

Oxygen Permeability of Films Made from CO₂-Precipitated Casein and Modified Casein[†]

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Oxygen permeabilities (OP) of CO₂-casein (CO₂CN), calcium caseinate (CaCN), and acylated casein (AcCN) films were determined as functions of % relative humidity (% RH), temperature, and plasticizer type. Tensile properties and water vapor permeabilities (WVP) were also measured. Plasticizers were glycerol (GLY) or a 3:1 ratio of GLY:poly(propylene glycol) (PPG), a hydrophobic plasticizer. OP of the CO₂CN:GLY film was almost twice that of films containing either plasticizer at 35% RH, but its OP approached that of the other films at 70% RH. OP and WVP of films plasticized with GLY were greater than that for films plasticized with PPG. Plasticizer type had little impact on the tensile strength of CO₂CN films while tensile strength of CaCN-GLY:PPG (3:1) films approximately doubled. Results show that structural dissimilarities in the films contribute to differences in OP only under conditions of low RH where the plasticizing effects of water are not significant.

KEYWORDS: Films; edible films; casein; water vapor permeability; oxygen permeability; tensile properties; carbon dioxide

INTRODUCTION

Films prepared from proteins are generally hydrophilic in nature and their barrier properties tend to deteriorate with increasing relative humidity (1). At conditions of low relative humidity, many protein films transmit oxygen at low levels (2), but their oxygen permeability is not as low as that of some synthetic films. Because they are less linear and possess many amino acid side groups, proteins have large free volume relative to that of synthetic polymers.

The free volume may be adjusted through proper selection of plasticizer or by chemical or enzymatic modification of the protein. Lieberman and Gilbert (3) showed that the gas permeation properties of collagen films could be modified by water and polyols to reduce internal hydrogen bonding and increase molecular spacing. McHugh and Krochta (4) modified the free volume of whey protein isolate films, as well as the mechanical properties of these films, through proper selection of plasticizer. Sothornvit and Krochta (5) modified the oxygen permeability and mechanical properties of whey protein isolate films by hydrolyzing the protein, thereby reducing intermolecular forces and free volume. Anker et al. (6) showed that the microstructure of WPI films depends on protein concentration, plasticizer type, and pH, and adjustment of these causes changes in microstructure affecting water and oxygen permeabilities.

Unlike most dairy-protein based films, films prepared from CO₂-precipitated casein (CO₂CN) have hydrophobic character, most likely because of its greater concentration of calcium and phosphorus relative to that found in calcium caseinate (7). This property is a result of precipitation carried out at a higher pH, which leaves some of the micellar calcium phosphate structure intact, and the higher precipitation temperature at 38 °C, which may increase protein-protein interactions as well (8). CO₂CN films with added glycerol plasticizer were stronger and stiffer than similar calcium caseinate-glycerol (CaCN:GLY) films and have lower water vapor permeability and solubility in water (7). Calcium caseinate is prepared by addition of Ca(OH)₂ to acid casein (9). Most of the native micellar calcium phosphate structure found in milk is lost in the preparation of acid casein. The calcium in calcium caseinate is bound electrostatically and does not support casein micelle structure.

Acylation agents, such as acetic anhydride, react with residues that contain the positively charged free amino groups, such as the epsilon-amino group of lysine (10), to form an amide. Acylation of CaCN and CO₂CN with acetic anhydride was described in Santos and Tomasula (11). At pH > 3, acylation was shown to increase the solubilities of both caseins in water. However, acylation did not improve the solubility of CO₂CN to the same extent as CaCN because although the majority of the lysine groups are on the exposed surface of the protein and in contact with water, residues associated with the hydrophobic segments are located in the micelle core.

The objective of this work was to show that structural differences in films made from CO₂CN, CaCN, and acylated

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calcium caseinate (AcCN) impact their oxygen permeability and tensile properties and that these values may be adjusted through choice of plasticizer, temperature, or relative humidity.

