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Modification of Physical and Barrier Properties of Edible Wheat Gluten-Based Films¹

ARISTIPPOS GENNADIOS,² CURTIS L. WELLER,² and ROBERT F. TESTIN³

ABSTRACT

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Edible films were produced from wheat gluten-based film-forming solutions. One film was produced as a control. Other types came from subjecting control films to three different soaking treatments. Three additional films were also produced by modifying the control film-forming solution. For all films, selected physical properties and permeability to water vapor and oxygen were measured. Comparisons indicated possible ways to improve the control film. All films were good oxygen barriers but limited water vapor barriers. Films containing hydrolyzed keratin

had lower oxygen permeability (83%) and lower water vapor permeability (23%). Films containing mineral oil had lower water vapor permeability (25%). Films soaked in calcium chloride solution and in buffer solution at the isoelectric point of wheat gluten had higher tensile strength (47 and 9%, respectively) and lower water vapor permeability (14% and 13%, respectively). Addition of a reducing agent increased tensile strength (14%). Soaking in lactic acid solution did not improve the standard film properties.

Edible films and coatings formulated from protein, polysaccharide, and lipid substances have been suggested as a means of food protection and preservation. A few examples have already found commercial use: meat casings from collagen (Hood 1987), waxes for fruits and vegetables (Kaplan 1986), and corn zein-based coatings for nutmeats and candies (Alikonis 1979).

Research in the field is active, and the concept is promising for new applications. Kester and Fennema (1986) and Guilbert (1986, 1988) have written technical reviews on edible films and coatings, summarizing past research and offering insights on the film-forming mechanisms. Reviews concentrating on film-forming abilities of wheat, corn, and soy proteins have been published by Gennadios and Weller (1990, 1991). Daniels (1973) reports a number of U.S. patents related to edible films and coatings.

Proteins from several plant sources, such as corn, wheat, soybeans, peanuts, and cottonseed, have been studied because of their film-forming capabilities. Poor water vapor barrier ability of protein films due to their hydrophilic nature constitutes their main limitation.

Film production from wheat gluten, a mixture of proteins accounting for about 80–85% of wheat flour proteins, has also been studied. Wall and Beckwith (1969) and Okamoto (1978) reported important information on the chemistry of the phenomenon, but the resulting films were weak and brittle. Stronger but readily water-soluble films were produced from gluten hydrolysates (Krull and Inglett 1971, Gutfreund and Yamauchi 1974).

Anker et al (1972) developed a method to produce strong and flexible films by casting heated gluten dispersions. Using this method, Aydt et al (1991) and Park and Chinnan (1990) produced wheat gluten films and evaluated several properties.

Gennadios et al (1990) adapted the procedure of Aydt et al (1991) with minor changes to produce a control wheat gluten film and characterized it by measuring a number of mechanical and barrier properties. The effect on these properties of modifying the film-forming solution was subsequently studied.

In the present study, the control wheat gluten film was prepared following the procedure of Gennadios et al (1990). Six alternative films were obtained: three by modifying the film-forming solution and three by applying treatments to the control film. A comparative study of all seven types of film was then carried out to measure physical properties (thickness, surface density, tensile strength,

and percentage elongation at break) and barrier properties (water vapor and oxygen permeability).

MATERIALS AND METHODS

Reagents

Wheat gluten (DO-PEP) was donated by ADM Arkady, Olathe, KS. Hydrolyzed keratin (CRODA K) was donated by Croda Inc., New York, NY. Glycerol, sodium sulfite, calcium chloride, and calcium nitrate, all of ACS grade, were purchased from Fisher Scientific, Pittsburgh, PA. Buffer solution (pH 7.5) was prepared by using preset pH crystals (TRIZMA, Sigma Chemical Co., St. Louis, MO.). Mineral oil (heavy, USP), lactic acid (USP), lithium chloride (purified), ammonium hydroxide, and ethanol were also purchased from Fisher Scientific.

Preparation of Control Film-Forming Solution

Film-forming solutions for the control film were prepared using the formula of Gennadios et al (1990). The mixture consisted of 15 g of wheat gluten, 72 ml of 95% ethanol, and 6 g of glycerol. The latter was added as a plasticizer. Gluten was dispersed in the solution by heating and stirring for 10 min on a magnetic stirrer-hot plate and slowly adding 48 ml of distilled water and 12 ml of 6*N* ammonium hydroxide. Heating rate was adjusted so that temperature of the solutions was 75–77°C at the end of preparation time.

