Effects of Polymerized Whey Proteins on Consistency and Water-holding Properties of Goat's Milk Yogurt

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ABSTRACT: The effects of polymerized whey proteins (PWP) on functional properties of goat's milk yogurt were investigated. PWP were prepared by heating whey protein isolate (WPI) dispersion (8.0% protein, pH 7.0) at 90 °C for 30 min. Three reconstituted goat milk (RGM) (12% total solids [TS] as control; RGM with 2.4% unheated WPI; and RGM with 2.4% PWP) and 1 RGM with 16.7% TS were prepared and inoculated with 0.04% yogurt starter culture. Inoculated milk was incubated at 43 °C for 5 h, cooled to 4 °C in an ice-water bath, and then placed at refrigerator (4 °C) overnight before testing. Incorporation of PWP significantly ($P < 0.001$) increased the viscosity (by 80%) and decreased the syneresis (by 25%) of the yogurt samples, whereas addition of unheated WPI did not significantly affect the viscosity and syneresis compared with the control. There were no changes in pH, TS, ash, fat, protein, and lactose contents among yogurt samples except the solids fortified control. Yogurt with 16.7% TS had the lowest syneresis but did not improve in viscosity. Transmission electron microscopy micrographs demonstrated that the microstructure of the goat's milk yogurt gel with PWP was denser than the control. Results of this study indicate that polymerized whey proteins may be a novel protein-based thickening agent for improving the functional properties of goat's milk yogurt and other similar products.

Keywords: polymerized whey proteins (PWP), goat's milk, yogurt, viscosity, syneresis

Introduction

Goat's milk has been described as having a higher digestibility and lower allergenic properties than cow's milk. Goat's milk products, such as yogurt and cheese, are becoming increasingly popular in the United States as specialty products and as substitutes for cow's milk products for those who having allergies against cow's milk (Park 1994a; Haenlein 1996). However, it is difficult to produce goat's milk yogurt with consistency comparable to cow's milk yogurt (Abrahamsen and Rysstad 1991), mainly due to the low level of α_{sl}-casein and seasonal changes in chemical composition of the milk (Guo 2003). α_{sl}-Casein, 1 of major caseins in cow's milk, is a structural component of the casein micelle and plays a major role in milk coagulation (Walstra and others 1984). Depending on breeds, α_{sl}-casein is found in relatively low or undetectable amounts in goat's milk (Guo 2003).

Traditional methods used commercially to improve the texture of yogurt include increasing the total solids in the milk and in the case of stirred yogurt adding stabilizers, for example, pectin and gelatin (Lucey and Singh 1998). Emerging approaches to modifying the texture of cultured dairy products include: novel stabilizers, various types of milk-derived ingredients, use of various types of membrane concentrate or fractions, specific cultures (for example, producing a specific exopolysaccharide type and content), enzymatic cross-linking of milk proteins, for example, transglutaminase, use of high hydrostatic pressure (for example, >200 MPa) to the milk to cause denaturation of whey proteins or of the yogurt to prevent postacidification, and very high pressure homogenization (Lucey 2004).

The unique functional and nutritional properties of whey proteins make them useful food ingredients for a wide array of applications (Morr and Ha 1993; de Wit 1998). In particular, the ability of whey proteins to form gels capable of holding water and other components, and providing textural properties is very important to the consumer acceptability of many foods such as processed meat, dairy, and bakery products (Ju and Kilara 1998).

It has been suggested that use of polymerized whey proteins (PWP) can improve the yogurt firmness and syneresis (Britten 2002). According to Vardhanabhuti and others (2001), the term "polymerized whey proteins" referred to soluble whey protein aggregates that are formed when heated at temperature and protein concentration that would normally form a gel but do not due to the low salt condition. An important functional property of PWP is their ability to form a gel under ambient temperatures (cold-set gelation). Cold-set whey protein gelation has been described by Barbut and Foegeding (1993), McClements and Keogh (1995), Nakamura and others (1995), and Sato and others (1995). In these studies, PWP were prepared and cooled to room temperature, at which gelation was induced by the addition of $CaCl_2$ or NaCl. Acidification with glucono-δ-lactone (Kawamura and others 1993) or acid naturally produced via fermentation using lactic acid bacteria (Li and Chen 2001; Alting and others 2004) have also been applied to achieve cold-set gelation following a thermal pretreatment.

