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Drying Temperature and Relative Humidity Effects on Wheat Gluten Film Properties

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The mechanical and physical properties of glycerol-plasticized wheat gluten films dried at different temperatures (20, 50, and 80 °C) and relative humidities (35 and 70% RH) were investigated. Dispersion of wheat gluten was prepared at pH 11 in aqueous solution. Films were obtained by casting the wheat gluten suspension, followed by solvent evaporation in a temperature and relative humidity controlled chamber. Decreasing relative humidity altered most of the mechanical properties. At 35% RH, tensile strength increased when drying temperature increased. However, at 70% RH, tensile strength decreased when temperature increased. Thickness of the films decreased by increasing temperature. Hypothetical coating strength increased with increasing drying temperature at 35% RH. However, at 70% RH, a maximum value was observed at 50 °C. Films produced at 80 °C exhibited low solubility in aqueous solution. Addition of 1.5% (w/v) sodium dodecyl sulfate increased solubility of all of the films except the film dried at 50 °C and 70% RH. Overall, drying temperature and relative humidity affected mechanical and physical properties of the wheat gluten films. However, the effect of drying temperature was more pronounced than the effect of relative humidity.

KEYWORDS: Wheat gluten film; drying conditions; water vapor transmission rate; solubility; mechanical properties

INTRODUCTION

Edible films and coatings offer alternative packaging to synthetic packaging materials. Because of their renewable natural resources (such as protein, lipids, and polysaccharides) and biodegradability properties, they have received great interest in recent years. Although they cannot completely substitute for synthetic packaging materials, they can extend shelf life and improve the quality of food by providing good mechanical and barrier properties. The properties of edible films and coatings have been extensively reviewed by Guilbert (*1*), Gontard and Guilbert (*2*), Krochta and De Mulder-Johnston (*3*), and Debeaufort et al. (*4*). Because proteins are widely available and relatively easy to handle, many cereal and vegetable proteins (such as wheat gluten and corn and soy proteins) and animal proteins (such as milk proteins, collagen, gelatin, and myofibrillar proteins) are commonly used to form packaging materials (*5*). Among those, wheat gluten based films have gained much interest, and their physical properties have been investigated extensively due to their unique cohesive and elastic properties. Wheat gluten films are traditionally obtained by mixing, casting, and drying of dispersion (*6*−*22*). The film preparation technique affects the mechanical and water barrier properties of edible films (*23*). In most of the studies, the influences of variables involved in film formation (e.g., pH, solvent type, and heat treatment) on film properties have been examined. Heat treatments have been applied to wheat gluten film solutions in different ways. In some studies, heat was applied during stirring of the dispersion (*7*−*11*, *13*, *14*, *16*, *17*, *20*). Others applied heat for a short time just before casting to remove gas bubbles from the film solution (*12*, *15*, *22*). Heat has also been applied to dried films (*19*, *21*). Applying pressure during heating to homogeneous blends of wheat gluten and glycerol was another method of film formation (*24*). A comparative assessment of these results was not easy due to different conditions of the wheat gluten preparation and film formation process. Whatever technique is used, it is well-known that heat treatment affects protein conformation and improves some film properties depending on heating conditions.

Drying is a simultaneous heat and mass transfer process used during film production. Alcantra et al. (*25*) remarked that drying conditions are important for future applications when the film will be used to cover food materials. Menegalli et al. (*26*) suggested that for gelatin biofilms, the film solution has to be dried under extremely controlled mild conditions such as near room temperature and relative humidity due to the strong influence of drying conditions on the functional characteristics of the film such as strength, elasticity, and cohesivity.

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Few studies have been carried out for the effect of drying conditions on film. Some studies analyzed only the effect of drying temperature (*27*, *28*), whereas others (*25*, *29*, *30*) investigated the effects of both drying temperature and relative humidity on film properties and morphology during drying. Microwave drying has also been an alternative drying process applied on film dispersion (*31*).

The common practice in wheat gluten film formation has involved changing the solvent pH, applying heat treatment, and evaporating the solvent at uncontrolled room conditions or at several temperatures (20−55 °C) in a ventilated oven. Film formation by drying is caused by increased polymer concentration in the medium, inducing bonds and forming three-dimensional networks (*5*). However, less attention has been given to the study of the effect of drying conditions of wheat gluten films until now. The main objective of this study was to determine the effect of drying temperature and relative humidity on mechanical and solubility properties of wheat gluten based films. However, the water vapor transmission rates of the films were also determined.

MATERIALS AND METHODS

Materials. Wheat gluten containing 76.5% protein, 11.8% starch, 5% lipids, and 0.7% ash (dry basis) was supplied by Amylum (Aalst, Belgium). Glycerol was obtained from Chemproha Chemical Distributor (Dordrecht, The Netherlands). Silica gel, sodium dodecyl sulfate, and reagents for the Lowry method were obtained from Sigma-Aldrich (Taufkirchen, Germany). Sodium hydroxide, sulfuric acid, and hydrochloric acid were obtained from Merck (Darmstadt, Germany).

