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# Properties of Films Made from $CO_2$-Precipitated Casein

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Tensile properties, water vapor permeabilities, and water solubilities were determined for casein films plasticized with glycerol. The casein was precipitated from milk that was sparged with carbon dioxide ($CO_2$). The films were compared to those made from commercial calcium caseinate. Glycerol was added to plasticize the films. At 20% (w/w) glycerol content, $CO_2$–casein films were >50% stronger than the calcium caseinate films. They were significantly stiffer than the caseinate films and were approximately as elastic. The water vapor permeabilities were ~20% less than those of the calcium caseinate films. $CO_2$–casein films were only 7% soluble in water compared to 100% solubility for the calcium caseinate films. The differences in properties of the films indicate structural dissimilarities.

**Keywords:** *Films; edible films; casein; tensile properties; water vapor permeability; carbon dioxide; water solubility*

## INTRODUCTION

The use of edible films as an alternative to polymeric, nonbiodegradable films has recently been the subject of many review articles and books (Kester and Fennema, 1986; Guilbert, 1986; Krochta and De-Mulder-Johnson, 1997). In the food industry, nonedible polymeric films are used in packaging as barriers to prevent moisture loss or uptake, to prevent oxidation, for flavor retention, and to prevent or retard microbial spoilage. The major advantages of these films are that they are made from cheap and uniform raw materials, they are easily and inexpensively manufactured, and they are flexible and strong. Fusing two film types may enhance their properties. A major disadvantage of polymer films is that they are not biodegradable.

Whereas polymeric films are generally applied to the food as a wrap, edible films may be applied to foods by dipping or spraying, eliminating packaging waste. Krochta and De Mulder-Johnston (1997) list the advantages of edible films. They may be used to inhibit migration between components in a processed food, to carry food ingredients, and to improve the mechanical integrity or handling characteristics of the food. A major disadvantage is their sensitivity to relative humidity. Edible films and coatings are not a replacement for synthetic packaging materials for prolonged storage of food (Kester and Fennema, 1986).

Films have been prepared from polysaccharides, hydrocolloids, proteins, and various combinations of these materials (Ghorpade et al., 1995). Their hydrophilic nature, resulting in poor water vapor barrier properties, limits their use. Plasticizers are often added to reduce brittleness, but their addition usually results in films with a lowered tensile strength and increased water vapor permeability. Cross-linking agents such as lactic acid, tannic acid, and calcium chloride have been added to protein films to increase water resistance, but the resulting films are less flexible and less transparent. The taste of the film may also be affected (Guilbert, 1986). Chen (1995) compared the water vapor permeabilities of edible casein films and other milk-protein-based films, with added glycerol plasticizer, to those of commercial films. Water vapor permeabilities of caseinate films are several times higher than permeabilities for commercial polymeric films. McHugh and Krochta (1994) showed that even though the water vapor permeabilities of milk-protein-based films are several times higher than those of polymeric films, their oxygen permeabilities were equal to or lower than those for polymeric films.

Additional studies have been conducted to evaluate the water vapor barrier properties and tensile strength of casein-based films. Avena-Bustillos and Krochta et al. (1993) found that the water vapor permeability of caseinate-based films improved with calcium addition, pH adjustment, and addition of lipid compared to that of caseinate films. Parris et al. (1995) found that addition of sodium caseinate to various hydrocolloids and plasticizers improved film water vapor barrier properties compared to those of hydrocolloid films without compromising strength.

Tomasula et al. (1995, 1997) precipitated casein in batch and continuous processes using high-pressure carbon dioxide at 38 °C. Use of carbon dioxide is attractive because the precipitant is eliminated from the whey upon release of pressure. It does not contaminate the curd and whey as acids do. The residual whey has a pH of only 6.0; after acid precipitation, the associated whey has a pH of 4.6. Because precipitation occurs at a higher pH, the calcium content of the casein is ~1.5%. This is about the same as the calcium content of calcium caseinate. Calcium caseinate is made by dissolving acid casein in water. This step is followed by the addition of calcium hydroxide to replace the calcium.

In this study, films that were prepared using carbon dioxide-precipitated casein were compared to films prepared from calcium caseinate, with and without added plasticizer. The objective of this work was to show that structural differences of the caseins affect the

tensile properties, water vapor barrier properties, and solubility properties of the films.