Milk proteins that are able to mimic the thickening functionality of gelatin, hydrocolloids, and other thickeners such as starches in food systems have been discussed by Hoch (1997) and Hudson and others (2000). The use of whey proteins in formulated foods has been increased in recent years. Controlled aggregation of whey proteins is used to increase the viscosity and improve the mouth feel of liquid products (Britten 2002). Similar to hydrocolloid functionality, the bacterially acidified cold gelation property of whey proteins can be exploited to improve the body texture and enhance

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the water-binding property of fermented dairy foods, such as yogurt products. The objective of this study was to examine the effects of polymerized whey proteins on consistency and water holding properties of goat's milk yogurt.

Materials and Methods

Starter and ingredients

The starter culture, Yo-Fast 10 (Chr Hansen, Milwaukee, Wis., U.S.A.), was a blend of strains of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp *bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium*, and *Lactobacillus* subsp *casei*. The cultures were stored at –70 °C in a concentrated form before use. Meyenberg instant powdered goat milk (whole milk, pasteurized, and spray-dried) was supplied by Jackson-Mitchell, Inc. (Turlock, Calif., U.S.A.). Whey protein isolate powder (WPI, ALACEN® 895) derived from cow's milk was obtained from NZMP (North America) Inc. (Santa Rosa, Calif., U.S.A.). According to the manufacturer, the WPI contains 93.0% protein, 0.3% fat, 0.5% lactose, 2.2% ash, and 4.0% moisture.

Preparation of polymerized whey proteins

Polymerized whey proteins (PWP) were prepared using a thermal denaturation method. WPI dispersion (8.0% protein) was prepared in distilled water and left overnight at 4 °C to equilibrate. The dispersion was brought to room temperature, adjusted to pH 7.0 using a 2.0 *M* NaOH solution, and divided into 2 portions. One portion of the dispersion was subjected to heating (90 °C, 30 min) in a water bath and cooled at room temperature for 2 h. Another portion of the dispersion (unheated) was used for comparison.

Yogurt preparation

Three reconstituted goat milk (RGM) (about 12% total solids [TS] as control; RGM with 2.4% unheated WPI dispersion; and RGM with 2.4% PWP) and 1 RGM with about 16.7% TS (as solids fortified control) were prepared and inoculated with 0.04% Yo-Fast 10 yogurt starter culture. Inoculated milk was incubated at 43 °C for 5 h, cooled in an ice-water bath with slow overhead stirring (50 rpm) for 10 min, and then placed at refrigerator (4 °C) overnight before testing. Stirred yogurts prepared from the above different treatments were coded A, B, C, and D, accordingly. Incorporation of 2.4% whey protein dispersion (8.0% protein) into RGM is equivalent to addition of about 0.2% of whey proteins, which is within the range of the common usage level (0.1% to 0.4%) in cultured products for gum stabilizers (Lucey 2004). Testing of each yogurt formulation was done in duplicates. The experiment was duplicated.

Physicochemical analysis

Total solids of the yogurt were measured by the forced-draft oven method, while the protein and fat contents of the samples were determined by the Kjeldahl and Babcock Methods (AOAC 2002). Ash content and titratable acidity (TA) were measured according to AOAC (2002) and lactose content was determined by the difference of TS minus other solid components as described by Guzman-Gonzalez and others (1999). The pH values of the yogurt samples were measured with a pH meter (model 240; IQ Scientific Instrument, Inc., San Diego, Calif., U.S.A.).

