Edible Films from Rice Protein Concentrate and Pullulan

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ABSTRACT

Edible films were prepared using a combination of rice protein concentrate and the polysaccharide pullulan. The protein-pullulan mixture with up to 50% protein concentrate could be cast on a glass plate into films with tensile strength of about 18 MPa and water vapor permeability of 40 g × mil/m² × day × mmHg. Film strength and water vapor resistance were improved by the addition of small amounts of propylene glycol alginate under alkaline condition. Oils were also incorporated into the film for improved water vapor resistance.

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Rice protein concentrate is a desirable food ingredient because the protein it contains is highly nutritional and hypoallergenic. However, because of the protein's lack of functional properties such as solubility in water, rice protein concentrate is underutilized as compared with other plant protein products (Hansen et al 1981). One way to improve its food-use functionality and to develop new products is to combine the protein concentrate with functional polysaccharides such as pullulan and alginate.

Rice protein concentrate can be prepared by the alkaline extraction of rice flour or of the broken rice kernels that are a by-product in the milling of rice. An alternative is to treat the flour or kernels with enzymes to partially remove the starch component (Chen and Chang 1984). On the other hand, many functional polysaccharides can be conveniently generated by fermentation of sugar and starch with microorganisms. Thus, alginate can be produced by *Azotobacter vinelandii* (Fyfe and Govan 1982) and pullulan by *Aureobasidium pullulans* (Catley 1979). Rice flour and broken rice kernels are a good source of sugar and starch. Therefore, both rice protein concentrate and the polysaccharides pullulan and alginate could be obtained as value-added products from under-utilized by-products of the rice manufacturing industry.

Research on edible films has been active in recent years, and many reviews on this new technology are available in the literature (Guilbert 1986, Kester and Fennema 1986). Edible films can be used as a carrier for flavor and nutritional additives or as a barrier for separation, protection, and preservation purposes. For example, yuba film from soy protein, which is a popular food ingredient in Asian countries, has a desirable texture and mouthfeel when soaked in water and is often used as an edible food wrapper. Rice protein concentrate does not form yuba-like films, whereas both pullulan and alginate, like most hydrocolloids, form films readily. However, films from starch, polysaccharide, or protein, including yuba film, are normally sensitive to humidity changes. When these polymers are combined, they could interact physically and chemically and result in films with improved properties.

Of particular interest is the macromolecular interaction involving the alginate derivative propyleneglycol alginate (PGA). In addition to being a good film-forming polysaccharide, PGA can cross-link with amino groups in protein and hydroxyl groups in starch or other polysaccharides (McDowell 1970). As a result, the presence of small amounts of PGA could significantly modify and often improve the performance of the protein-polysaccharide films (Shih 1994).

In this research, edible films were prepared using rice protein concentrate and pullulan as the main components. The effects of cross-linking with PGA and incorporation of oil on film properties including tensile strength and water vapor permeability were investigated.

MATERIALS AND METHODS

Reagents

Rice protein concentrate with 53% protein (N × 6.25), prepared by the treatment of rice flour with enzymes to partially remove the starch component, was provided by California Natural Products (Lathrop, CA). Pullulan was obtained from Hayashibara Co. (Okayama, Japan), polyethylene glycol (Carbowax 300) from Union Carbide Chemicals (Danbury, CT), and PGA from the Kelco Division of Merck & Co. (Rahway, NJ). All other reagents and chemicals were of ACS reagent grade.

Protein Analysis

Nitrogen of the rice protein concentrate was determined using the FP-428 nitrogen determinator (LECO, St. Joseph, MI). Subunits of the protein were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis according to Laemmli (1970). The Coomassie blue R-250 stained gels (4–20% gradient) were analyzed by densitometry and image analysis using the GDS200 gel documentation system (UVP, San Gabriel, CA).