MATERIALS AND METHODS

Materials. Carbon dioxide-precipitated casein (CO₂CN) was prepared as described previously in Tomasula et al. (8) by injecting CO₂ into milk at 38 °C in a batch reactor until pressure reached 5.5 MPa. The contents of the reactor were held for 5 min. After depressurization, the casein and whey were removed from the reactor and separated. The casein was washed with distilled water to remove excess whey and then freeze-dried. Alanate 310 Ca caseinate (CaCN) was purchased from New Zealand Milk Products, Inc. (Santa Rosa, CA) and used to make films for comparative purposes. CaCN was also modified with acetic anhydride to make acetylated Ca caseinate (AcCN) films as described in Santos et al. (11). The degree of acetylation was 88.2%. Glycerol (GLY) and poly (propylene glycol), (PPG), average MW 425, used as plasticizers in the films, were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Film Formation. Aqueous solutions containing 4% (w/w) total solids of either CO₂CN, CaCN, or AcCN, and plasticizer, were prepared so that the resulting films contained 30% (w/w) plasticizer. The plasticizers used were either GLY or a 3:1 ratio of GLY:PPG (GLY:PPG (3:1)). The films were prepared as described previously (7). Films were cast by pipetting approximately 20 mL of the solution into 100-mm wide × 15-mm high polystyrene Petri dishes (Fisher Scientific Co., Pittsburgh, PA). Five films were cast from each solution. The solutions were allowed to dry to films at room temperature of approximately 23 °C and relative humidity (RH) of approximately 50%. The resulting films were stored in a desiccator with RH of 50% maintained by using saturated NaHSO₄ solution. Film thickness was measured as described previously (7) using a model 3 micrometer (B. C. Ames Co., Waltham, MA). Film thickness was determined as the mean of 10 measurements selected over the face of the film with a precision of measurement of ±5%.

Water Vapor Permeability (WVP) Measurements. The method used to measure WVP has been described previously (12). It is based on American Society of Testing and Materials Method E96-80 (13) as modified by McHugh et al. (14). Four replicates each of CO₂CN, CaCN, or AcCN, containing 4% (w/w) total solids and 30% (w/w) of either plasticizer, GLY, or GLY:PPG (3:1), were tested. Temperature was controlled to 30 ± 2 °C.

Oxygen Transmission Measurement. Oxygen transmission rates through the films were measured using an Ox-Tran 2/20 modular system (Modern Controls, Inc., Minneapolis, MN). The instrument operates according to ASTM Standard Method D-3985-81 (15) at 0% RH and over the range from 30 to 75% RH. Films were double-side masked with aluminum foil having 5 cm² openings. Masked films were placed in the test cell and exposed to the test gas (oxygen) on one side and the carrier gas (nitrogen) on the other side. Oxygen transmission rates were measured with the smooth side of the films facing outside of the test cell. Tests were carried out by setting temperature to 23 °C and then varying RH from 30% to 70%. Readings were also taken at 0% RH. Oxygen transmission rates were also carried out at constant RH of 40% and temperatures in the range from 10 to 40 °C. Temperature and relative humidity of both gases were controlled by the Ox-Tran 2/20 system. Oxygen permeability is reported in units of cc-μm/kPa-d-m². The experiments were carried out in quadruplicate.

Oxygen permeability was calculated by dividing the oxygen transmission rates by the differential partial pressure of oxygen across the film (1 atm) and multiplying by the film thickness.

Tensile Property Measurements. The tensile properties, tensile strength (TS), elongation to break (ETB), and initial modulus (IM), were obtained as described previously (7). An Instron model 1122 tensile tester equipped with a 2000 g load cell was used to obtain the data. A gauge length of 25 mm and an extension rate of 5 mm/min were used. Samples were stored at 50% RH before testing. Five replicates were run for each film using 5-mm wide specimens. Standard deviation was calculated using version 6.0 of the Instron software.

