Interaction and Stabilization of Acidified Casein Dispersions with Low and High Methoxyl Pectins

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The ability of low (LMP) and high methoxyl pectins (HMP) to stabilize acidified sodium caseinate dispersions was evaluated. Samples of 2.5% casein, 1% pectin, and 2.5% casein−1% pectin were hydrated in a 0.1 constant ionic strength buffer and pH was adjusted to 3.8, 4.8, 5.8, and 6.8. Without pectin, casein precipitated at pH 3.8 and 4.8. In single-component dispersions, pH had little influence on turbidity, *L* values, sedimentation, and apparent viscosity. *L* values for dispersions of LMP−casein and HMP−casein at pH 3.8 and 4.8 were about twice those at pH 5.8 and 6.8 and were higher than those for pectin. Apparent viscosity of LMP−casein and HMP−casein dispersions was not influenced by pH as were turbidity and *L* values. At low pH, dispersions were less Newtonian and had greater consistency index. Increased stability and apparent viscosity were consistent with fewer interactive sites between HMP and casein than between LMP and casein.

Keywords: *Pectin−protein interactions; acidified dairy drinks; viscosity; light scattering; turbidity; low methoxyl pectin; high methoxyl pectin*

INTRODUCTION

Pectin stabilizes acidified dairy beverages, drinkable yogurts, fruit-juice-containing milks, and fruit-flavored, protein-fortified drinks. When milk is heated and slowly acidified by lactic fermentation or glucono-δ-lactone (GDL), the size and composition of the casein micelle and particles are altered (Glahn, 1982; McMahon and Brown, 1984; Amice-Quemeneur et al., 1996). A slow rate of acidification and smaller particles are favored at low temperatures of acidification. Generally, larger particle sizes result in more unstable dispersions, which are prone to syneresis and "wheying off" (Glahn, 1982). In acidified milks with sufficient pectin levels, the average size of the casein particle is decreased to <1 μm, size distribution is more uniform, and the flow behavior is more Newtonian, less thixotropic (Parker et al., 1994; Kravtchenko et al., 1995). In pectin-containing acidified milks, Brookfield viscosity measurements indicate that an initial sharp increase in viscosity at lower pectin levels is followed by a sharp decline. A second, smaller increase in viscosity at higher pectin levels coincides with an increase in turbidity and stability of dispersions (Glahn, 1982).

The mechanism of pectin stabilization of acidified milk is not clear. Haylock et al. (1995) proposed that at greater than critical pectin levels (CPL), unbound pectin increased the viscosity of the serum and prevented protein precipitation. Adsorption of pectin to casein particles and stabilization by electrostatic repulsion were proposed by Glahn (1982). Kravtchenko et al. (1995) estimated that a pectin layer of approximately 55 nm on the surface of casein prevented precipitation by steric hindrance.

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Previous research on pectin stabilization of acidified milks has used yogurts made from low-fat milk or skim milk powder (Parker et al., 1994; Kravtchenko et al., 1995), skim milk powder acidified with GDL (Glahn, 1982; Glahn and Rolin, 1994), or sodium caseinates directly acidified with HCl (Pedersen and Jorgensen, 1991). Caseins represent about 80% of the milk proteins, and pectin interaction with casein was proposed to be a key factor in stabilization of acidified milk products.

Although most researchers associate electrostatic interactions of milk proteins with pectin, there has been no comparison of low methoxyl (LMP) and high methoxyl (HMP) pectin. The greater potential number of carboxylic acid groups on LMP will influence electrostatic interactions and, possibly, the stability of acidified milks. In this study, we compare LMP and HMP interaction with sodium caseinate in a multicomponent buffer. Physical and chemical properties of casein dispersions with LMP and HMP at pH values between 3.8 and 6.8 were measured.

MATERIALS AND METHODS

Materials. Sodium caseinate was obtained from New Zealand Milk Products (Santa Rosa, CA) and tested for protein content (88.66% Dumas combustion, NA 1500 N2 analyzer). Unstandardized citrus LMP (ref 78/512) and HMP (ref 91/278) were donated by Citrus Colloids (Hereford, U.K.). The degree of esterification was determined according to the method of Voragen et al. (1986). The molecular weight (MW) was determined by high-performance size exclusion chromatography (HPSEC) with refractive index, viscometric, and light scattering detection (Viscotek Europe, Oss, The Netherlands). Pectin was applied to Biogel TSK 60-40-30XL columns in series and eluted at 0.8 mL/min in sodium acetate buffer at pH 3.0. The dye binding protein assay kit was obtained from BioRad Laboratories (Richmond, CA).