Preparation of Modified Film-Forming Solutions

Film 1. Heavy mineral oil (3.5 g) was added to the control solution at the beginning of the heating period. Use of mineral oil as a component of food coatings is permitted by the Food and Drug Administration (CFR 1980).

Film 2. Sodium sulfite (0.2 g) was added to the control solution at the beginning of the heating period to facilitate dispersion of gluten. Sodium sulfite is a reducing agent with the ability to cleave intramolecular and intermolecular disulfide bonds developed between wheat gluten protein chains (Krull and Wall 1969).

Film 3. Of the 15 g of gluten used in the control solution, 2 g (about 13.33%) was replaced by hydrolyzed keratin protein.

Treatments on the Control Film

Film 4. Control films were soaked in 15% (w/w) lactic acid solutions for 20 sec to introduce a tanning effect on the films.

Film 5. Control films were soaked in 1*M* aqueous calcium chloride (CaCl₂) solution for 20 sec and then immediately submerged in distilled water for 10 sec to remove excess solution. This bonded the divalent calcium cations with pairs of negatively charged sites on polypeptide chains, promoting crosslinking in the film structure.

Film 6. Control films were soaked for 20 sec in a buffer solution with a pH of 7.5, a value corresponding to the isoelectric point of wheat gluten (Wu and Dimler 1963). Insolubilization of the

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wheat gluten protein at its isoelectric point was the reason for applying this treatment.

Casting of Film-Forming Solutions

Upon removal from the hot plate, mixtures were kept at room conditions for 2–3 min to allow bubbling to cease before casting on flat glass plates. Spreading of mixture on the plates was performed with a thin-layer chromatography spreader bar (Brinkman Co., New York, NY). Thick layers of masking tape were attached to the glass plates parallel to the spreader-moving direction, leaving an available casting area of 40 cm × 30 cm. The tape layers restrained movement of cast solutions perpendicular to the casting direction, contributing to efforts for even film-forming solution distribution and control over film thickness. Film-forming solutions for films 1 and 3 were passed through a hand homogenizer (Chase-Logeman, Hicksville, NY) before casting to ensure incorporation of the added substances.

Drying of Cast Film-Forming Solutions

Glass plates with cast solutions were placed in an air-circulating oven (Isotemp, model 338F, Fisher Scientific) maintained at 32±2°C. After 15 hr, the plates were removed from the oven, films were peeled off the glass surface, and testing specimens were cut.

Drying of Soaked Films

Immediately after soaking, specimens from films 4, 5, and 6 were hung by metal clips in the air-circulating oven and left to dry for about 5 hr at 32±2°C.

Conditioning Before Tests

Before measurements of thickness, surface density, tensile strength, and percentage elongation at break were made, the film was conditioned in a desiccator maintained at 50±5% rh and 23±2°C. The rh was controlled at that level by using a saturated solution of calcium nitrate. Another desiccator, maintained at 11±5% rh and 23±2°C, was used for film conditioning before measuring water vapor and oxygen gas transmission rates. The rh was controlled by using a saturated solution of lithium chloride.

Thickness and Surface Density

A hand-held micrometer (B. C. Ames Co., Waltham, MA) was used for measuring film thickness to the nearest 2.54 μm (0.1 mil) from 5-cm × 5-cm samples. For each type of film, four samples were taken from each of four separately cast films. Five micrometer readings were taken from each of these samples, one at the center and four around the perimeter. Therefore, a total of 80 thickness measurements were collected for each film type.

These 5-cm × 5-cm samples were also weighed on a balance to the nearest 1 mg. Balance readings were divided by the area of the samples (25 cm²) to calculate surface density. For each type of film, 16 surface density values were obtained.

Tensile Strength and Percentage Elongation at Break

Film tensile strength and percentage elongation at break were determined using an Instron Universal Testing instrument (model

4201, Instron Engineering, Canton, MA) operated according to the ASTM standard method D 882-88 (ASTM 1989). Initial grip separation and crosshead speed were set at 50 mm and 500 mm/min, respectively.