Rheological measurements

The viscometry measurements of yogurt samples were performed in shear mode by a procedure adapted from Keogh and O'Kennedy (1998). Tests were carried out at 4 °C using a controlled strain rheometer (model AR-1000; TA Instrument, New Castle, Del., U.S.A.), equipped with a 40-mm stainless-steel cone and plate geometry with a cone angle of 2°. A shear rate sweep from 1/s to 200/s was carried out to determine the relationship

$$\eta = k\gamma^{n-1}$$

where η is the viscosity (Pa.s), k is the consistency index (Pa.s^n), γ is the shear rate (/s), and n is the power law factor (Keogh and O'Kennedy 1998). The solvent trap was always used to prevent evaporation.

More empirical measures of viscosity were obtained using a Brookfield viscometer (model DV-II+; Brookfield Engineering Labs, Inc., Middleboro, Mass., U.S.A.) with a nr 4 spindle rotating at 100 rpm. The sample temperature was 4 °C. For relative comparison between treatments, viscosity reading was taken at the point of 30th s and torque was maintained at all times between 10% and 100%. The viscosity determined with the Brookfield viscometer is known as the Brookfield viscosity in the following text of this article to distinguish it from the apparent viscosity measured by the rheometer.

Syneresis measurement

Syneresis of the yogurt was determined by the centrifugation procedure of Keogh and O'Kennedy (1998) with modifications. A sample of about 200 g of yogurt (Y) was prepared in a centrifuge cup and centrifuged for 10 min at 2500 rpm (average 640 × *g*) at 4 °C. The whey expelled (W) was removed and weighed. The syneresis was calculated as:

$$\text{Syneresis (\%)} = (W/Y) \times 100\%$$

Transmission electron microscopy

Microstructure of the yogurt samples were examined by transmission electron microscopy (TEM). The sampling was carried out by embedding the yogurt samples into agar as described by Schellhaass and Morris (1985). The agar cubes were fixed overnight at 4 °C in 2.5% glutaraldehyde in cacodylate buffer (pH 7.2), washed 3 times in fresh cacodylate buffer (pH 7.2), then post-fixed in 1% osmium tetroxide for 2 h at room temperature followed by 3 rinses in fresh buffer. After dehydration using a graded series of ethanol (10% to 100% 2×), the samples were transferred into propylene oxide (2–), then into a mixture of 50:50 propylene oxide and epoxy resin (Embed 812/Araldite 502, Electron Microscopy Science, Hatfield, Pa., U.S.A.) and allowed to infiltrate overnight at room temperature. After infiltration, the samples were transferred through 3 changes of freshly prepared epoxy resin and finally polymerized in a 60 °C oven for 48 h. Pieces of the polymerized blocks were cut and mounted onto plastic stubs with super glue and trimmed and sectioned (60 to 70 nm thick) and stained with uranyl acetate and lead citrate. The samples were examined using a Philips CM10 transmission electron microscope at 80 kV.

Statistical analysis

Data were analyzed by a general linear model procedure of the Fisher's protected-least-significant-difference test using SAS (SAS Inst., Cary, N.C., U.S.A.). This test combines analysis of variance (ANOVA) with comparison of differences between the means of the treatments at the significance level of $P < 0.05$.

Results and Discussion

Physicochemical characteristics

Gross composition, TA, and pH of goat's milk yogurts are listed in Table 1. No significant differences ($P > 0.05$) in chemical composition between control yogurt (A) and samples with whey proteins (B

Table 1—Gross composition, titratable acidity (TA), and pH of goat's milk yogurt samples[a]

	Goat's milk yogurt[b]			
Parameters	A	B	C	D
Total solids (TS, %)	11.94 ± 0.06b	11.95 ± 0.07b	11.89 ± 0.02b	16.68 ± 0.02a
Protein (%)	3.44 ± 0.01b	3.53 ± 0.03b	3.52 ± 0.04b	4.80 ± 0.01a
Fat (%)	3.38 ± 0.09b	3.27 ± 0.13b	3.29 ± 0.08b	5.00 ± 0.38a
Ash (%)	0.79 ± 0.01b	0.78 ± 0.01b	0.79 ± 0.01b	1.12 ± 0.01a
Lactose (%)[c]	4.33 ± 0.10b	4.37 ± 0.12b	4.29 ± 0.07b	5.77 ± 0.37a
TA (%)	0.787 ± 0.006c	0.796 ± 0.003c	0.806 ± 0.005b	1.036 ± 0.008a
pH	4.36 ± 0.01b	4.36 ± 0.02b	4.35 ± 0.02b	4.53 ± 0.02a