Sample Preparation. Samples of 2.5% casein, 1% pectin, and 1% pectin−2.5% casein were hydrated with a three-component buffer consisting of 0.05 M acetic acid, 0.05 M 2-(*N*-morpholino)ethanesulfonic acid (MES), and 0.1 M tris-(hydroxymethyl)aminomethane (Tris). Thus, pH could be

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Table 1. Protein Precipitation of LMP−Casein and HMP−Casein Dispersions Hydrated in a Three-Component Buffer after Centrifugation at 450*g*[a]

	LMP−casein		HMP−casein	
pH	P-ppt[b]	P-spt[b]	P-ppt	P-spt
3.8	6.09^{A}	17.34^{B}	no ppt[c]	19.54^{B}
4.8	2.11^{B}	20.58^{A}	no ppt	20.07^{B}
5.8	no ppt	23.35^{A}	no ppt	27.37^{A}
6.8	no ppt	19.45^{AB}	no ppt	25.41^{AB}

[a] The same upper case letters within columns indicate means are not significantly different. Values are means of 15 replicates. [b] P-ppt indicates protein in precipitate in mg/mL. P-spt indicates protein in supernatant in mg/mL. [c] No ppt indicates that no precipitate was observed.

adjusted between 6.8 and 3.8 with minimal effect on the ionic strength of 0.1 (Ellis and Morrison, 1982). Dispersions were homogenized using a Sorvall Omni mixer (Newtown, CT) at speed 4 for 4 min at 40 °C and rehydrated overnight at 4 °C. The next day, the pH was adjusted from an initial pH of 6.9−7.0 to 3.8, 4.8, 5.8, and 6.8 with 2 N HCl and 2 N NaOH.

Protein, Turbidity, and Sedimentation. After 24 h, samples were equilibrated at room temperature and 50 mL of each was centrifuged at 450*g* (IEC Centra-7 centrifuge). The supernatant was decanted, and if a pellet was present, an equal weight of buffer was added. Protein in the supernatant and resuspended pellet was determined by dye binding assay (Bradford, 1976) with IgG as standard. The turbidity of the supernatant was measured as optical density at 650 nm (Glahn, 1982). Pectin−casein dispersions at pH 3.8 and 4.8 were diluted 1:50 with buffer before turbidity reading. Colorimetric parameters were obtained as L values using a white standard ($L = 95.47$, $a = -0.5$, $b = 1.0$) (Johnston and Murphy, 1995) on a HunterLab HD-25-2 in the reflectance mode (Hunter Associates, Inc., Reston, VA). Sedimentation was measured according to a modified method of Hinds et al. (1994). A 10 mL pipet was sealed with parafilm after it was filled with the dispersion. The volume of precipitate was measured after 24, 48, and 72 h at 4 °C.

Apparent Viscosity. Apparent viscosity was measured at 4 °C using a Haake RV 20 concentric cylinder viscometer with an NV sensor. Shear stress was determined over a shear rate range of 1−1000 s^{-1}. Apparent viscosity was calculated at a shear rate of 150 s^{-1} (Wayne and Shoemaker, 1987).

Statistical Analysis. Values shown are means of three replications. The data were analyzed using SAS data analysis system (SAS, 1985). Proc ANOVA was performed to evaluate the effect of treatment and pH on response variables. The Duncan test was performed to evaluate the effect of pH within treatments and the effect of treatment within pH.

RESULTS AND DISCUSSION

At pH 5.8 and 6.8, pectin alone, pectin−casein, and casein formed stable dispersions, which did not precipitate during centrifugation. Casein dispersions coagulated at pH 3.8 and 4.8, and no further study could be done. Unless stated otherwise, results describing casein alone refer only to casein at pH 5.8 and 6.8.