Peak loads and extension at break point were recorded for tested film specimens (100 mm long and 25.4 mm wide). Tensile strength was calculated by dividing peak load by cross-sectional area. Thickness of individual specimens, required to calculate cross-sectional area, was determined as an average of five micrometer readings taken on each specimen before testing. Dividing extension values by the initial grip separation (50 mm) and multiplying by 100 yielded percentage elongation at break. A total of 40 specimens were tested for each film type (10 specimens from each of four separately produced films).

Water Vapor Permeability

A water vapor transmission instrument (Permatran-W600, Mocon Inc., Minneapolis, MN) was used to measure water vapor transmission rate through films. This is a six-station, computer-monitored system, and it was operated according to ASTM standard method F 1249-89 (ASTM 1989). Test conditions were 23°C and 11.1% rh gradient. Testing and calculations for determining water vapor permeability values were performed as described by Gennadios et al (1990). Four samples were tested for each film type, each one obtained from a separately produced sheet.

Oxygen Permeability

An Ox-Tran 1000 instrument (Mocon, Inc.) was used to measure oxygen transmission rate through films. This is a 10-station computer-monitored system, and it was operated according to the ASTM standard method D 3985-81 (ASTM 1989). Testing at 23°C and 0% rh and calculations to determine oxygen permeability values were carried out as described by Gennadios et al (1990). Four samples were tested for each film type, each one obtained from a separately produced sheet.

RESULTS AND DISCUSSION

Calculated mean values and standard deviations for each measured physical property (thickness, surface density, tensile strength, and percentage elongation at break) are presented in Table I. Mean values and standard deviations for water vapor permeability and oxygen permeability are presented in Table II. Duncan's multiple-range test (Steel and Torrie 1980) was applied to determine significantly different ($P < 0.05$) means for each property.

Physical Properties

Control over film thickness is required for film uniformity, reliability of measured properties, and validity of comparisons between thickness-dependent properties of different films. During a previous study by Gennadios et al (1990), wheat gluten films were cast on glass plates in a manner similar to that used in this study. The only difference was that movement of the cast solutions on the glass surface was not restricted. Consequently,

TABLE I
Mean and Standard Deviation Values for Thickness, Surface Density,
Tensile Strength, and Elongation at Break of Various Wheat Gluten-Based Films^a

Treatment Number	Film Treatment	Thickness (μm)	Surface Density (mg/cm ²)	Tensile Strength (MPa)	Elongation at Break (%)
	Wheat gluten (control)	127 ± 11 b	14.9 ± 1.2 b	2.6 ± 0.2 c	237.9 ± 21.9 d
1	Wheat gluten with mineral oil	125 ± 11 b	15.0 ± 1.0 b	2.2 ± 0.3 d	267.2 ± 40.1 c
2	Wheat gluten with sodium sulfite	128 ± 8 b	14.7 ± 1.0 b	2.9 ± 0.4 b	192.3 ± 24.9 f
3	Wheat gluten with hydrolyzed keratin	119 ± 9 c	15.2 ± 1.1 b	1.7 ± 0.2 e	313.5 ± 34.5 b
4	Control soaked in 15% (w/w) lactic acid	136 ± 10 a	17.0 ± 0.7 a	1.4 ± 0.3 f	417.0 ± 41.5 a
5	Control soaked in 1M calcium chloride	127 ± 10 b	15.2 ± 0.8 b	3.8 ± 0.9 a	162.2 ± 40.3 g
6	Control soaked in pH 7.5 buffer	127 ± 9 b	14.9 ± 1.1 b	2.8 ± 0.4 b	215.0 ± 30.3 e

^aMeans followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.

the viscosity of cast solutions played an important role in film thickness. The less viscous a solution, the more it spread during casting and drying. In this study, spreading was prevented by layers of masking tape attached to the plates, resulting in lower thickness variability among and within films. Thickness variability within the same type of film was attributed to the manual operation (slightly variable casting speed of the spreader) and a lack of completely level drying-oven shelves. Rough spots on the film surface could have also yielded increased micrometer readings and thickness variability. A few rough granular spots were noticeable on films produced in this study. They probably originated from starch in the commercial wheat gluten that gelatinized upon heating and subsequently recrystallized on cooling.