[a] Values with different letters within a row differ significantly ($P \leq 0.05$); each value is the mean ± standard deviation; $n = 4$.
[b] A = control; B = yogurt prepared from reconstituted goat milk (RGM) with 2.4% unheated whey protein isolate (WPI); C = yogurt prepared from RGM with 2.4% polymerized whey proteins (PWP); D = solids fortified control.
[c] Calculated by difference.

and C) were observed. Percentage of TS, protein, fat, ash, and lactose of the yogurt samples were similar (11.90%, 3.45%, 3.30%, 0.79%, and 4.30%, respectively).

The 3 goat's milk yogurts (A, B, and C) prepared in this study had similar total solids, ash, and lactose, but lower protein and higher fat contents when compared with commercial U.S. goat's milk yogurts (Park 1994b). Chemical composition of yogurt varies depending on the type of raw materials used, type of yogurt manufactured, fortification methods, and so forth. As expected, enrichment of goat milk powder resulted in a significantly higher TS content in yogurt product (D) and accompanied by increase in protein, fat, ash, lactose, and TA compared with the control (Table 1).

There was no significant differences in pH among A, B, and C, whereas a significantly higher pH was noted in D (Table 1), presumably due to higher buffering capacity from increasing protein content in the milk. The pH of the control yogurt (4.36; Table 1) was higher than the pH (4.25) of the other goat's milk yogurt formulation reported by Posecion and others (2005). In the present study, the RGM was used, whereas in the Posecion and others (2005) study, the goat's milk was concentrated before fermentation; the resultant concentration of the natural acids in the milk and/or the nature and activity of the starter culture used in this study may account for the difference in pH.

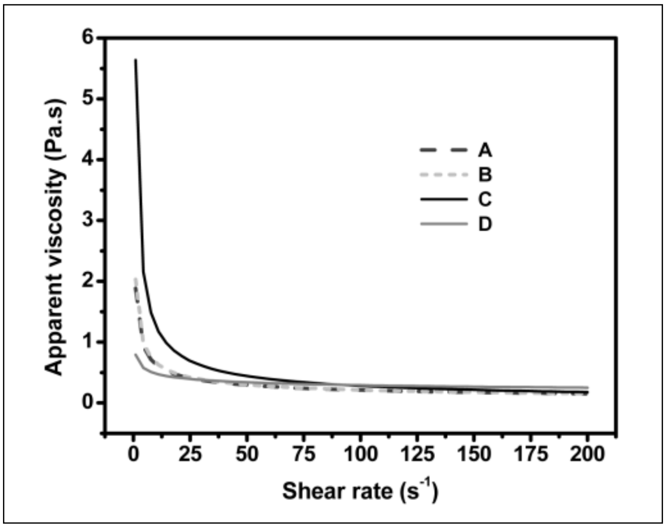

Figure 1—Flow properties of goat's milk yogurt samples prepared from reconstituted goat milk (RGM) with about 12% total solids (TS) (a; control), RGM with 2.4% unheated WPI (b), RGM with 2.4% PWP (c), and RGM fortified with 4.7% solids (d).

Rheological properties

The shape of the typical flow curves (Figure 1) of the goat's milk yogurts (stirred) resembles the shape of the curves that is usually found for weakly aggregating dispersions (de Rooij and others 1993; Potanin 1993). The apparent viscosity of yogurt continuously decreases with increasing shear rate due to breakup of the aggregates. Eventually, when all aggregates are broken up and only colloidal particles are present, hydrodynamic forces dominate all other forces and the sample becomes Newtonian, that is, has a constant viscosity. The flow behaviors of Figure 1a and 1b were similar; Figure 1c had the highest apparent viscosity values, whereas Figure 1d had the lowest values at the low shear rate (<15/s) region (Figure 1).