Statistical Analyses. Sigma Plot 2001 (SPSS Inc., Chicago, IL) was used to analyze all results statistically. Linear regression was carried

out using the Regression Library. Differences between samples were determined using the student t-test. Differences between means were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Oxygen Permeability of Films. The CO₂CN films were made from casein that was precipitated from milk using high-pressure CO₂ at 5.5 MPa. This casein precipitates at a higher pH of 5.4 (8, 16) than acid casein precipitated from milk at pH 4.8 using a mineral acid such as HCl. Because of the higher precipitation pH, some of the micellar calcium phosphate structure from milk remains intact. The Ca content of CO₂CN is 1.6% and the phosphorus content is 0.5%. The commercial CaCN used to prepare the CaCN films has a Ca content of 1.6% but contains ~60% as much phosphorus as the CO₂CN. The Ca is in the ionic form and no micellar structure is present. AcCN films were prepared from acetylated commercial CaCN.

All films were prepared with added plasticizer because the nonplasticized films were too brittle to handle. Plasticizers, such as polyols, and water reduce internal hydrogen bonding and increase molecular spacing in films (3). The films prepared from CO₂CN-GLY were milky in appearance, but still transparent, in contrast to the clear, transparent films prepared from CaCN-GLY or AcCN-GLY. The CO₂CN-GLY and CaCN-GLY films did not crack upon folding in half as the more brittle AcCN-GLY films did. All films prepared with the GLY-PPG (3:1) were almost opaque, caused by some crystallization of the plasticizer on the surface of the film. Although the films were flexible enough to fold, a score mark was left behind after folding.

The effects of added GLY on the tensile properties of CO₂CN-GLY and CaCN-GLY films of various thicknesses were reported in Tomasula et al. (7). Results indicated that tensile strength (TS) and initial modulus (IM) were greatest for films containing 20% GLY and elongation to break (ETB) was greatest for films containing 30% GLY. WVP was measured for films containing 30% GLY. In this study, oxygen permeability (OP) data is reported only for casein films containing 30% GLY or 30% GLY:PPG (3:1). All films used in this study were approximately 0.1-mm thick. We limited our study to films prepared from 4% (w/w) solutions because as indicated in our previous study, solutions containing the least amount of protein are most desirable for commercial applications.

A semilog plot of OP of the films at 23 °C as a function of increasing % RH is shown in **Figure 1**. At 0% RH, OP of the CO₂CN-GLY film is significantly greater than that of the other films. OP of the CO₂CN-GLY:PPG (3:1) and CaCN and AcCN films, with added GLY or GLY:PPG (3:1), are not significantly different ($p < 0.05$). At each value of RH ≥ 35%, OP of the CO₂CN-GLY film is significantly greater than OP of the other films and OP of the AcCN-GLY film is significantly lower. OP of the other films increased in the order CaCN-GLY:PPG (3:1) < CO₂CN-GLY:PPG (3:1) < CaCN-GLY. The log of OP for the CO₂CN-GLY film is a linear function of % RH from 0% to 40% and shows a slight negative change in slope as % RH is increased above 40% RH. The CaCN-GLY:PPG (3:1), the CaCN-GLY, and the CO₂CN-GLY:PPG (3:1) films show a positive change in slope at 40% RH and the AcCN film shows a positive change in slope at 35% RH.

These results indicate that differences in OP at 0% RH for films plasticized with the hydrophilic plasticizer, GLY, are due to differences in casein structure. The added GLY behaves much like water and competes for hydrophilic sites on the protein, causing the protein to become more flexible. Because oxygen

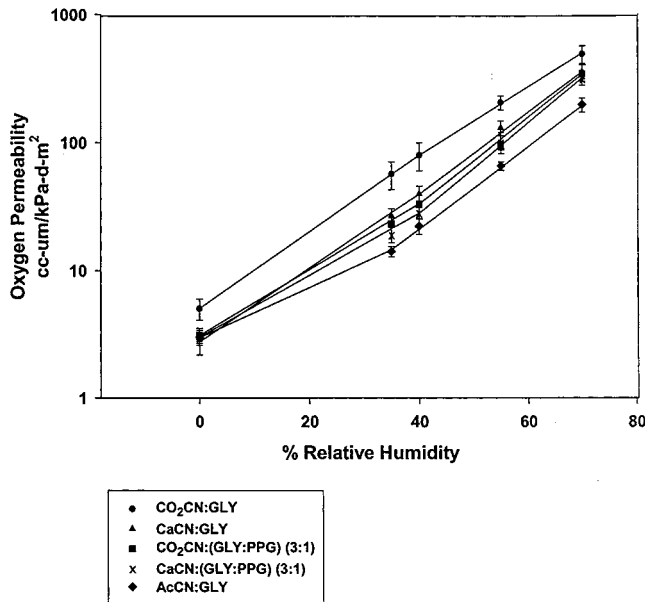


Figure 1. Semilog plot of oxygen permeability (OP) for the films at 23 °C as a function of increasing % relative humidity.