Protein-Pullulan Films

A solution was prepared by dissolving 0.10 g of PGA, 0.25 g of Carbowax 300, and 0.50 g of glycerol in 22 ml of deionized water. To the solution was added a total of 6.00 g of various ratios of rice protein concentrate and pullulan. The mixture, with a pH of 6.8–7.0, was homogenized at 20,550 rpm for 3 min using an Utra-Turrax T25 homogenizer (IKA, Staufen, Germany). The resulting slurry was cast on a glass plate with a film spreader at a 1-mm clearance. The films were air-dried at room temperature overnight, removed from the plate, and stored in a chamber at 65% rh for 48 hr before analysis.

Films were also prepared by adjusting the slurry to pH 8.5 with 0.6*N* ammonium hydroxide (≈1 ml) before films were cast. Controls without PGA, but at pH 7 and 8.5, were also conducted.

Water-resistant additives, including canola oil and lecithin, were incorporated by two methods. In one procedure, 0.80 g of the additive was added to the slurry during the film preparation. In the other procedure, a thin layer of the additive, at add-ons of 0.78–0.83 g, was cast onto the surface of the preformed protein-pullulan film.

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Tensile Testing

Tensile strength and percent elongation at break of the films were analyzed (ASTM 1993) with a universal testing machine (model 4201; Instron Engineering Corp., Canton, MA). Films were preconditioned at 21.1°C and 65% rh for 48 hr before analysis (a modification from the 25°C and 50% rh normally used). Film thickness was determined as an average of seven measurements with a bench comparator (B.C. Ames, Waltham, MA). The tensile strength in megapascals was calculated by dividing the maximum load by the cross-sectional area of the film. The percent elongation was calculated from the maximum elongation during testing verses the original gauge length of the film.

Water Vapor Permeability

Water vapor permeability (g × mil/m^2 × day × mmHg) of the film was determined by the "cup method" (ASTM 1993) with some modifications. Film specimens were conditioned for 48 hr in a chamber at 21.1°C and 65% rh before being analyzed in a Carson dish (No. 305-8; Thwing-Albert, Philadelphia, PA). The film was positioned on top of the cup and sealed with beeswax. Anhydrous calcium chloride was used in the cup as the desiccant.

Solubility

Solubility of the film was determined by soaking and gently stirring about 1 g of thin strips of film in 10 ml of water for 0.5 hr. After the removal of water by centrifugation, the residue of the film strips was recovered and dried in an oven. Solubility was calculated as the percent weight loss of the film strips from the soaking.

RESULTS AND DISCUSSION

Film-Forming Components in Rice Protein Concentrate

Rice protein concentrate consists of protein and carbohydrate as the main components. According to the manufacturer, total carbohydrates (39%) include sugars (6–22%), dietary fiber (7%), and polysaccharides (11–27%). As macromolecular interaction is often involved in the development of film-forming properties, the fraction of 11–27% polysaccharides appears to be the most capable of enhancing the formation of films.

Rice protein (53%) is by far the most prominent film-forming polymer in the system. The protein content was confirmed in our laboratory by nitrogen analysis, and the protein concentrate was found to contain very little nonprotein nitrogen compounds. Subunits of the protein were analyzed by electrophoresis. Scanning of the electrophoretic profile (Fig. 1) indicated that the protein has five major subunits, including three with molecular weights of 35,500, 25,500, and 16,500, which are typical subunits of rice endosperm glutelin (Juliano and Boulter 1976). These protein subunits are obviously the ingredients most likely to interact by physically complexing or chemically forming covalent bonds with pullulan in the formation of edible films.