## MATERIALS AND METHODS

**Materials.** Carbon dioxide ($CO_2$)-precipitated casein was prepared as described previously (Tomasula et al., 1995) by injecting $CO_2$ into milk at 5520 kPa and 38 °C in a batch reactor. The reactor contents were held for 5 min. After precipitation, the casein was washed with distilled water to remove whey proteins, lactose, and minerals. The casein was then freeze-dried. Alanate 310 calcium caseinate (New Zealand Milk Products, Inc., Santa Rosa, CA) was used to make films for comparative purposes. Proximate analysis of the caseins was determined in our laboratory according to methods described previously (Tomasula et al., 1995). Glycerol (GLY), used as a plasticizer, was purchased from Aldrich Chemical Co. (Milwaukee, WI).

**Film-Making Procedure.** Aqueous solutions of 2, 4, 6, and 8% (w/w) $CO_2$–casein and Alanate 310 calcium caseinate were prepared. Twenty milliliters of each was then pipetted into 100 mm wide × 15 mm high polystyrene Petri dishes (Fisher Scientific Co., Pittsburgh, PA) to cast films of the pure caseins.

Aqueous solutions with total GLY concentration and either Alanate 310 or $CO_2$–casein of 6% (w/w) were then prepared so that the resulting films contained either 20, 30, 40, or 50% (w/w) GLY. The solutions were stirred vigorously using a hand-held stirrer for 2 min. A light vacuum was applied to each solution to remove bubbles. Five films were cast from each solution. The films were allowed to dry overnight at ~23 °C and 50% relative humidity (RH) and then were stored in a desiccator at ~50% RH and 23 °C. Storing the films at 50% RH prevents the films from shrinking, warping, or developing cracks and permits easy removal from the plates. RH was maintained in the desiccator using a saturated $NaHSO_4$ solution.

Thicker films were prepared from the same solutions containing either casein and 30% (w/w) GLY by pipetting ~28 mL of solution into a Petri dish and following the film-making procedure described above.

**Film Thickness.** A model 3 micrometer (B.C. Ames Co., Waltham, MA) was used to measure film thickness. Reported values of film thickness are the mean of 10 measurements selected randomly over the face of the film. The precision of the thickness measurements was ±5%.

**Water Vapor Permeability (WVP) Measurements.** The apparatus and method used to measure WVP have already been described (Parris et al., 1995). The method is based on ASTM E96-80 (ASTM, 1980) as modified by McHugh et al. (1993). Four replicates each of $CO_2$–casein or Alanate 310 films containing 6% (w/w) total solids and 30% (w/w) GLY were tested. Air velocity was maintained at 150 m/min across the films. Temperature was controlled at 30 ± 2 °C. WVP for all films was determined with the shiny side down facing the vapor source.

**Tensile Property Measurements.** An Instron model 1122 tensile tester equipped with a 2000 g load cell was used to measure tensile strength (TS), elongation to break (ETB), and initial modulus (IM). Five replicates were run for each film composition using 5 mm wide specimens. A gauge length of 25 mm and an extension rate of 5 mm/min were used. Samples were stored at 50% RH for at least 24 h before testing. Standard deviation was calculated using version 6.0 of the Instron software. Scatter plots of the data were prepared using SigmaPlot 4.0 for Windows, Chicago, IL.

**Solubility Measurements.** The procedure used to determine the solubility of the films in water is similar to that described in Gontard et al. (1992). Water solubility was determined for $CO_2$–casein and Alanate 310 films containing 6% (w/w) total solids and either 0 or 30% GLY. A 4 cm diameter disk was cut from each of the films, weighed, and then immersed in water at room temperature for 24 h with stirring. The nondissolved film was then dried at 100 °C for 24 h and weighed. The percentage solubility was defined as the mass of casein in the film that dissolved divided by the initial mass of casein in the film. The experiments were performed in triplicate.

