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Chitosan/lactic acid films were cast upon pectin films with either glycerol or lactic acid as plasticizer to give clear laminated films with dynamic mechanical properties similar to those for pectin films alone. To prevent fungal growth, lactic acid was used to replace glycerol in the pectin film without significant change in dynamic mechanical properties. The storage modulus and loss modulus of chitosan/pectin laminated films were significantly greater than respective moduli of chitosan films alone. Water vapor permeation of pectin or chitosan films, made with lactic acid, was unchanged by lamination.

Keywords: *Films; chitosan / lactic acid; pectin*

INTRODUCTION

One objective of research at this Center is to make biodegradable films from renewable, stable agricultural sources. These films can then be applied to replace some petroleum-based films or to new specialized niche areas made available by increasing environmental concerns. To this end, biodegradable films from milk proteins and polysaccharides (Parris et al., 1995) and from blends of pectin with starch, plasticized with glycerol, have been investigated (Coffin and Fishman, 1993, 1994). Considerable attention has been given to pectin because it is widely available from underutilized agricultural waste material and is readily modified, through demethylation, to give preparations that can form excellent films. Pectin and cellulose are the major cell wall structural polysaccharides of higher plant cells. Pectin is a family of heterogeneous branched polysaccharides consisting mostly of variably methylated galacturonan segments separated by rhamnose residues, some of which may be linked to short neutral sugar side chains. The rhamnose residues redirect the orientation of galacturonan segments to produce kinks which, upon aggregation, ensure open structures favorable for gel formation. Isolation of pectin from plant cell walls is achieved by breaking up the gel structure, usually stabilized by calcium cations, to solubilize large aggregates of pectin. Various grades of pectin are commercially available in different degrees of methyl esterification and in different ranges of molecular weights, or more accurately, different degrees of disaggregation. Pectin and starch blends can be used to make a range of films with very good properties (Coffin and Fishman, 1993). The scope of films made with pectin combined with other polysaccharides was widened to include chitosan for several reasons. First, chitosan is derived from chitin, the second most abundant polysaccharide on the earth, after cellulose (Lezica and Quesada-Allue 1990), and is commercially available from a stable, renewable source, that is, waste from the shellfish industry (Knorr, 1991). Second, chitosan forms good films and membranes. In Japan, films from composites of chitosan and cellulose have been made by casting dispersions on steel or chrome plates at elevated temperatures (70–100 °C; Nishiyama, 1993). Some of these films contained glycerol and had good tensile strength. They were readily biodegradable either in sea water or in soil (Hoskawa et al., 1990; Nishiyama, 1993). Third, the cationic properties of chitosan offer the film-maker an opportunity to take advantage of electrostatic interactions with anionic, partially demethylated pectins. Chitosan is a partially N-acetylated 2-deoxy-2-amino-α-glucan. Some heterogeneity is introduced by the distribution of free amino groups that result from hydrolysis of some of the acetyl groups of the homopolysaccharide, chitin (Errington et al., 1993).

Chitosan membranes can be formed by making films rigid with cross-linking agents, such as glutaraldehyde (Uragami et al., 1994) or divalent metal ions, or with polyelectrolytes (Dutkiewicz et al., 1992), including anionic polysaccharides, such as pectin. Chemically modified chitosan membranes can be used for separating ethanol from water by pervaporation (Lee, 1993). Chitosan membranes are being applied to water purification as well (Muzzarelli et al., 1989). Chitosan coatings applied to fruits and vegetables reduce water loss and extend shelf life (El Ghaouth et al., 1991). Chitosan films have been investigated for controlled release of pharmaceuticals (Bonvin and de Bertorello, 1993).