It has been known for some time that whey proteins become associated with casein micelles during the heat treatment of milk (Sawyer 1969). The whey protein association with casein micelles during heating increases the surface hydrophobicity of the micelles and favors micelle connections during the gelation by acidification (Mottar and others 1989). It has also been shown that the whey protein association with casein micelles favors the association of gel fragments after stirring during the recovery in gel structure (Cayot and others 2003). The flow behavior of Figure 1c suggests that preheated whey proteins (that is, PWP) were able to associate with casein micelles during the fermentation. The hydrophobicity of the particles of stirred firm gels (set gel fragments) in C is increased by the high hydrophobicity of the constituted casein micelles from PWP-added milk. It consequently enhances the ability of these particles to aggregate (or combine) after stirring. This tendency to aggregate would be retained in stirred gel and would facilitate the recovery in a gel state (Cayot and others 2003) and thus results in a stronger gel (resistance to initial shearing).

The consistency index (k) and power law factor (n) for the control goat's milk yogurt were 1.691 Pa.s^n and 0.534, respectively (Table 2). Incorporation of PWP significantly ($P < 0.001$) increased the k value (by 3.449 Pa.s^n) and decreased the n value (by 0.177) of the yogurt samples, whereas addition of unheated WPI did not significantly affect the values of k and n compared with the control (Table 2). On the other hand, yogurt enriched with goat milk powder (Table 2, yogurt D) had the lowest k value and the highest n value. Gum stabilizers such as gelatin and xanthan gum/locust bean gum have been shown to increase the consistency index k and decrease the power law factor n of yogurts (Keogh and O'Kennedy 1998). Many polymeric materials, and particularly yogurt, display greater shear-thinning (reduced n value) as k increases (Ramaswamy and Basak 1991).

Effects of various treatments on the Brookfield viscosity of stirred goat's milk yogurt were compared (Figure 2). Methods of adding unheated WPI and increasing TS of the milk did not improve the yogurt viscosity. In contrast, addition of PWP increased

Table 2–Consistency index (k) and power law factor (n) of goat's milk yogurt samples[a]

Yogurt[b]	k (Pa.s^n)	n
A	1.691 ± 0.222b	0.534 ± 0.011b
B	1.679 ± 0.544b	0.529 ± 0.019b
C	5.140 ± 0.855a	0.357 ± 0.021c
D	0.733 ± 0.083b	0.793 ± 0.013a

[a] Values with different letters within a column differ significantly ($P \leq 0.05$); each value is the mean ± standard deviation; $n = 4$.

[b] A = yogurt prepared from reconstituted goat milk (RGM) with about 12% total solids (TS) (control); B = yogurt prepared from RGM with 2.4% unheated whey protein isolate (WPI); C = yogurt prepared from RGM with 2.4% polymerized whey proteins (PWP); D = yogurt prepared from RGM fortified with 4.7% solids.

($P < 0.001$) the viscosity of yogurt by 80% in comparison to the control (Figure 2).

Stabilizers are commonly added to control textural defects and increase consistency in stirred-type yogurt (Lucey and Singh 1998). Results of the present study show that PWP could be used as a novel protein-based thickening agent in the production of goat's milk yogurt.

Syneresis

Syneresis shown in Figure 3c was reduced ($P < 0.05$) by 25% compared with that in Figure 3a (control). No significant differences in syneresis were observed between Figure 3b and 3a. On the other hand, enrichment of milk with goat milk powder resulted in yogurt (Figure 3d) with minimal syneresis. Our results confirmed the statement of Britten (2002) where use of polymerized whey proteins in yogurt formulations reduces syneresis. In contrast, Schorsch and others (2001) reported that gels formed from the mixture of preheated whey proteins and casein micelles are more prone to syneresis.

Whey separation can be defined as the appearance of whey (serum) on the gel surface (for example, of a set yogurt). Syneresis is the shrinkage of the gel, which then leads to whey separation.