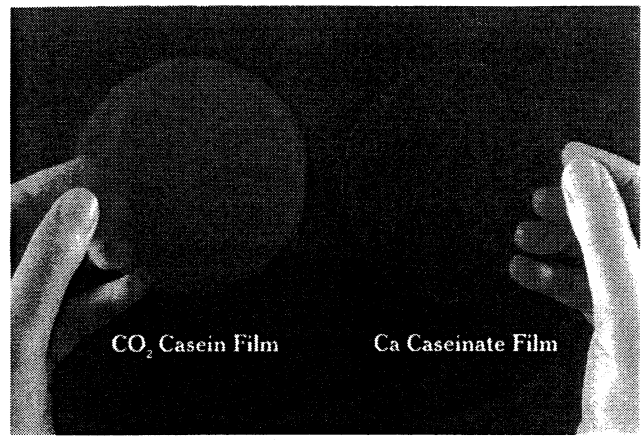


**Figure 1.** Films prepared from $CO_2$–casein and calcium caseinate. Both films contain 30% (w/w) glycerol.

**Scanning Electron Microscopy.** Strips of dry films were immersed in 1% glutaraldehyde–0.1 M imidazole–HCl solution at pH 6.8 for 48 h at room temperature. After washing in imidazole buffer for 1 h, these strips were immersed in 2% $OsO_4$–0.1 M imidazole solution for 2 h, washed in distilled water, dehydrated in a graded series of ethanol solutions, and embedded in an epoxy resin mixture. Thin sections were cut with diamond knives, stained with solutions of 2% uranyl acetate and lead citrate, and examined in a model CM12 scanning transmission electron microscope (Philips Electronics, Mahwah, NJ) operated in the bright field mode at an instrumental magnification of 22000×.

**Statistical Analyses.** Microsoft Excel 97 SR-1 (Microsoft Corp., Redmond, WA) was used for all statistical analyses. The data were analyzed with ANOVA, and means were compared using the $F$ test. Differences were considered to be significant at $P < 0.05$.

## RESULTS AND DISCUSSION

**Qualitative Film Properties.** $CO_2$–casein and calcium caseinate films prepared from the protein solutions without added GLY were brittle. $CO_2$–casein films prepared from 2% (w/w) solutions were brittle and difficult to peel from the Petri dishes. Calcium caseinate films prepared from either the 2 or 4% (w/w) solutions were difficult to remove from the dishes. To facilitate comparison between the properties of the two films, the films were prepared from solutions containing 6% (w/w) total solids. Films prepared from 8% (w/w) solutions were qualitatively comparable to films prepared from the 6% (w/w) casein solutions. Only the calcium caseinate films were tested for tensile properties; they performed similarly to the films cast from 6% (w/w) solutions. These films were not subjected to water vapor barrier property testing or solubility testing. We limited our study to films prepared from the 6% (w/w) solutions because films prepared from solutions containing the least amount of protein are most desirable in commercial applications to keep costs low.

Films prepared from $CO_2$–casein were slightly milky in appearance, but transparent, as shown in Figure 1. The milky appearance may be due to the presence of intact casein micelles. Some of the $CO_2$–casein films dried to almost a matte surface. The calcium caseinate films were transparent and had smoother surfaces as shown in Figure 1. Added plasticizer did not affect the appearance of the films. The $CO_2$–casein films ap-