That chitosan films might be laminated to pectin films is suggested by the finding of Lehr et al. (1992) that, of all the natural polysaccharide wet films tested, including pectin, only chitosan films had mucoadhesive capability with standard pig intestinal mucosa. Therefore, a combination of hydrogen bonding, electrostatic forces between carboxylate groups of pectin and protonated amino groups of chitosan, and compatible water activities might make possible a stable interface between a pectin film and a chitosan film. Very strong electrostatic interactions can be expected to produce a precipitate or a thin membrane, as suggested by Dutkiewicz et al. (1992). For this reason a moderately unesterified pectin [65% degree of methylation (DM)], which forms good films with glycerol as plasticizer (Coffin and Fishman, 1993), was selected for investigation with a readily available commercial chitosan. A chitosan film laminated to a pectin film could be expected to alter water vapor permeability (WVP) and water solubility. Chitosan films are compatible with animal tissue (Balassa and Prudden, 1977), including the human eye, and are resistant to microbiological growth (Allan and

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Hadwiger, 1979). When attacked by natural fungi, chitosan films have a built-in source of nitrogen to enhance biodegradation. They also offer the potential of membrane construction through cross-linking of amino groups or insolubilization through alteration of pH (Yang et al., 1984).

MATERIALS AND METHODS

Materials. Lime pectin was obtained from Grinsted Products, Inc. (Kansas City, KS) and was 65% methylated (Type 1400). Chitosan was obtained from Pronova Biopolymer, Inc. (Raymond, WA,) and was 15% acetylated (Seacure 240). Lactic acid (85%) and glycerol were of ACS reagent grade. Distilled water was used to prepare all solutions.

Film Preparation. Pectin (2–4 g) was added to rapidly stirred water (200 mL) containing either glycerol (1%) or lactic acid (1%). After 4 h, the viscous solution was filtered through glass wool to remove air bubbles. Filtrate (25–30 mL) was poured directly into a plastic Petri dish or onto a chitosan film preformed in a similar plastic Petri dish. Chitosan (2–4 g) was added to rapidly stirred water (200 mL) containing the same amount of lactic acid, according to the manufacturer's recommended procedure. After 1 h, the clear solution was filtered through glass wool to remove undissolved bits of material. The chitosan lactate, bubble-free filtrate (30 mL), was poured either directly into a polystyrene Petri dish or onto a pectin film preformed in a similar Petri dish. After air-drying for at least 72 h, the films were dried at room temperature in a vacuum oven for 30 min. Each film was easily peeled from the Petri dish by inserting a razor blade at the film–rim interface and pulling up around the edge with forceps. A 25 mL volume of polysaccharide solution was finally adopted to completely cover the bottom of the Petri dish. Good reproducibility was attained by using fixed volumes within a uniform casting environment. Each solution thereby was constrained to lose nearly the same amount of water during film formation.

Mechanical Testing. Dynamic tests were performed with a Rheometrics (Piscataway, NJ) RSA II solids analyzer using the appropriate set of jaws for holding films. Liquid nitrogen was used for testing at below room temperature. A nominal strain of 0.1% and an applied frequency of 10 rad/s (1.59 Hz) were routinely used. Film strips, 7.0 mm × 38.1 mm, were excised from the center of the circular films with a razor blade. Sample thickness was measured with a Model 3 Dial Comparator micrometer (B. C. Ames Co., Waltham, MA). The gap between jaws holding the film strip was set at 23.0 mm. Test results were analyzed with Rheometrics RHIOS v3.0.1 software run on an IBM PC MSDOS platform.

Water Vapor Permeation. The method of McHugh et al. (1994) as adapted by Parris et al. (1995) was used. Films were equilibrated in an environmental chamber (National Appliance Co., Portland, OR) maintained at 50% relative humidity and 25 °C prior to measurement.