Film 4, which was soaked in lactic acid solution, was the thickest. This may have resulted from lactic acid deposition on the film surface. Surface density data also support an indication of lactic acid uptake by film 4. Film 4 has surface density that is significantly higher than those of all other films.

Soaking in aqueous calcium chloride solution (film 5) produced films with the highest tensile strength and the lowest elongation (an increase of almost 50%). Adding sodium sulfite (film 2) or soaking in pH 7.5 solution (film 6) also produced films that were stronger than the control film. Soaking in lactic acid had a plasticizing effect on films. Films that received this treatment were the weakest (lowest tensile strength) and the most stretchable (greatest elongation).

Barrier Properties

Water vapor permeabilities were evaluated for the films by applying a small rh gradient (11.1%) across film samples. As explained in Gennadios et al (1990), this was necessary to avoid swelling, non-Fickian behavior of films, and overranging of the Permatran-W600 sensor.

Addition of mineral oil (film 1) and partial substitution of gluten with hydrolyzed keratin (film 3) reduced water vapor permeability by about 25% compared to that of the control film. Smaller reductions (about 15%) were observed by soaking in a calcium chloride solution (film 5) and in pH 7.5 solution (film 6). Conversely, adding sodium sulfite (film 2) yielded films with increased water vapor permeability.

Oxygen permeability of all films prepared in this study was low. Film 3, which contains keratin, was the only one with lower oxygen permeability (by about 80%) than that of the control film.

Film Formation

Polymerization during film formation of wheat gluten is believed to proceed through intermolecular disulfide, hydrophobic, and hydrogen bonding (Wall and Beckwith 1969). Sulfhydryl groups are responsible for the formation of disulfide bonds.

Denaturation of protein by heating in an alkaline environment is necessary to disrupt protein structure, break existing disulfide intramolecular bonds, and expose sulfhydryl and hydrophobic groups, making them available for bonding. Upon casting and drying, sulfhydryl groups lead to disulfide bonding by air oxida-

tion and chain-type sulfhydryl-disulfide interchange reaction.

Incorporation of mineral oil in the film-forming solution (film 1) was meant to take advantage of its nonpolar hydrophobic nature and increase film water resistance. A reduction of water vapor permeability (about 25%) was noticed. However, films produced in this manner had lower tensile strength.

Film 2 was produced by adding sodium sulfite to the film-forming solution. Sodium sulfite is a reducing agent that has the ability to cleave disulfide bonds. Therefore, the purpose of incorporating it in the solution was to increase the number of sulfhydryl groups and, subsequently, the intermolecular disulfide bonds formed after casting. Reduction in polypeptide chain lengths may also be beneficial, leading to higher mobility of the chains and increasing chances of bonding upon drying. Higher tensile strengths of the resulting films offers evidence of some increase in bonding, but permeability to both oxygen and water vapor is higher.

Partial substitution of wheat gluten with hydrolyzed keratin (film 3) resulted in films with better water vapor and oxygen barrier properties than all others prepared in this study. This may indicate that linkages developed between the two proteins.

Control films were soaked in lactic acid solution to induce a tanning effect on films and reduce their water vapor permeability. Guilbert (1986, 1988) observed that treating films with lactic acid and tannic acid (another tanning agent) improved water barrier abilities of protein films made from gelatin, casein, serum albumin, and ovalbumin. Submerging wheat gluten films in lactic acid during the present study (film 4) did not result in any improvement in water barrier ability. Furthermore, tensile strength of films treated with lactic acid was decreased. Strength reduction was expected because lactic acid has plasticizing ability, and its use as a plasticizer in edible films has been suggested (Krull and Inglett 1971).

The purpose of soaking films in aqueous CaCl₂ solutions (film 5) was to promote crosslinking in the protein structure by the divalent calcium cations (Ca²⁺). According to Wall and Beckwith (1969), magnesium divalent cations (Mg²⁺) increase wheat gluten consistency by eliminating negative charges and bridging together protein chains.

Guilbert (1986, 1988) soaked films produced from gelatin, casein, serum albumin, and ovalbumin in aqueous CaCl₂ solutions (20%, w/w). No significant improvement in water vapor barrier properties was noticed. Another study by Krochta et al (1988) concluded that soaking casein films in 0.75M aqueous CaCl₂ solution, followed by soaking in distilled water, lowered water permeability and solubility of films.