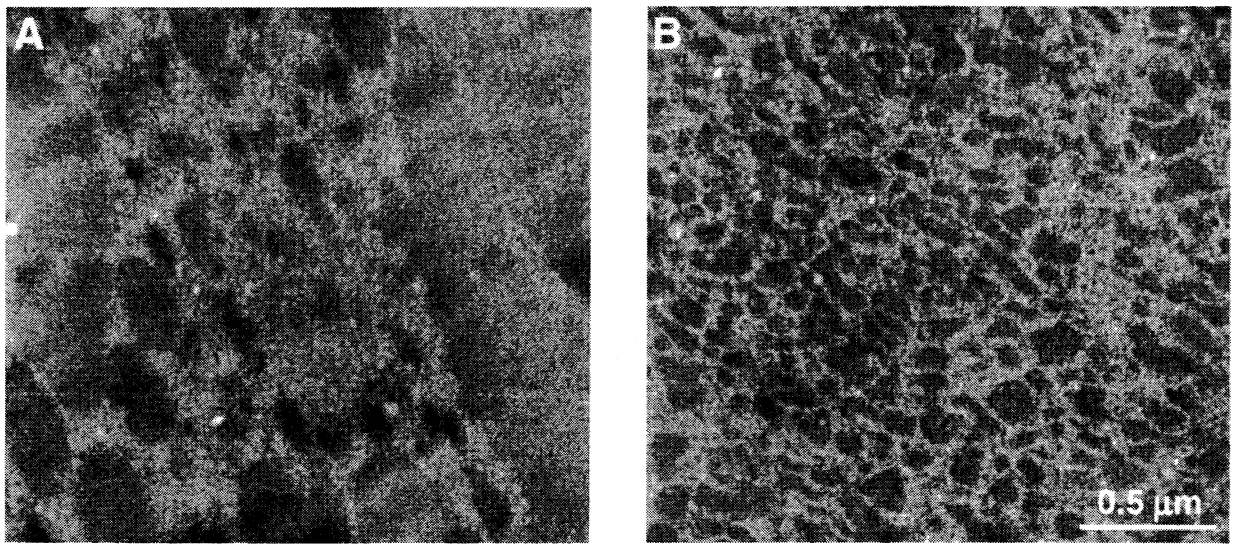


**Figure 2.** Scanning electron micrographs of calcium caseinate (A) and $CO_2$–casein (B). Both films contain 30% (w/w) glycerol.

**Table 1. Proximate Analysis of $CO_2$–Casein and Commercial Calcium Caseinate (Reported on Moisture-Free Basis)**

| casein | ash (%) | protein (%) | fat (%) | lactose (%) | Ca (%) | P (%) | Na (%) |
|---|---|---|---|---|---|---|---|
| $CO_2$–casein | 3.89 | 94.1 | 1.52 | 0.5 | 1.6 | 0.5 | 0.2 |
| calcium caseinate[a,b] | 4.44 | 92.3 | 0.5 | 2.7 | 1.6 | 0.3 | 0.8 |
| calcium caseinate[a,c] | 4.3 | 94.5 | 1.1 | 0.1 | 1.3 | 0.8 | 0.2 |

[a] Alanate 310, New Zealand Milk Products, Inc. (Santa Rosa, CA). [b] Analysis performed in our laboratory. [c] Analysis supplied by the manufacturer.

peared to have more "depressions", which were almost pore-like, compared to the caseinate films. The surface depressions in both films may be a result of localized phase separations during drying of the film. Observation by SEM (Figure 2) indicates that the casein micelles in the calcium caseinate films (A) are large and randomly distributed throughout the film. Casein micelles in the $CO_2$–casein films (B) are much smaller and located in a more ordered arrangement. The smaller micelles in the $CO_2$–casein can be attributed to the higher precipitation pH, which was sufficient to disrupt only some of the larger micelles.

**Tensile Properties.** Results of the proximate analyses of the $CO_2$–casein and the calcium caseinate used in this study are shown in Table 1. Analytical results obtained in our laboratory for the two caseins showed equivalent amounts of calcium. Calcium caseinate contains ~60% as much phosphorus as does $CO_2$–casein.

Tensile properties for blends of $CO_2$–casein and calcium caseinate films with GLY (average film thickness = 0.15 mm) are plotted in Figure 3. Films containing 0 and 10% GLY were too brittle for testing. Tensile strength (TS), elongation to break (ETB), and initial modulus (IM) were determined. TS is a measure of film strength. ETB is a measure of the flexibility of the film, and IM is a measure of the stiffness of the film.

For both films, TS decreased with increasing GLY content. At 20% (w/w) GLY content, TS was >30% greater for the $CO_2$–casein films, but the difference in TS decreased with increasing GLY content. There was no significant difference between the values of ETB for the $CO_2$–casein films and calcium caseinate films over the entire range of GLY content. The values of ETB

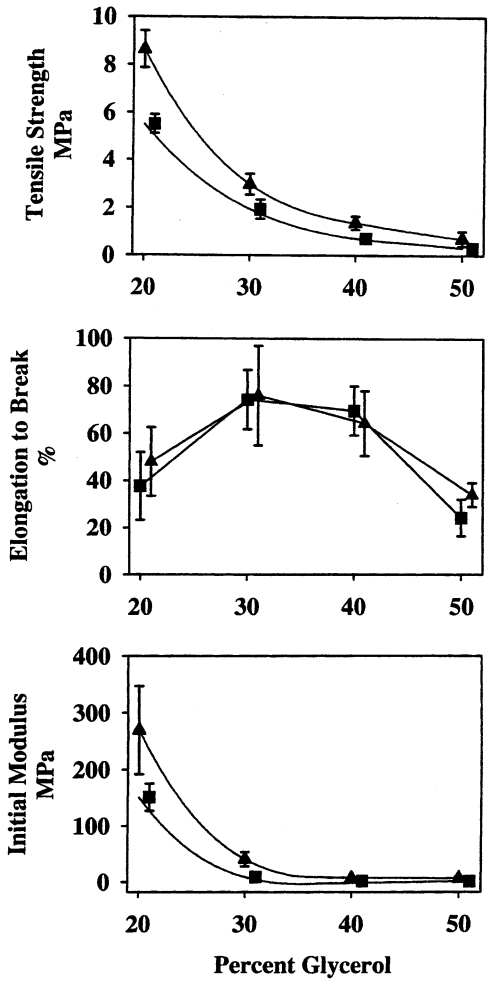


**Figure 3.** Effect of GLY concentration on tensile properties of (▲) $CO_2$–casein and (■) calcium caseinate films.

dropped with GLY content >40%. IM for the $CO_2$–casein films is greater than IM for the calcium caseinate films over the entire range of added GLY.