is barely soluble in glycerol or water, diffusion of oxygen occurs through the free volume of the protein. The hydrophilic CaCN and AcCN films have more sites available for binding of GLY than the hydrophobic CO₂CN film, less free volume available for diffusion of oxygen, and therefore lower values of OP than CO₂CN. Both water and GLY compete for the hydrophilic sites on the protein at RH > 0%. Above 35% RH for the AcCN-GLY film and 40% RH for the CO₂CN and CaCN-GLY films, a phenomenon hypothesized by Lieberman and Gilbert (3) for gas permeation of collagen films appears to apply to the casein films of this study. According to Lieberman and Gilbert (3), clustering of water molecules occurs after plasticizer and water molecules are sorbed on the hydrophilic sites and only when all of these sites are occupied at higher water concentrations does the clustering occur. The attraction between the plasticizer and water molecules and the hydrophilic sites is stronger than that between the plasticizer or water. The clustering of plasticizer or water molecules is accompanied by an increase in the available free volume of the protein and therefore the permeability of the casein films. This would account for the positive change in slope observed for the most hydrophilic films as % RH is increased. It is hypothesized that this clustering effect is less for the CO₂CN-GLY film because of its hydrophobic character as shown by the slight negative change in slope. Liebermann and Gilbert (3) observed that water clustering of polyol plasticized collagen films occurred at approximately 30% RH. In this study, as % RH is increased, the AcCN and CaCN films solubilize in water to a greater extent than the CO₂CN films. Tomasula et al. (7) showed that CO₂CN films have a limited solubility in water compared to CaCN films.

Addition of PPG to the GLY plasticizer significantly lowered values of OP for the CO₂CN film because of binding of PPG. OP for the CaCN film was lowered to a lesser extent, indicating either binding to CaCN or more likely a decrease in clustering of the water and GLY molecules leading to a decrease in free volume of the protein. With an increase in % RH, the effects of added PPG diminished for both films most likely because of increased clustering effects introduced with the addition of more water molecules.

Increasing the relative molecular dimensions of a plasticizer between polar groups of the protein would also increase the

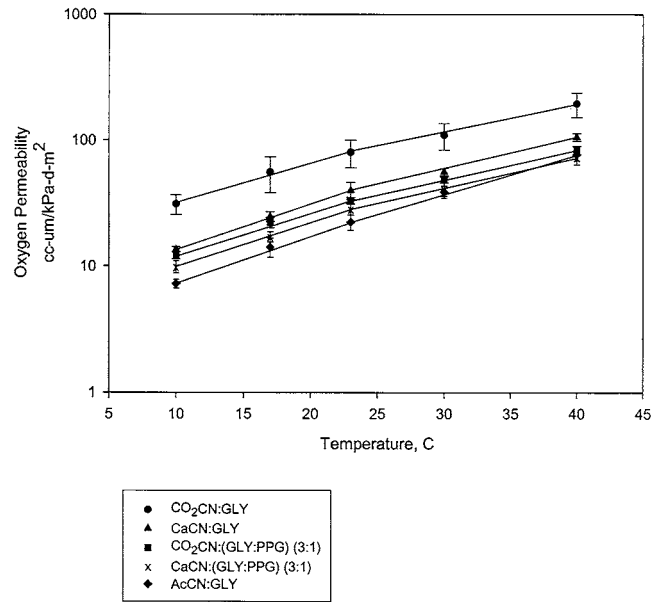


Figure 2. Semilog plot of oxygen permeability (OP) for the films as a function of temperature.

free volume with an additive effect on gas permeability (3). The impact of the larger molecular weight and hydrophobic PPG (MW = 425) did not increase OP for the CO₂CN films but decreased it. This is most likely because the free volume of the CO₂CN film is already large because of its partial micellar structure so that the PPG has little effect.