RESULTS

Pectin films cast on chitosan films had surface irregularities due to uneven shrinkage. Nevertheless, chitosan films could be cast on pectin films to give clear films without any noticeable features, including haze, at the interface. These clear, double-cast films are called here laminated films, and the first film cast is called the foundation film. Compositions of solutions used to cast films are in units of grams per deciliter. Laminated film thickness fell within the range of 0.10–0.15 mm. When glycerol was added to pectin to reduce brittleness, fungal growth was occasionally observed at the interface of the pectin/chitosan film. Stereomicroscopic examination of individual fungal colonies revealed that the mycelia extended into the lower pectin level of the laminated film. After a colonized film

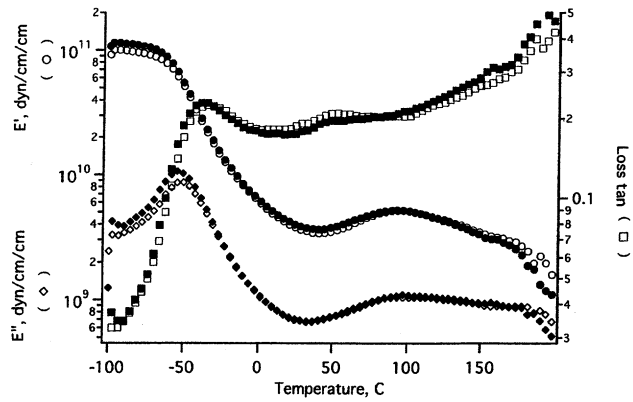

Figure 1. Effect of replacement of glycerol (solid markers) with lactic acid (open markers) in pectin films on storage (E') and loss (E'') moduli and loss tan.

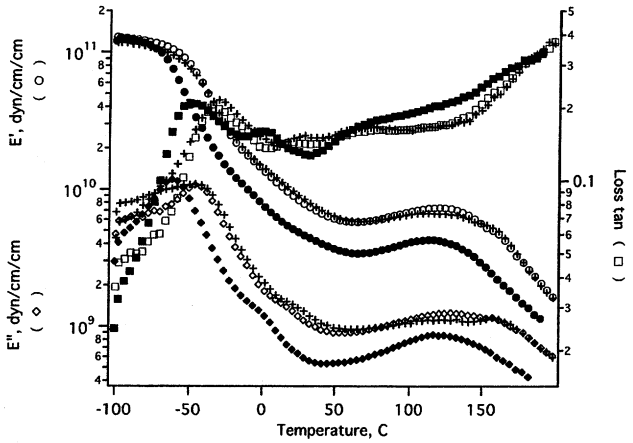

Figure 2. Effect of replacement of glycerol (solid markers) with lactic acid (open markers) in pectin/chitosan laminate films on storage (E') and loss (E'') moduli and loss tan. Responses from a laminate with an equal proportion of lactic acid and glycerol in the pectin foundation film are indicated by crosses.

completely dried, the growth of each fungal colony stopped. Lactic acid was substituted for glycerol in the pectin films, and no further fungal growth was observed. The pectin films with lactic acid (2:1 g/dL) had properties similar to those for pectin films with glycerol and lactic acid (2:0.5:0.5 g/dL; Figure 1). No significant differences in storage moduli, a measurement of stiffness, were observed when a low level of glycerol in pectin/glycerol/lactic acid/chitosan/lactic acid (2:0.5:0.5:1:1 g/dL) film was replaced with lactic acid (Figure 2). Laminated films with a high level of only glycerol in the pectin foundation film had lower storage and loss modulus values than those films made with lower levels of either glycerol/lactic acid or lactic acid alone in the pectin film layer (Figure 2). Loss modulus is a measure of slippage resulting in irreversible stretching. Good storage modulus values of 8×10^{10} to 9×10^{10} dyn/cm^2 near room temperature were obtained for laminated films of pectin/lactic acid/chitosan/lactic acid (2:1:1:1 g/dL) and were significantly higher than the values for films from pectin/glycerol/chitosan/lactic acid (2:2:1:1 g/dL; Figure 2). As shown in Figure 3, the lamination of a chitosan/lactic acid film to a pectin/lactic acid film showed some increase in both storage and loss moduli within the temperature range of −100 to ca. 200 °C compared to the film cast from just pectin and lactic acid (unlaminated). As can be seen in Figure 4, the storage modulus value for a film made from only chitosan and lactic acid dropped precipitously from near