TS results for both films are in general agreement with the values listed in Chen (1995) for caseinate films containing GLY. TS values for other protein film types are also of similar magnitude (Gnanasambandam et al., 1997; Ghorpade et al., 1995).

**Table 2. Variation of Tensile Properties with Film Thickness for $CO_2$–Casein and Calcium Caseinate Films Containing 30% (w/w) Glycerol**

| film type | film thickness (mm) | TS[a] (MPa) | ETB[a] (%) | IM[a] (MPa) |
|---|---|---|---|---|
| $CO_2$–casein | 0.11 | 1.2[a] | 50.2[a] | 9.6[a] |
| | 0.15 | 3.0[b] | 74.2[a] | 40.9[b] |
| calcium caseinate | 0.11 | 1.6[ac] | 66.6[a] | 10.8[a] |
| | 0.15 | 1.9[c] | 76.0[a] | 8.9[a] |

[a] Within each category, means with no superscript in common are significantly different ($P < 0.05$).

The differences in the tensile properties of the two films, especially at lower GLY content, may be related to the manner in which calcium and phosphorus are bound to the caseins. $CO_2$–casein is precipitated at pH 5.4 (Tomasula et al., 1995) instead of the isoelectric pH 4.6 used to isolate acid casein. The higher precipitation pH is associated with higher calcium content. Because aggregates are formed at this pH, it is assumed that some of the micellar calcium phosphate, which maintains the casein micelle structure, is dissolved in the whey. In acid casein manufacture, most of the micellar calcium phosphate dissolves. Commercial calcium caseinate is made by dissolving acid casein in water followed by the addition of calcium hydroxide to replace calcium. The casein coagulate is broken down upon addition of calcium hydroxide, weakening hydrophobic protein interactions. $CO_2$–casein film may be stronger because more of the micellar calcium and phosphate linkages are intact. It was concluded that the functional properties of $CO_2$–casein differ from those of calcium caseinate most likely because of the manner in which calcium and phosphorus are associated with the caseins (Strange et al., 1998).

The flexibility of the films is not significantly different over the entire range of GLY content (Figure 3). ETB for the $CO_2$–casein film declined with GLY content >30% and for the calcium caseinate film declined with GLY content >40%. GLY reduces intermolecular forces in films by inserting itself between the protein chains. There may be an electrostatic attraction between calcium and the hydroxyl groups of GLY. GLY may also establish hydrogen bonding with amino acid residues of casein.

Tensile properties of polymeric films are not affected by film thickness. In our study, small but significant differences in TS and IM were noted with increasing film thickness, as shown in Table 2. For the $CO_2$–casein films, TS increased slightly with increasing film thickness. ETB for both film types did not vary with the change in film thickness. IM for the $CO_2$–casein film increased with film thickness but did not vary significantly for the calcium caseinate film. Differences are most likely an artifact of making and drying the films in Petri dishes and possibly would not be observed if the film were made in a different manner. All films had the same surface area exposed to 50% RH, but the thicker films took longer to dry, as expected. As the films were drying, it was observed that film formed and adhered to the sides of the Petri dishes while the rest of the film was reduced in height by evaporation. The height of the film was approximately evenly reduced from its center to its perimeter. The films shrank away from the sides of the dish when dry, with some puckering observed at the edges of the films. This drying pattern may have affected the distributions of protein, glycerol, and water molecules, leading to the small differences in measured tensile properties.