If sediment was detected after the mild centrifugation, protein in the precipitate was quantified (Table 1). Precipitation was observed only in LMP−casein dispersions at pH 3.8 and 4.8. No precipitation was found in LMP−casein dispersions at pH 5.8 or 6.8, in pectin at any pH, or in casein alone at pH 5.8 and 6.8. No precipitation was observed in HMP−casein dispersions at any pH. Lower protein concentrations in the supernatant of HMP−casein dispersions were obtained at pH 3.8 and 4.8 than observed at pH 5.8 and 6.8. This may be attributable to pectin interference with the protein assay (Alkorta et al., 1994) at the lower pH range. We observed an underestimation of protein with increasing amounts in the protein assay, especially at pH 3.8 and 4.8. Therefore, the statistical difference in protein content estimated for HMP−casein dispersions is likely due to greater interference of the protein assay by HMP at low pH values. Nevertheless, the sedimentation results clearly indicate that HMP more effectively stabilized casein dispersions at lower pH than LMP.

Table 2. Turbidity (OD_{650}) and L Values of LMP (1%), Casein (2.5%), and LMP−Casein Dispersions (1:2.5) Hydrated in a Three-Component Buffer[a]

	turbidity			L values		
pH	LMP	LMP−casein[b]	casein[c]	LMP	LMP−casein	casein[c]
3.8	0.38^{Ab}	22.90^{Aa}	nd[d]	31.33^{Ab}	71.77^{Aa}	nd
4.8	0.37^{Ab}	23.40^{Aa}	nd	30.40^{Ab}	63.47^{Ba}	nd
5.8	0.38^{Ab}	1.10^{Ba}	0.21^{Ab}	28.53^{Bb}	31.43^{Ca}	31.50^{Aa}
6.8	0.38^{Ab}	0.90^{Ba}	0.19^{Ab}	29.93^{ABb}	29.60^{Cb}	31.83^{Aa}

[a] Values are means of three replicates. The same upper case letters within columns indicate means are not significantly different. The same lower case letters within rows of a single variable indicate means are not significantly different. [b] LMP−casein dispersions were diluted 1:50 with buffer before measurements. [c] Casein alone coagulated at pH 3.8 and 4.8. [d] nd, no data.

Table 3. Turbidity (OD_{650}) and L Values of HMP (1%) and HMP−Casein Dispersions (1:2.5) Hydrated in a Three-Component Buffer[a]

	turbidity		L values	
pH	HMP	HMP−casein[b]	HMP	HMP−casein
3.8	0.75^{Ab}	44.40^{Aa}	29.26^{Ab}	80.33^{Aa}
4.8	0.67^{Ab}	35.50^{Aa}	29.76^{Ab}	75.33^{Aa}
5.8	0.67^{Ab}	1.70^{Ba}	30.10^{Aa}	30.80^{Ba}
6.8	0.65^{Ab}	1.23^{Ba}	29.00^{Ab}	30.50^{Bb}

[a] Values are means of three replicates. The same upper case letters within columns indicate means are not significantly different. The same lower case letters of a single variable within rows indicate means are not significantly different. [b] HMP−casein dispersions were diluted 1:50 with buffer before measurements.

Sedimentation due to gravitational force was not observed. Samples of casein, LMP, LMP−casein, HMP, and HMP−casein left in a 10 mL sealed pipet at 4 °C showed no sedimentation after 24, 48, or 72 h.

Turbidity and L Values of LMP Dispersions. Because the size of casein micelle particles influences light scattering (Walstra and Jenness, 1984), turbidity and L values were evaluated. The effect of pH on turbidity (OD_{650}) and lightness values (L values) of LMP, casein, and LMP−casein dispersions was determined (Table 2). The pH had no effect on turbidity of LMP, stable casein dispersions (pH 5.8 or 6.8), or LMP−casein dispersions at pH 5.8 or 6.8. In LMP−casein dispersions at pH 3.8 and 4.8, about 20-fold higher turbidity was observed compared to LMP−casein dispersions at higher pH values. Further, about 60-fold higher turbidity was observed in LMP−casein dispersions at pH 3.8 and 4.8 compared to LMP dispersions at any pH. At pH 5.8 and 6.8, the turbidity of LMP−casein dispersions was approximately 2−5 times higher than LMP or casein alone at the same pH.

At pH 3.8 and 4.8, L values for LMP−casein dispersions were more than double the value of the same dispersions at pH 5.8 and 6.8 or of LMP dispersions at any pH (Table 2). L values for stable casein dispersions were not significantly different. The effect of pH on L values for LMP was slightly different at lower pH compared to higher pH values.