Semilog plots of OP for the films at 40% RH as a function of temperature in the range from 10 to 40 °C are shown in **Figure 2**. Over the entire temperature range studied, OP for the CO₂CN-GLY films is significantly greater than OP for the other films. OP for the AcCN-GLY film is significantly lower up to 30 °C. OP for the other films at temperature 30 °C increase in the order CaCN-GLY:PPG (3:1) < CO₂CN-GLY:PPG (3:1) < CaCN-GLY. At 23 °C, with the exception of the curve for AcCN-GLY, there is a change in slope or break for each of the curves and OP rises less sharply with increasing temperature. At 40 °C, OP of the films plasticized with GLY:PPG (3:1) and the AcCN-GLY films converges. The breaks in the curves occur at the point where initiation of swelling of the films was observed as temperature was increased and is indicative of structural transition. However, that change is less apparent for the CO₂CN-GLY film. Gennadios et al. (17) attributed this behavior in protein films to a change in structure from a crystalline to an amorphous state, similar to the glass-transition temperature phenomena observed for many polymeric films.

Oxygen Permeability and Water Vapor Permeability. The effects of protein type and plasticizer type on OP and WVP of the films at 50% RH and 23 °C is shown in **Figure 3**. OP for the films plasticized with GLY decreases in the order CO₂CN-GLY > CaCN-GLY > AcCN-GLY but OP for the CO₂CN and CaCN films plasticized with GLY:PPG are not significantly different. OP for the CaCN-GLY, CO₂CN-GLY:OOG (3:1), and the CaCN-GLY:PPG (3:1) films are not significantly different. OP of the CO₂CN-GLY film (143.8) is almost twice that of the CO₂CN-GLY:PPG (3:1) film (73.6) but the difference in OP between the CaCN-GLY film (85.6) and the CaCN-GLY:PPG (3:1) film (67.9) is not as large. WVP for all films plasticized with GLY are not significantly different. CO₂CN and CaCN films plasticized with GLY:PPG (3:1) have lower WVP than films plasticized with GLY alone. After the

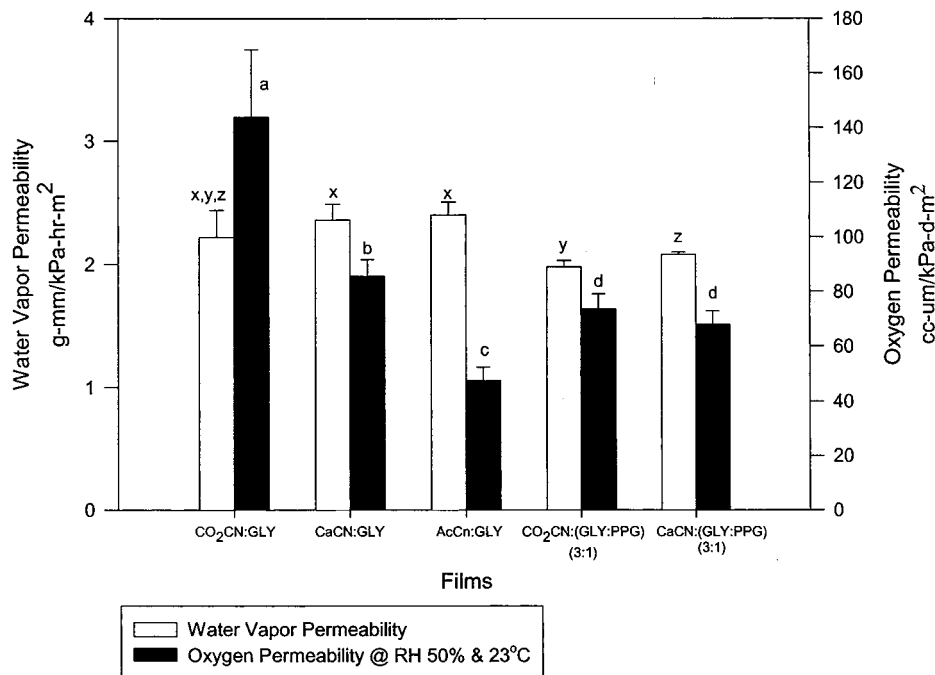


Figure 3. Effect of protein type and plasticizer type on the oxygen and water vapor permeabilities of casein films. Means with different letters are significantly different ($P < 0.05$).