Effect of Film Composition

Protein or polysaccharide films are normally either brittle in texture at low relative humidities or weak in strength at high relative humidities. Efforts have been made to modify film strength and water sensitivity by changing the composition and processing procedures. Figure 2 shows the tensile strength and water vapor permeability of protein-pullulan films as a function

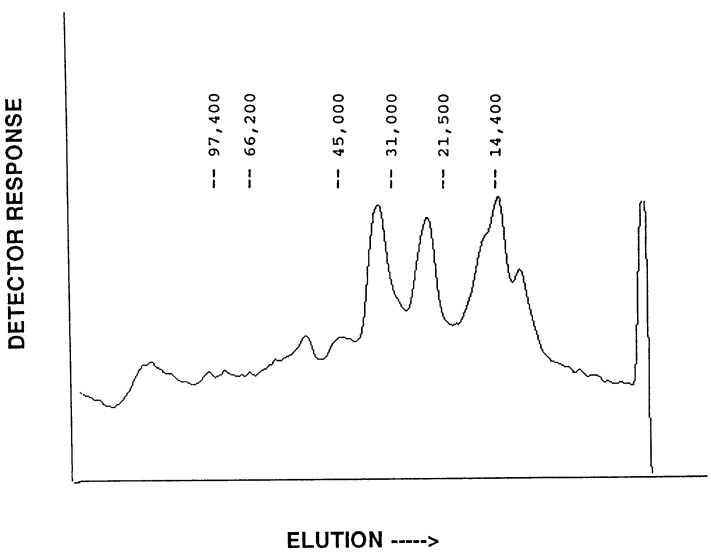

Fig. 1. Densitometry scanning of the sodium dodecyl sulfate polyacrylamide gel electrophoresis profile of rice protein concentrate

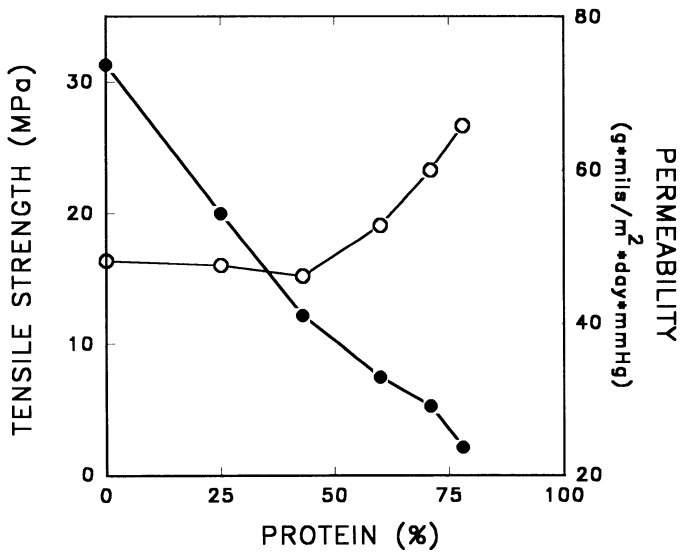

Fig. 2. Plots of tensile strength (•) and water vapor permeability (○) as a function of protein composition in protein-pullulan films.

TABLE I
Effect of Increased Cross-Linking by Propyleneglycol Alginate (PGA) at Alkaline pH on Tensile Strength, Elongation at Break (*E*), Water Vapor Permeability (WVP), and Solubility of Protein-Pullulan Films

Film[a]	Protein-Pullulan Ratio (w/w)	Thickness (mil)	Tensile Strength (MPa)	*E* (%)	WVP (g × mil/m^2 × day × mmHg)	Solubility (%)
1A	0:1	3.2 ± 0.1	34.2 ± 0.5	2.9 ± 0.2	32.6 ± 0.4	60.3 ± 1.6
1B	0:1	3.4 ± 0.1	31.1 ± 0.5	2.9 ± 0.3	48.2 ± 0.6	76.4 ± 1.4
2A	3:4	4.8 ± 0.1	18.1 ± 0.3	3.8 ± 0.2	38.4 ± 0.3	35.4 ± 0.8
2B	3:4	4.5 ± 0.2	12.0 ± 0.1	3.9 ± 0.4	46.2 ± 0.6	59.3 ± 1.6
3A	7:2	10.0 ± 0.5	2.0 ± 0.1	4.1 ± 0.4	45.9 ± 0.7	35.3 ± 0.8
3B	7:2	10.0 ± 0.4	1.2 ± 0.1	4.0 ± 0.2	65.8 ± 1.0	51.4 ± 0.9