The effect of pH on turbidity and L values was similar for HMP and HMP−casein dispersions (Table 3). About 25-fold higher turbidity was observed in HMP−casein dispersions at pH 3.8 and 4.8 compared with HMP−casein dispersions at pH 5.8 and 6.8. Approximately

Table 4. Viscosity (mPa·s) of LMP (1%), LMP−Casein, HMP (1%), HMP−Casein, and Casein Hydrated in a Three-Component Buffer, at 150 s^{-1} Shear Rate[a]

pH	LMP	LMP−casein	casein[b]	HMP	HMP−casein	casein[b]
3.8	18.43^{Ab}	40.40^{Aa}	nd[c]	40.92^{Bb}	95.57^{Aa}	nd
4.8	19.50^{Ab}	55.85^{Aa}	nd	62.68^{Ab}	96.73^{Aa}	nd
5.8	20.27^{Ab}	35.38^{Aa}	2.66^{Ac}	68.75^{Ab}	103.01^{Aa}	2.98^{Ac}
6.8	18.70^{Ab}	35.24^{Aa}	2.79^{Ac}	64.53^{Aa}	55.15^{Bb}	2.91^{Ac}

[a] The same upper case letters within columns indicate means are not significantly different. The same lower case letters within rows indicate means are not significantly different. Values are means of three replicates. [b] Casein alone coagulated at pH 3.8 and 4.8. [c] nd, no data.

60-fold higher OD_{650} was observed in HMP−casein dispersions at pH 3.8 and 4.8 compared to HMP dispersions at any pH value. At pH 3.8 and 4.8 in HMP−casein dispersions, a 2−3-fold higher increase in *L* values was observed compared to HMP−casein dispersions at pH 5.8 and 6.8. *L* values of HMP dispersions were not significantly different at any pH.

Generally, pH did not affect the turbidity or reflectance of pectin or casein (at pH 5.8 or 6.8) dispersions alone but did affect dispersions of LMP−casein or HMP−casein. Thus, electrostatic interactions play a key role in pectin−casein interaction. Turbidity was related to particle size, concentration, and optical properties of the individual milk protein constituents during acidification (Banon and Hardy, 1991). Changes in turbidity were correlated with a collapse of the outer "hairy" layer of the micellular structure (Banon and Hardy, 1992). In GDL acidified skim milk, an increase in turbidity with an increase in pectin concentration was reported by Glahn (1982). He suggested that dispersions of smaller casein particles required more pectin for stabilization. A decrease in *L* values after 6 kbar hydrostatic pressure treatment was related to limited reaggregation of fragments and presence of large, nonsedimentable milk proteins (Johnston et al., 1992). In this study, the dramatic increase in turbidity and *L* values after acidification of dispersions of LMP−casein or HMP−casein suggests the formation of smaller, more numerous particles.

The apparent viscosity at 150 s^{-1} of LMP, casein (at pH 5.8 and 6.8), or LMP−casein dispersions was not affected by pH (Table 4). The apparent viscosity of HMP at pH 3.8 was significantly lower than apparent viscosity at higher pH values. It is possible that the pK_a of the HMP is near the pH range, 3.8−4.8. The significant difference in apparent viscosity of HMP−casein dispersions between 5.8 and 6.8 is not likely to be related to changes in ionization of pectin, but could be related to changes in ionization of casein and subsequent interaction.

There was a greater than additive increase in apparent viscosity at 150 s^{-1} in LMP−casein dispersions at pH 5.8 or 6.8 compared to LMP or casein (Table 4). Greater than additive apparent viscosity at 150 s^{-1} was also observed in HMP−casein dispersions at pH 5.8. HMP−casein dispersions at 6.8 had lower apparent viscosity than HMP alone. The weight-average molecular weights (M_w) of HMP and LMP were determined to be 243 000 and 158 200, respectively. Estimation of molecular weight by capillary viscometry indicated these pectins had identical intrinsic viscosities. At higher concentrations, the apparent viscosity of HMP was nonlinear, indicating the presence of some pectins of large MW. This heterogeneity in MW of HMP probably accounts for some of the differences in apparent viscosity between dispersions of LMP, HMP, LMP−casein, and HMP−casein.