Common reasons for the occurrence of syneresis include the use of a high incubation temperature, excessive whey protein to casein ratio, low solids content, and physical mishandling of the product during storage and retail distribution (Lucey 2004). Yogurt, which is an acid milk gel formed by gradual acidification with a lactic starter, has some problems of syneresis with a change of temperature or physical impact. Common means of preventing whey syneresis include enrichment of dry matter and/or of protein content as well as addition of hydrocolloids such as gelatin and starch. Use of PWP may also have comparable effects due to its unique acid-induced cold-set gelation property. As whey protein gel has strong water-holding capacities, the yogurt made from milk added with PWP has a greater capacity for immobilization of the aqueous phase in the yogurt network, thus reducing susceptibility to syneresis. On the other hand, as evidenced by the electron micrographs reported in this study, PWP were able to attach to the surface of casein micelle, and this in turn favors the formation of bridges between the casein particles leading to a narrow pored mixed casein/PWP network. As pore size reduces, the protein network will result in smaller syneresis (Farnsworth and others 2006).

For cultured dairy products, the challenges include controlling the texture and reducing the whey separation with lower milk solids (due to their high cost) and fewer stabilizers (due to growing consumer interest in cultured products like yogurt as a healthy food) (Lucey 2004). Our results suggest that PWP may be used as a novel dairy-based alternative to replace conventional stabilizers (for example, gelatin, hydrocolloids, and starches), as well as to minimize the use of milk solids. Therefore, a cost reduction by a reduction in the nonfat solid content especially for nonfat goat milk powder is made possible.

Microstructure analysis

Electron micrographs (× 38750) of yogurts with the same total solids contents (a, b, and c) are compared (Figure 4). The microstructures of Figure 4a and 4b were similar by TEM. Casein micelles were aggregated together in large protein clusters and showed less branched (Figure 4a and 4b), resembling casein micelle network of plain unfortified yogurt gels made from unheated cow's milk (Harwalkar and Kalab 1980). The microstructure of Figure 4c was significantly different from those of Figure 4a and 4b. Much smaller fat globules were observed in Figure 4c compared with other yogurts. In addition, TEM micrographs also revealed that most casein micelles in Figure 4c appeared in the form of small individual entities sur-

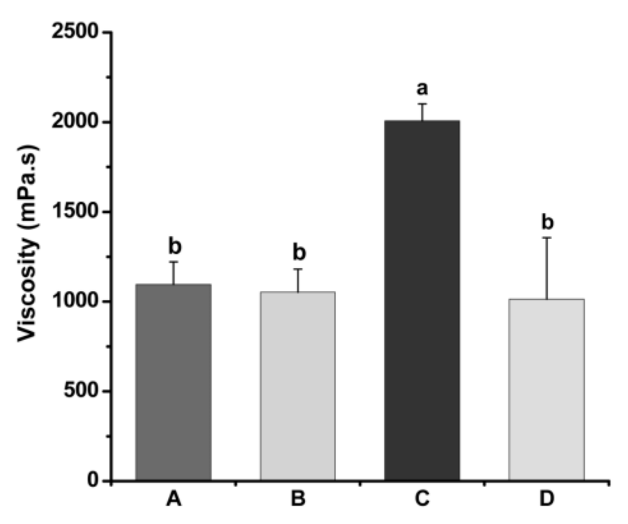

Figure 2—Viscosity of goat's milk yogurt samples prepared from reconstituted goat milk (RGM) with about 12% total solids (TS) (a; control), RGM with 2.4% unheated whey protein isolate (WPI) (b), RGM with 2.4% polymerized whey proteins (PWP) (c), and RGM fortified with 4.7% solids (d). The bars with different letters are significantly different ($P < 0.05$).