[a] Films 1A, 2A, and 3A were prepared at pH 8.5, which enhanced the PGA cross-linking; films 1B, 2B, and 3B were prepared at neutral pH, which did not enhance the PGA cross-linking.

of rice protein concentrate content. Pure pullulan films or those with a low content of rice protein concentrate were fragile under normal conditions and had relatively high tensile strengths of about 30 mPa. With the addition of more protein concentrate, film strength decreased and so did the brittleness. Water vapor permeability remained unchanged for films with protein concentrate as high as 50%. For films with higher than 50% protein concentrate, the permeability increased, or the resistance to water vapor decreased, with increasing protein concentrate. For films with about equal ratios of protein concentrate and pullulan, improvements could be achieved with a decreased but acceptable film strength and practically no sacrifice in water vapor resistance.

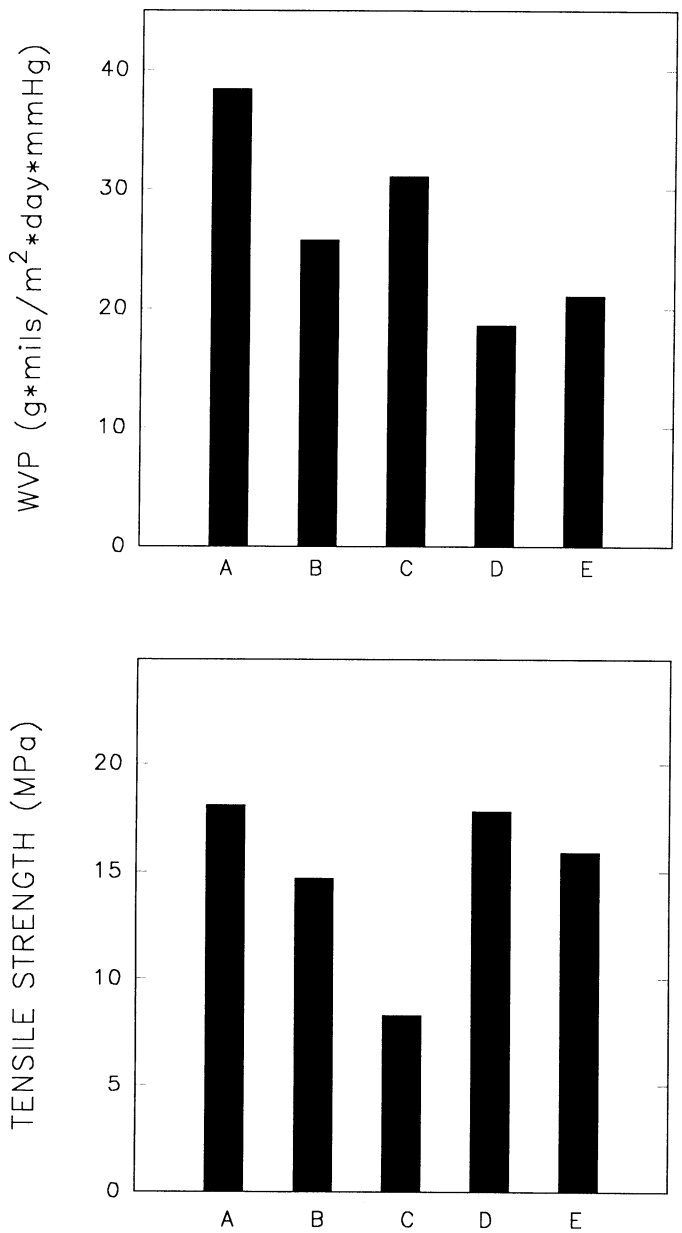

Fig. 3. Effect of lipids incorporated by different methods on water vapor permeability (WVP) and tensile strength of protein-pullulan films. The films consisted of rice protein concentrate and pullulan at a 3:4 (w/w) ratio. Film A, with no lipids added, served as a reference. Films B and C were prepared by the emulsifying method with the incorporation, at 10% each, of canola oil and lecithin, respectively. Films D and E were prepared by the coating method with the incorporation, at 10% each, of canola oil and lecithin, respectively.

