetrasodium pyrophosphate has a long history of use in process cheese products where it is often used to reduce melt (Berger et al., 1998). Tetrasodium pyrophosphate is a permitted stabilizer in foods according to the US Code of Federal Regulations (CFR, 2003). It appears possible, therefore, that very low levels of TSPP could be used to reduce the whey separation defect in yogurts. Compared with the control yogurt, the use of 0.1% TSPP reduced whey separation by 35%. In some countries (e.g., Japan), low levels (<0.1%) of TSPP are permitted in flavored fermented milk products as acidity regulators. Codex Alimentarius is currently revising its draft standard for flavored fermented milks and there are proposals to implicitly include TSPP as a permitted additive up to a level of 970 mg/kg (Codex Alimentarius, 2008).

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