Soaking films in CaCl₂ solutions and then in distilled water during this study yielded statistically significant higher tensile strength and lower water vapor permeability than those of the control film. Therefore, Ca²⁺ may have provided some additional crosslinking in the film structure.

Protein insolubility at pH values equal to their isoelectric point suggests that soaking protein films in buffer solutions at these pH values could tighten film structure and improve water barrier ability. Krochta et al (1988) reported a reduction in water vapor

TABLE II
Mean and Standard Deviation Values for Water Vapor Permeability (WVP) and Oxygen Gas Permeability (O₂GP) of Various Wheat Gluten-Based Films^a

Treatment Number	Film Treatment	WVP ^b (× 10 ⁻¹¹ g/m·sec·Pa)	O ₂ GP ^c (amol/m·sec·Pa)
	Wheat gluten (control)	5.6 ± 0.3 c	2.0 ± 0.2 b-d
1	Wheat gluten with mineral oil	4.1 ± 0.1 e	1.7 ± 0.2 d
2	Wheat gluten with sodium sulfite	6.1 ± 0.4 b	2.1 ± 0.2 bc
3	Wheat gluten with hydrolyzed keratin	4.3 ± 0.3 e	0.3 ± 0.1 e
4	Control soaked in 15% (w/w) lactic acid	5.4 ± 0.3 c	2.1 ± 0.2 bc
5	Control soaked in 1M calcium chloride	4.8 ± 0.4 d	1.9 ± 0.1 cd
6	Control soaked in pH 7.5 buffer	4.8 ± 0.2 d	2.3 ± 0.3 b

^a Means followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.

^b Evaluated at 23°C with 11.1% rh on one side of the films and 0% rh on the other.

^c Evaluated at 23°C and 0% rh, where 1 amol = 10⁻¹⁸ mol.

permeability of about 50% after soaking casein films in buffer solutions adjusted to the isoelectric point of casein (pH 4.6).

According to Wu and Dimler (1963), the isoelectric points of the gliadin and the glutenin fractions of wheat gluten are pH 8.1 and pH 7.1, respectively; the isoelectric point of the whole gluten is pH 7.5. Soaking wheat gluten films in a solution adjusted to pH 7.5 during the present study (film 6) increased tensile strength and reduced water vapor permeability.

CONCLUSIONS

Edible films were consistently produced from various wheat gluten-based film-forming solutions. These films were characterized by measuring selected physical and barrier properties and comparing them to those of a control wheat gluten film. Adding a nonpolar hydrophobic substance (mineral oil) to the film-forming solution reduced water vapor permeability by about 25%. A film produced from wheat gluten and hydrolyzed keratin protein in a 6.5:1 ratio (weight basis) resulted in films that were not as strong as the control. However, they did exhibit lower oxygen permeability (about 83%) and water vapor permeability (about 23%). Using a reducing agent (sodium sulfite) to aid dispersion of wheat gluten in the film-forming solution yielded stronger films, but no improvement in water vapor and oxygen barrier ability was noticed. Treating films with calcium cations promoted cross-linking in the film structure, as evidenced by higher tensile strength measurements and lower water permeability of treated films. Treating films with a tanning agent (lactic acid) did not improve their barrier characteristics and also had a plasticizing effect on them. Soaking films in a solution adjusted to the isoelectric point of wheat gluten (pH 7.5) increased both strength and water barrier ability of films.

Efforts to reduce water vapor permeability of wheat gluten films had limited success. Nevertheless, the good oxygen barrier properties of such films can be utilized in food packaging and preservation. As an example, coating food items susceptible to lipid oxidation with protein films in combination with an external conventional moisture-barrier material appears possible. Another possible application is the use of wheat gluten films in multilayer laminates, replacing moisture-sensitive polymeric oxygen barriers such as polyamide and ethylene vinyl alcohol. Future research should explore possibilities of improving wheat gluten film properties by promoting crosslinking through enzymatic and chemical protein treatments. Aldehydes and other tanning agents employed in the leather industry for crosslinking of collagen could probably enhance wheat gluten film properties. However, the toxicity of such tanning agents would result in loss of film edibility, leaving only nonfood packaging applications to be considered.

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