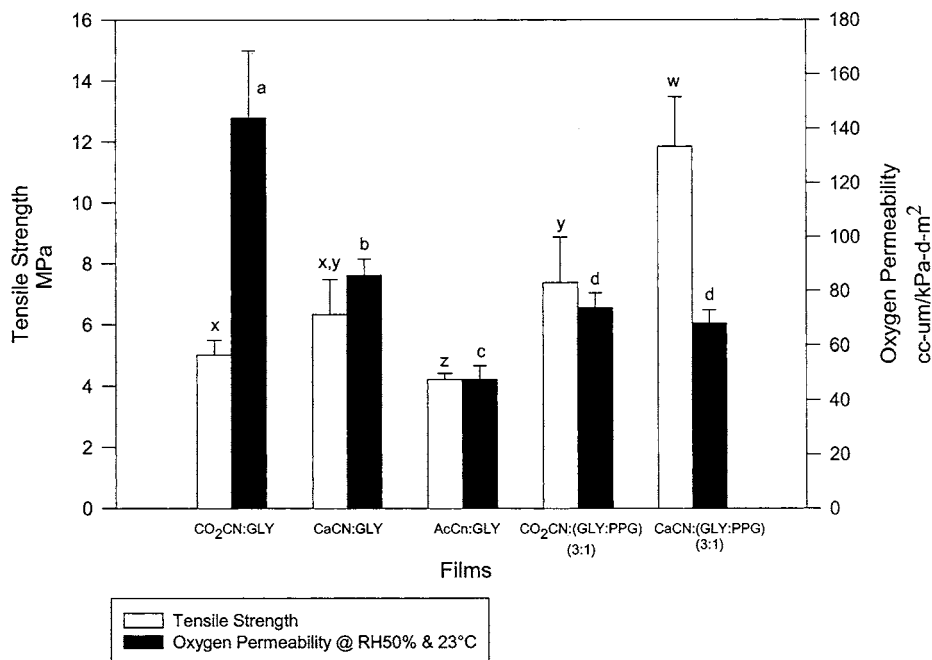


Figure 4. Tensile strength (TS) and oxygen permeability (OP) of the films as a function of casein type and plasticizer type. Means with different letters are significantly different ($P < 0.05$).

WVP experiments, the thickness of the AcCN and the CaCN films containing either plasticizer increased in thickness by 8–13%. The thickness of the CO₂CN films increased only up to 4%. As discussed previously, OP properties of films plasticized with GLY are determined by protein structure—oxygen is barely soluble in water and diffusion occurs through the free volume of the protein—while WVP properties depend on plasticizer type and at high %RH, the solubility of the protein in water (7). OP and WVP of films plasticized with GLY:PPG (3:1) are lower because of its lower solubility in water.

Only the CO₂CN and CaCN—GLY:PPG (3:1) films offer the combination of low OP and WVP while the other films have approximately the same WVP and varying OP.

Tensile Properties and Oxygen Permeability. The tensile properties—tensile strength (TS), elongation to break (ETB), and initial modulus (IM)—were measured for films at 23 °C and 50% RH. TS is a measure of film strength, ETB is a measure of the flexibility of the film until it breaks, and IM is a measure of the stiffness of the film. Previous results (7) indicated that films made from both caseins containing approximately 20% GLY had greater TS and IM compared to the same films containing up to 50% GLY. ETB was not variable with GLY content.