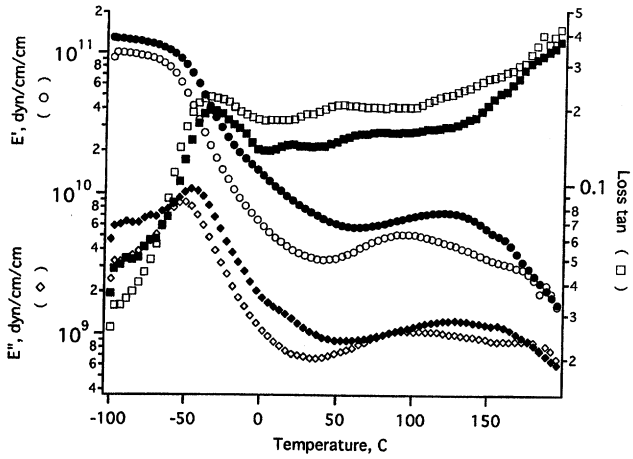

Figure 3. Effect of lamination of chitosan/lactic acid (solid markers) film onto pectin/lactic acid film (open markers, unlaminated) on storage (E') and loss (E'') moduli and loss tan.

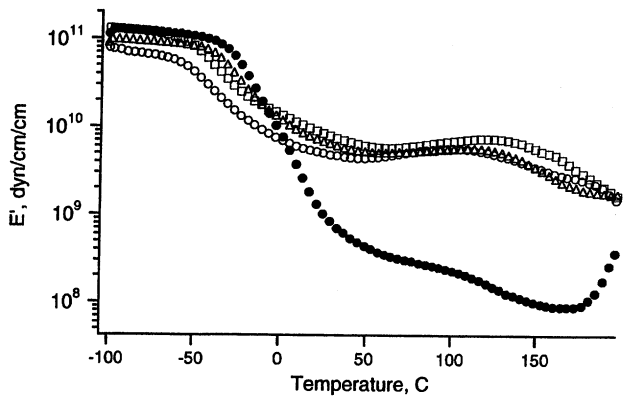

Figure 4. Storage modulus for pectin (P)/lactic acid (LA) film (P:LA = 2:1, open circles), for chitosan (C)/lactic acid film (C:LA = 2:2, solid circles), and for laminates (P:LA:C:LA = 2:1:1:1, squares, and P:LA:C:LA = 1:0.5:1:1, triangles).

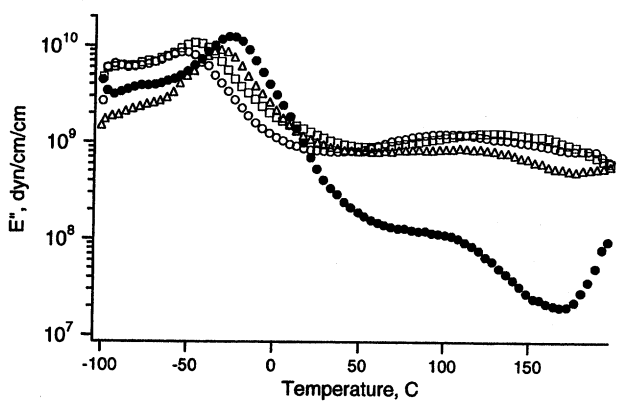

Figure 5. Loss modulus (E'') for pectin (P)/lactic acid (LA) film (P:LA = 2:1, open circles), for chitosan (C)/lactic acid film (C:LA = 2:2, solid circles), and for laminates (P:LA:C:LA = 2:1:1:1, squares, and P:LA:C:LA = 1:0.5:1:1, triangles).