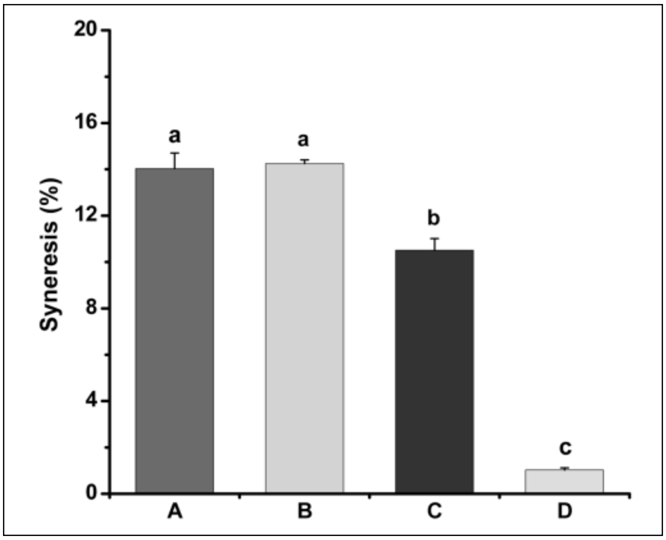

Figure 3—Syneresis of goat's milk yogurt samples prepared from reconstituted goat milk (RGM) with about 12% total solids (TS) (a; control), RGM with 2.4% unheated whey protein isolate (WPI) (b), RGM with 2.4% polymerized whey proteins (PWP) (c), and RGM fortified with 4.7% solids (d). The bars with different letters are significantly different ($P < 0.05$).

rounded and linked by finely flocculated protein (Figure 4c). These fine protein floccules, which occurred only in Figure 4c, were probably composed of PWP added into the initial milk mix before inoculation with yogurt starter cultures. It is also probable that the PWP added to the milk mix reacted with the casein micelles during fermentation and formed an insoluble complex. This would explain accumulation of most of the protein floccules at casein micelle surfaces and their participation in the formation of linkages between the micelles (Figure 4c). Further studies are needed to confirm these findings. Fine protein floccules were also observed in yogurt gels made from heated milk fortified with whey protein concentrates (Modler and Kalab 1983). In contrast, whey protein was not disclosed by electron microscopy in the yogurt prepared with unheated WPI (Figure 4b), suggesting that its gel formation results mainly from aggregation of the casein micelles, with "native" whey proteins acting only as "inactive" filler (Schorsch and others 2001). The effect of PWP on the yogurt microstructure is quite clear: the binding of PWP to the micelle surface favors the formation of bridges between the casein particles leading to a narrow pored mixed casein/PWP network, thus results in improved consistency and less syneresis.

Conclusions

Polymerized whey proteins seem to be an effective thickening agent to improve the consistency and water holding properties of the goat's milk yogurt. Incorporation of PWP significantly increased the viscosity by 80% and decreased the syneresis by 25% of the yogurt samples compared with the control. As shearing speed increased, the apparent viscosity of the goat's milk yogurts decreased, indicating a shear thinning property. At a high shearing speed, shearing time had little effect on the apparent viscosity. TEM micrographs demonstrated that the microstructure of the goat's milk yogurt gel with PWP appeared denser than the control samples. Results of this study indicate that PWP addition may be a useful mean in the production of goat's milk yogurt and other similar products.

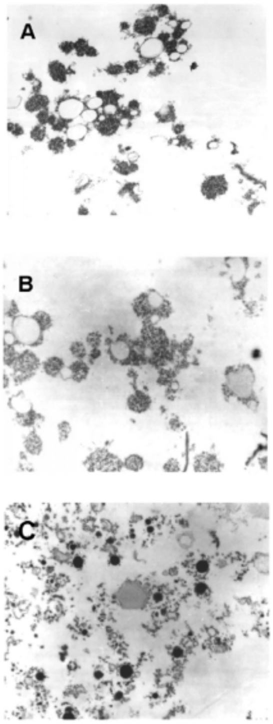

Figure 4—Transmission electron microscopy (TEM) micrographs (× 38750) of goat's milk yogurt samples prepared from reconstituted goat milk (RGM) with about 12% total solids (TS) (a; control), RGM with 2.4% unheated whey protein isolate (WPI) (b), and RGM with 2.4% polymerized whey proteins (PWP) (c).

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