Figure 4 shows TS and OP of the films as a function of protein and plasticizer type. IM is not reported. TS of the CaCN and the CO₂CN films plasticized with GLY are not significantly

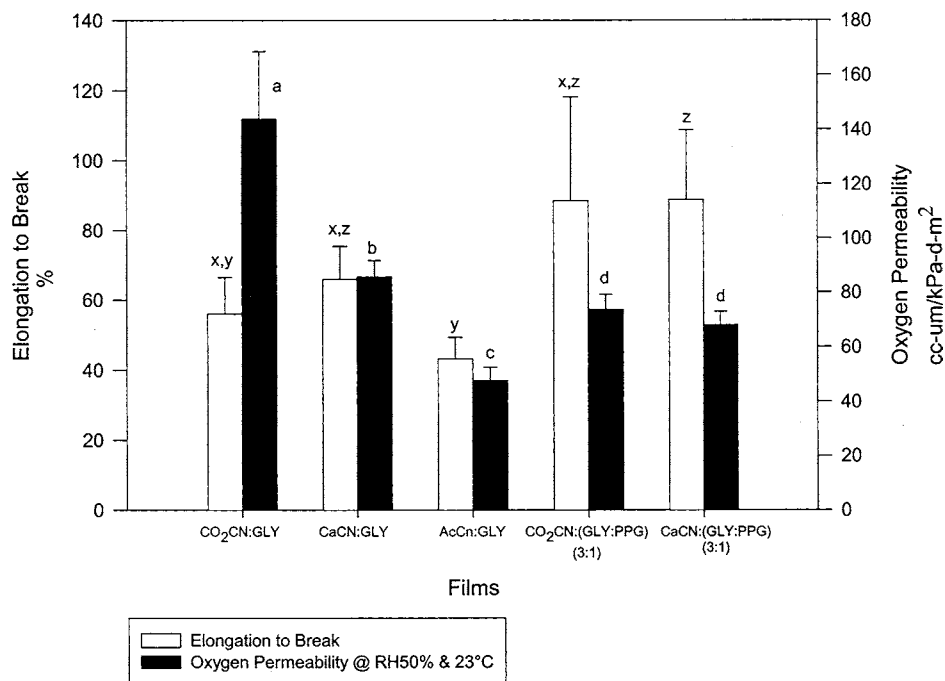


Figure 5. Elongation-to-break (ETB) and oxygen permeability (OP) of the films as a function of casein type and plasticizer type. Means with different letters are significantly different ($P < 0.05$).

Table 1. Comparison of Oxygen Permeabilities and Tensile Properties for Protein-Based Films and Synthetic Films

| film ^a | test conditions | oxygen permeability cc- μ m/kPa-d-m ² | tensile strength (MPa) | elongation to break (%) | reference |
|--|-----------------|---|---------------------------|----------------------------|----------------------------|
| CO ₂ CN-GLY | 23 °C; 50% RH | 144 | 5 | 56 | this study |
| CO ₂ CN-GLY | 10 °C; 40% RH | 30 | | | this study |
| CaCN-GLY | 23 °C; 50% RH | 86 | 7 | 66 | this study |
| CO ₂ -GLY:PP G (3:1) | 23 °C; 50% RH | 74 | 7 | 88 | this study |
| CaCN-GLY:PP G (3:1) | 23 °C; 50% RH | 68 | 12 | 88 | this study |
| AcCN/GLY | 23 °C; 50% RH | 48 | 4 | 43 | this study |
| WPI:G (5.7:1) | 23 °C; | 18.5 | 29.1 | 4.1 | McHugh et al. (4) |
| 15%GLY | 50% RH | | | | |
| WPI:GLY(2.3: 1) | 23 °C; 50% RH | 76.1 | 13.9 | 30.8 | McHugh et al. (4) |
| 30% | | | | | |
| hydrolyzed WPI 5.5% DH WPI/GLY 3:1-1.8:1 | 23 °C; 50% RH | 42.2-111.9 | | | Sothornvit and Krochta (5) |
| hydrolyzed WPI 10% DH WPI/GLY 3:1-1.8:1 | 23 °C; 50% RH | 35.6-89.1 | | | Sothornvit and Krochta (5) |
| LDPE | 23 °C; 50% RH | 1870 | | | Salame (18) |
| HDPE | 23 °C; 50% RH | 427 | 13 | 500 | Salame (18) |
| PVDC-based films | 23 °C; 50% RH | 0.4-5.1 | 26 | 300 | Salame (18) |
| EVOH (70% VOH) | 23 °C; 0% RH | 0.1 | | | Salame (18) |
| EVOH (70% VOH) | 23 °C; 95% RH | 12 | | | Salame (18) |