−30 to 50 °C. Near room temperature the storage modulus was approximately 1×10^9 dyn/cm^2. The film from pectin/lactic acid (2:1 g/dL) had a storage modulus that stabilized from 0 to well over 100 °C and was from 5×10^{10} to 8×10^{10} dyn/cm^2 (Figure 4). The loss modulus for films made from chitosan/lactic acid (2:2 g/dL) dropped below that for pectin/lactic acid (2:1 g/dL) above ca. 20°C (Figure 5). The laminated films of chitosan/lactic acid (1:1 g/dL) on pectin/lactic acid films at compositions of either 2:1 or 1:0.5 g/dL had similar storage modulus–temperature profiles. Some small structural irregularities were observed for laminated films made with the thinnest foundation film (pectin/lactic acid 1:0.5 g/dL).

Replacement of glycerol by lactic acid in the foundation pectin film of the laminate resulted in an increase of water vapor permeability (WVP) of from 1.88 ± 0.07 for pectin/glycerol/chitosan/lactic acid (2:1:1:1 g/dL to 2.36 ± 0.14 g mm/kPa h m^2) for pectin/lactic acid/chitosan/lactic acid (2:1:1:1 g/dL). Unlaminated films of either pectin/lactic acid (2:1) or chitosan/lactic acid (2:2 g/dL) had comparable WVP values of 2.23 ± 0.20 and 2.25 ± 0.15 g mm/kPa h m^2, respectively.

DISCUSSION

Glycerol was first used as a plasticizer for pectin foundation films used for chitosan film laminates because Coffin and Fishman (1993) found that the brittleness of lime pectin and lime blended pectin/starch films could be effectively reduced by reasonable levels of glycerol. Lactic acid was selected for chitosan films to dissolve the chitosan and to control brittleness. The chitosan/lactic acid proportion was maintained at 1:1 because of limited solubility of chitosan at higher or lower proportions of acid. A number of films made with chitosan and acetic acid became hazy within a month. Apparently, in these films, chitosan formed local precipitates as acetic acid slowly left the film. No such haze was observed in any of the films made with lactic acid over a period of up to 4 months.

Pectin/glycerol and/or lactic acid films cast on chitosan/lactic acid films gave highly irregular laminates. In a few instances, the pectin film shrank away from the wall of the polystyrene Petri dish toward the center. This shrinkage occurred over the surface of the chitosan film and indicated, therefore, that there was very little ionic interaction between the protonated amino groups of the chitosan and the negatively charged carboxylate groups of the 65% methylated lime pectin. The irregular drying patterns of pectin films upon chitosan films might be attributable to differential water activity gradients set up by water loss from the pectin/glycerol solution through the chitosan film, in addition to loss directly to air above the solution. Water loss through the chitosan film could be expected to follow known fractal percolation pathways (Adler, 1989) that could possibly contribute to uneven drying of the pectin/glycerol film. That chitosan films have lower WVP values than pectin films, discussed below, may be a significant factor. Films from water soluble polysaccharides and/or proteins have usually been cast on water impenetrable surfaces such as glass, plastics, or stainless steel. It is not fully understood at this time why chitosan films cast on pectin films were optically clear and dried without structural irregularities.

A problem with some of the laminated films made with glycerol developed when fungal colonies were occasionally observed. These colonies all had similar morphologies and appeared to grow in the middle of the laminated films during the first 72 h as the chitosan/lactic acid film dried. Since the mycelia were observed to extend down into the pectin/glycerol layer, it was concluded that the pectin/glycerol region within the laminate presented conditions favorable for growth up to the point that the chitosan/lactic acid film completely dried. Thereafter, growth was arrested and each fungal colony became fixed. When a section of laminated film containing such a colony was left in water, the whole film was enveloped by the fungus within 24 h. In one sense, this laminated film had built-in biodegradability.

All fungal growth could be avoided by replacing the glycerol in the pectin film by lactic acid and by using pectin solutions of at least 2 g/dL. Whether or not this was an effect of pH and/or pectin concentration is not at present known.