Table 5. Flow Behavior (*n*) and Consistency Index (*m*) for LMP, LMP−Casein and HMP−Casein Dispersions, and Casein Hydrated in a Three-Component Buffer[a,b]

	LMP		casein		LMP−casein	
pH	*m*	*n*	*m*	*n*	*m*	*n*
3.8	0.02	0.98	nd[c]	nd	0.13	0.78
4.8	0.02	0.97	nd	nd	0.19	0.80
5.8	0.02	0.98	0.007	0.93	0.06	0.91
6.8	0.03	0.95	0.007	0.92	0.06	0.91
	HMP		casein		HMP−casein	
pH	*m*	*n*	*m*	*n*	*m*	*n*
3.8	0.07	0.90	nd	nd	0.37	0.76
4.8	0.17	0.80	nd	nd	0.39	0.73
5.8	0.17	0.81	0.005	0.90	0.35	0.76
6.8	0.16	0.81	0.015	0.95	0.07	0.94

[a] Casein alone coagulated at pH 3.8 and 4.8. Values are means of three replicates. [b] As *n* decreases from unity, the dispersion is more pseudoplastic. *m* is interpreted as relative thickness (Schmidt and Smith 1992). [c] nd, no data.

The apparent viscosity at 150 s^{-1} was the lowest shear rate possible to achieve stable viscosity readings. Flow behavior (*n*) and consistency (*m*) index (Table 5) were estimated from the slope and intercept of the flow curve to obtain further information on interactions (Schmidt and Smith, 1992). In pseudoplastic fluids, the flow behavior index (*n*) decreases from unity and is a measure of departure from Newtonian behavior (Sharma and Bhat, 1992). Consistency indices (*m*) were interpreted as relative thickness values (Schmidt and Smith, 1992) and are a measure of the consistency of the fluid (Sharma and Bhat, 1992).

Dispersions of LMP−casein or HMP−casein were less Newtonian and had higher consistency than LMP or HMP at pH 3.8, 4.8, and 5.8. In LMP−casein dispersions, the *m* values at pH 3.8 and 4.8 were nearly 3 times greater than at pH 5.8 and 6.8. Further, the *n* values at low pH were markedly less than at high pH values. At pH 3.8 or 4.8, dispersions of LMP−casein were less Newtonian and had higher consistency than LMP. The value for *m* in HMP at pH 3.8 was lower than at any other pH. HMP at pH 3.8 was also more Newtonian than at pH 4.8, 5.8, or 6.8. At pH 3.8, 4.8, and 5.8, HMP−casein dispersions were less Newtonian and had greater consistency indices than dispersions at pH 6.8.

The molecular weight differences of the two pectins probably account for the different apparent viscosities between LMP−casein and HMP−casein dispersions. However, pH did not affect apparent viscosity of LMP−casein or HMP−casein dispersions as greatly as it did turbidity and *L* values. Interchain hydrophobic interactions are reported in HMP via methoxyl groups (Oakenfull and Scott, 1984). The degree of esterification (% DE) was estimated to be 40.0% and 71.7% for LMP and HMP, respectively. LMP, with greater charge density, has more numerous electrostatic binding sites and may coat the casein particle leading to sedimentation. It is possible that LMP−casein dispersions have a greater heterogeneous distribution of size. Some aggregates are so large they precipitated during low-speed centrifugation. Other smaller aggregates contribute to high turbidity and reflectance values at low pH. With fewer interactive sites, a smaller region of HMP may interact with the casein particle and free a substantial portion of the pectin chain for solvent interaction, forming dispersions resistant to centrifugal sedimentation.

Conclusions. Turbidity and *L* values of pectin−casein dispersions increase dramatically at lower pH, suggesting the formation of numerous, smaller particles, which are less prone to sedimentation. The pH effect supports electrostatic interactions between pectin and casein. On the other hand, smaller differences in apparent viscosity of dispersions at different pH values were observed. In LMP−casein dispersions, greater intermolecular interactions are likely to occur. In casein dispersions containing HMP, fewer carboxylic acid sites are available and more of the linear pectin structure is available to interact with solvent. The greater interaction of LMP with the casein surface tends to decrease stability. Anchoring of HMP to the casein surface and interaction of HMP with solvent tend to increase stability.

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