**Table 3. WVP Values of $CO_2$–Casein and Calcium Caseinate Films Containing 30% (w/w) Glycerol**

| film type | average thickness (mm) | RH (%) | film swelling | WVP[a] (g·mm/kPa·h·m$^2$) |
|---|---|---|---|---|
| $CO_2$–casein | 0.112 | 85.8 | no | 2.22[a] (1.90) |
| | 0.163 | 87.7 | no | 2.58[b] (2.22) |
| | 0.184 | 87.9 | no | 3.21[c] (2.80) |
| | 0.277 | 89.7 | no | 3.80[d] (3.41) |
| calcium caseinate | 0.171 | 86.5 | yes | 3.18[c] |
| | 0.222 | 85.5 | yes | 4.45[e] |

[a] Values in parentheses for the $CO_2$–casein films were calculated without the water vapor permeability correction factor of McHugh et al. (1993). Within each category, means with no superscript in common are significantly different ($P < 0.05$).

The tensile properties of the casein films may be considered moderate (10–100 MPa) at the lower end of added GLY, in comparison to low-density polyethylene films (Krochta and De Mulder-Johnston, 1997). ETB is close to that observed in oriented polypropylene films with ETB of 60%.

**WVP.** WVP was determined for $CO_2$–casein and calcium caseinate films containing 30% (w/w) GLY. The values are reported in Table 3 for various film thicknesses. The WVP correction factor (McHugh et al., 1993) was used to correct for the effect of the water vapor partial pressure gradient in the stagnant air layer of the test cup. WVP for the $CO_2$–casein films was also calculated using ASTM Method E96 with the assumption of 100% RH (values shown in parentheses).

WVP values for the $CO_2$–casein films were less than those for the calcium caseinate films at a particular film thickness. As shown in Table 3, the increased WVP for calcium caseinate films is accompanied by a smaller RH inside the test cup. The decreased RH is due to absorption of water by the protein, resulting in swelling of the film. $CO_2$–casein films did not swell—an indication of greater resistance to moisture mass transfer. Differences in the WVP properties may be attributed to the more rigid structure of the $CO_2$–casein films.

Both films show an increase in WVP with increasing thickness that is indicative of hydrophilic films (McHugh et al., 1993; Ghorpade et al., 1995). However, the effects are not as pronounced as they are in McHugh et al. (1993) because the films in this study are thicker and the resulting RH covers a narrow range. WVP values are more likely a result of structural differences. WVP is not a function of film thickness for hydrophobic films.

WVP values reported in this study are greater than those reported by Avena-Bustillos and Krochta (1993) for calcium caseinate films with approximately half the thickness.

**Water Solubility.** Water solubility was determined for the various casein films, with and without 30% added GLY plasticizer. Results are reported in Table 4.

Calcium caseinate films were easily dispersed in water. Upon dispersal in water, the $CO_2$–casein-based films showed no loss of integrity but changed from a transparent film to white and developed some tackiness. The whitening of the films is because of the clustering of the casein proteins due to the repulsion of water molecules. When the films were vigorously stirred, they broke up but did not dissolve further. The films did not whiten during the WVP studies, though.

**Table 4. Water Solubility of $CO_2$–Casein Films and Comparison to Other Protein Films**

| protein film | water solubility (%) | reference |
|---|---|---|
| $CO_2$–casein | 7.1 | this study |
| $CO_2$–casein–30% GLY | 16.8 | this study |
| calcium caseinate | 90.0 | this study |
| calcium caseinate–30% GLY | 100 | this study |
| soy film | 37.7 | Ghorpade et al. (1995) |
| rice bran films | | Gnanasambandam et al. (1997) |
| pH 9 | 11 | |
| pH 3 | 9 | |

The increased solubility for the films containing GLY appears to be due to the presence of the plasticizer, because both films have about the same protein content. The presence of the plasticizer, in the range of 0–30% GLY, does not appear to significantly increase the solubility of the protein as seen by Stuchell and Krochta (1994) for edible soy films or by Mahmoud and Savello (1993) for whey films.

The results are compared to the solubilities of other protein film types reported in the literature in Table 4. The water solubility of the $CO_2$–casein film is comparable to that for rice bran films with GLY added as plasticizer (Gnanasambandam et al., 1997).

## CONCLUSIONS

Casein produced by sparging $CO_2$ into milk forms strong films that are highly hydrophobic. The properties are most likely a result of precipitation at a higher pH, which leaves some of the micellar calcium phosphate structure intact, and the higher precipitation temperature, which may increase protein–protein interactions as well. $CO_2$ films with added GLY plasticizer are stronger and stiffer than similar calcium caseinate/GLY films but have lower WVP and water solubility. The hydrophobic nature of these films may recommend them for uses that require better strength and moisture resistance.

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