The storage and loss modulus and loss tan temperature profiles in Figure 1 for pectin/glycerol and pectin/lactic acid demonstrate that replacement of glycerol by lactic acid had negligible effect on the flexibility of the film. These results suggested that it would be worthwhile to employ lactic acid in the pectin films for lamination studies in place of glycerol to avoid unnecessary complications due to fungal growth.

Laminated films with low levels of lactic acid in the pectin film (2:1 or 1:0.5 g/dL) had storage and loss modulus values as high as or slightly higher than those of their counterparts made with 50% or 100% glycerol plasticizer compositions (Figure 2). At present no particular flexibility advantage can be seen in using mixtures of glycerol and lactic acid for plasticizers in pectin foundation films, because, apparently, lactic acid alone preserves high storage and loss modulus values.

The small (<1 mm) structural irregularities observed in laminate films made with pectin/lactic acid at a level of 1:0.5 g/dL can be ascribed to insufficient thickness, since no such irregularities were seen when levels of pectin/lactic acid and/or glycerol at 2:1 were used in the foundation film. The loss of water from the chitosan/lactic acid solution that covers the pectin film was probably made complex by the assumed capture of some of this water by the pectin film before a final equilibrium condition (dry state) was obtained for the laminate. That even those thinner (ca. 0.10 mm) laminates with structural irregularities had storage and loss moduli comparable to thicker (ca. 0.13 mm), completely clear laminates is noteworthy (Figure 2) in that some irregularities might be tolerated for those film applications not dependent on high clarity.

The results from dynamical testing of both storage and loss moduli done on the laminated films support the following conclusions:

(1) Replacement of glycerol by lactic acid either in pectin films or in pectin/chitosan laminates does not significantly change the storage or loss modulus.

(2) When laminated to pectin films, lower storage and loss modulus chitosan/lactic acid film give laminates with properties similar to those for pectin films alone.

It may well be that the less stiff chitosan film was made more stiff when one of its surfaces was in intimate contact with a stiff pectin film. Otherwise, the storage and loss modulus values might have been proportional to the amount of chitosan film laminated to the pectin film. These results also suggest that chitosan films can be laminated to pectin or possibly blended pectin/starch films by direct casting and that the resulting laminated films will have properties similar to the pectin foundation film. The laminate would then have one surface with the biocompatible properties, especially skin contact, of a chitosan film and the overall strength and flexibility of a pectin or blended pectin/starch film. The nitrogen content of the chitosan layer can be expected to improve biodegradability. Pectin/chitosan laminate films shouild be more resistant to water dissolution than pectin or blended pectin/starch films, because chitosan and pectin in solution react to form insoluble precipitates.

Pectin films made with glycerol had a significantly lower WVP (1.88 g mm/kPa h m^2) than Parris et al. (1995) found for pectin alone. In the case of films made from whey proteins, glycerol increased the transferability of water, and Mahmoud and Savello (1992) attributed this effect to a loosening and expansion of the protein gel network of the film by glycerol. Fishman et al. (1993), on the other hand, found that in solution glycerol caused a breakup of pectin gel particles into smaller aggregates. Glycerol in a pectin film may therefore be expected to decrease WVP due to a partial collapse of the pectin gel network. This would also account for the decrease in pectin film brittleness observed by Coffin and Fishman (1993) with the addition of glycerol as a plasticizer. The high value of 2.36 g mm/kPa h m^2 measured for the WVP of the lactic acid laminate film suggests that lactic acid did not disrupt the pectin gel network to the extent that did glycerol. In the case of chitosan films, lactic acid is required for a stable noncrystalline network through initial solubilization of the polysaccharide. Apparently, lamination of chitosan/lactic acid film to pectin/lactic acid film does not have a significant effect on the WVP of either film. Perhaps the lactic acid in all of these films controlled the WVP. This possibility requires further investigation.

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