^a Abbreviations: WPI, whey protein isolate; LDPE, low-density polyethylene; HDPE, high-density polyethylene; PVDC, polyvinylidene chloride; EVOH, ethylene vinyl alcohol.

different and are greater than TS of the AcCN-GLY film. Use of the GLY:PPG (3:1) plasticizer significantly increased TS for the CO₂CN and CaCN films with TS of CaCN-GLY:PPG (3:1) > CO₂CN-GLY:PPG (3:1). ETB (Figure 5) of the CO₂CN and the CaCN-GLY films are not significantly different and are greater than that of the AcCN-GLY film.

In a previous study (7), the reported TS of CO₂CN-GLY films were significantly greater than those for the corresponding CaCN films containing 20% GLY. The difference in TS values between the two films decreased as the percentage of GLY was increased. In addition, the films were cast from solutions containing 6% (w/w) protein. The strength of the CO₂CN films relative to the CaCN films was attributed to the intact micellar calcium and phosphate linkages that are characteristic of CO₂-CN. In this study, the films were cast from 4% (w/w) protein solutions and the effects of the micellar calcium and phosphate linkages appear not to contribute to film strength.

The significant increase in TS, especially for the CaCN film, appears to confirm that addition of PPG decreases the free volume of CaCN increasing protein interactions, thereby strengthening the film. This effect is not as pronounced for the more hydrophobic CO₂CN film. Only the CaCN-GLY:PPG (3:1) film has high TS and ETB, low OP, and low WVP (Figure 3).

Comparison of Oxygen Permeability, Water Vapor Permeability, and Tensile Properties. OP, WVP, TS, and ETB of films from this study are compared in Table 1 to those for edible films cast from whey protein isolate (WPI), 5% or 10% hydrolyzed WPI plasticized with GLY, and various synthetic films. The synthetic films are also known to be moisture sensitive. The values of OP reported for the films from this study at 50% RH and 23 °C were extrapolated from Figure 1. OP of the CO₂CN-GLY film at 23 °C and 50% RH is greater than that reported for the WPI films. OP for the CO₂CN-GLY:

PPG (3:1) film is within the range of values reported for the WPI:GLY (30%) and both hydrolyzed WPI films. OP for the CaCN and AcCN-GLY films are within the range of OP reported for the hydrolyzed WPI films. OP for the films of this study are less than those reported for the LDPE and HDPE films but greater than OP reported for the PVDC or EVOH films.

The WVP reported for the films from this study are less than those reported for the untreated or hydrolyzed WPI films. However, taken as a group, the values of WVP for the protein films indicate that they are much less effective water vapor barriers than LDPE and HDPE films.

The TS of all films from this study are less than those reported for the WPI-GLY films (4) but the ETB are greater. ETB for the films of this study, though, are much less than that for LDPE or HDPE. TS of the CaCN-GLY:PPG (3:1) film is comparable to that of the LDPE film but ETB% is only 88 compared to a value of 500 for LDPE.

CONCLUSIONS

Oxygen permeabilities, water vapor permeabilities, and tensile properties were determined for films made from CO₂CN, a casein with hydrophobic character, and the more hydrophilic CaCN and AcCN. All but the AcCN films were plasticized with GLY or a 3:1 ratio of GLY: PPG. It was hypothesized that the OP, WVP, and tensile properties of the films were affected by changes in the free volume of the proteins induced by choice of plasticizer or increasing % RH, which introduced the plasticization effects of water. Because OP of the films from this study were comparable to or improved over those of synthetic polymers, they have excellent potential as protective barriers for foods and other products, either alone or as part of a multilayer laminate. However, their higher water vapor permeabilities and lower tensile strength may limit their use. Currently, their cost relative to that of synthetic polymers and the lack of developmental research for large-scale processing may limit their adoption in potential applications.

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