Effect of Cross-Linking

A small amount of PGA at 1.5% (w/w) of the total solid was added to the protein-pullulan films to improve overall performance. PGA interacts with protein or polysaccharide to form covalent bonds, particularly under alkaline conditions (McDowell 1970; Shih 1994). Control films without PGA at pH 7 and 8.5 showed no difference in performance (data not shown). Table I shows the effect of PGA on film properties when cross-linking was increased by having a pH of 8.5 instead of 7. Films 1A and 1B are pure pullulan films, prepared under alkaline (pH 8.5) and neutral (pH 7) conditions, respectively. Film 1A showed higher tensile strength and lower water vapor permeability than film 1B, both desirable improvements. Similarly, film 2A showed improvement as compared with 2B for films with a protein concentrate-pullulan ratio of about 3:4, and film 3A as compared with 3B for high protein (75% protein concentrate) films. Because both pure pullulan films and high-protein films responded positively to this PGA cross-linking reaction, PGA appeared to interact or cross-link effectively with both the amino groups in protein and the hydroxyl groups in pullulan.

Protein concentrate or polysaccharide films are normally highly hygroscopic, and disintegrate readily in water. The PGA treatment, particularly at alkaline pH, improved the integrity of the protein-pullulan films. Thus, as shown in Table I, with increased cross-linking, films 1A, 2A, and 3A were less soluble in water than 1B, 2B, and 3B, respectively. These alkali-treated films maintained their shapes when soaked and gently stirred in water.

Water-Resistant Additives

For further improvement in resistance to water vapor, attempts were made to introduce various water-resistant additives into the protein-pullulan films. In one procedure, canola oil and lecithin were added to the protein-pullulan slurry and homogenized before it was cast into films. In another procedure, the same fatty materials were used to coat the preformed protein-pullulan films. In both cases, the presence of protein appeared to enhance the incorporation of the oil additives and made the film less greasy-looking. The mixture of protein concentrate and pullulan at a 3:4 ratio was chosen for our investigation. Different oil additives were incorporated into films at comparable add-ons. Up to 15% oil can be incorporated into the film with no noticeable greasy appearance. Figure 3 shows the effect of oil additives on the water vapor permeability and tensile strength of the protein-pullulan films. Compared to the water vapor permeability for the oil-free film A, addition of canola oil using the emulsifying method (film B) and the coating method (film D) lowered the water vapor permeability value by 32.8 and 51.6%, respectively. The decreases were 19.0 and 45.1% for lecithin using the emulsifying method (film C) and coating method (film E), respectively. Apparently, in the development of water-resistant properties, oil-coating to form a bilayer film is significantly more effective than oil-emulsifying to form a homogeneous film. The result agrees with earlier findings by Schultz et al (1949), who found that low-methoxyl pectinate films were better moisture barriers when lipids, including natural waxes and fatty acids, were added as a surface coating than when they were suspended in the film-forming solvent system. Presumably, the superiority of the surface-coated forms results from greater continuity of the lipid layer.

Tensile strength of the film was also affected by the method of formation and the difference in additives. While strength was slightly lowered for the lecithin-coated bilayer film, it remained practically unchanged for the canola-coated one. On the other hand, tensile strength was lowered to a greater extent for films made by the emulsifying method, particularly for the lecithin-added film.

CONCLUSIONS

Edible films consisting of about a 1:1 ratio of rice protein concentrate and pullulan have a good balance of tensile strength and water vapor permeability, which is not achievable for films with either component alone. Addition of small amounts of PGA, which interacts with both protein and carbohydrates, further increases the film's tensile strength, integrity in water, and water vapor resistance. Films coated with oil are more effective barriers to the transfer of water vapor than those with oil incorporated by the emulsification method.

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