Preparation of Wheat Gluten Film. Wheat gluten (10% w/w) was dispersed through a sieve with stirring at low speed in water containing 2% w/w glycerol. The pH was controlled during stirring and adjusted to 11 with NaOH. After 30 min of stirring, the solution was put into a water bath at 70 °C for 10 min. Then 20 g of the resulting dispersion was pipetted into a Petri dish (∅ 9.0 cm). The cast films were placed in a large shallow box (35 × 35 × 10 cm) made of an insulating material to prevent conductive heating from the bottom surface. The boxes were covered with cheesecloth and put in a temperature and relative humidity controlled environmental chamber (Heraeus, -VÖTSCH GmbH, HC0020, Germany). To investigate the effects of a wide range of drying conditions on film properties, 20, 50, and 80 °C and 35 and 70% RH were used, respectively. Drying was performed until films could be easily removed. Drying time was in the range of 9−48 h. Films were stored at 20 °C and 60% RH before determination of mechanical and physical properties.

Determination of Water Content of Wheat Gluten Films. Preweighed wheat gluten films were dried in an oven at 40 °C and atmospheric pressure until constant weight was reached. The water content of the films was calculated from the weight difference of the films before and after drying.

Measurement of Film Thickness. Film thickness was measured using a micrometer (Mitutoyo, no. 293-521-30, Japan). Film strips were placed within the micrometer, and the gap was reduced until the first indication of contact was noted. Measurements were taken at five different locations of the films, and the mean value was used in the calculations for mechanical test measurements.

Tensile Test Measurement. Tensile test measurements were performed on samples of films according to ISO 527-3: 1995/5A/10 (*32*). According to the standards, wheat gluten films were cut into dumbbell-shaped specimens with a die (overall length = 75 mm; width = 4 mm; gauge length = 20 mm). Ends of film strips were mounted and clamped with steel grip jaws. Tensile properties were determined from three individual cast films, with two subsamples tested from each film replicate. A Lloyd mechanical testing machine (LS500, England) that was mounted with a 500 N static load supported with DAPMAT-1.4 software was used. The grip separation was set to 50 mm, with a crosshead speed of 20 mm/min. Tensile properties were determined from the curves of stress versus strain, using the software mentioned above. Tensile strength (σ_{max}) was calculated by dividing the peak load by the initial cross-sectional area of the specimen. Elongation (ϵ_b) was expressed as the percentage of the change of the original length of the specimen between the grips at break. The modulus of elasticity (E) was determined from the slope of the linear regression performed on the initial points (∼10) on the stress−strain curves. In addition to the properties mentioned above, the hypothetical coating strength (HCS) was represented as σ_{max}/E, which is the ratio between the tensile strength and the modulus of elasticity (*33*). Specimens were conditioned in the presence of saturated NaBr at 20 °C and 60% RH (selected on the basis of ISO standards) for at least 48 h prior to the tensile tests that were performed under the same atmospheric conditions used for conditioning the test specimens.

Corrected Water Vapor Transmission Rate (CWVTR). The test was performed as described by Kayserilioğlu et al. (*22*). Films (∅ 6.3 cm) were clamped on the top of polyethylene bottles. The bottles were filled with dry silica particles to maintain 0% RH inside the bottles. The air gap at the top of the bottle was very small. Therefore, the possibility of convection can be ignored (*34*). The bottles were placed in an environmental chamber at 23 °C and 85% RH (*22*). After 24 h, the increase in weight of the polyethylene bottles was monitored with time to assess the water vapor transmission rate of the films. Water vapor permeability is affected by the thickness of the films (*34*). Therefore, to eliminate the effect of thickness on permeability, CWVTR was calculated by multiplying the WVTR by the thickness of the films. Two samples were tested for each type, each one obtained from a separately produced film.

Protein Solubility. A piece of film sized 12 mm × 12 mm was cut and dried in an oven at 40 °C until constant weight was achieved and then weighed to obtain the initial film dry weight. The piece of film was placed into a test tube with 12 mL of Milli-Q water and 0.01% sodium azide to prevent microbial growth. The tubes were capped and incubated at 23 °C for 24 h with gentle agitation in a water bath. Soluble protein contents of film specimens in aqueous solution with or without 1.5% w/v SDS were determined according to the modified Lowry method (*35*). The protein contents of dried films were measured by Kjeldahl analysis. The percentage protein solubility was calculated as

$$\text{solubility (\%)} = (N_1/N_2) \times 100$$

where N_1 = weight of protein in 12 mL of solution, N_2 = (initial dry film weight) × (fraction of protein in dry film), fraction of protein in dry film = grams of protein in film/film sample (grams) used for Kjeldahl test.

Calculated protein solubility values were obtained by using three replicate samples from different films.

Statistical Analysis. To evaluate the effects of process parameters on mechanical and physical properties of the films (except CWVTR), statistical analysis was performed by two-way ANOVA test, using GraphPad Prism statistical package (GraphPad Prism Software Inc., San Diego, CA).

Scanning Electron Microscopy (SEM). Differences in surface film morphology were investigated by using a scanning electron microscope (JSM-6400, Noran Instruments). The samples were sputter-coated with gold target to a thickness of 250 Å prior to examination.

RESULTS AND DISCUSSION

Preparation of film dispersions was conducted under room conditions. Cast films were dried at different temperatures (20, 50, and 80 °C) and relative humidities (35 and 70% RH) in the environmental chamber. Film surfaces were uniform at the end of the drying process, and they were easy to peel off the casting surface. The films were strong and flexible enough to be tested.

Mechanical Properties. Mechanical properties of wheat gluten films were expressed as tensile properties and HCS. Tensile strength (σ_{max}) offers a measure of integrity and heavy-duty use potential for films, and elongation (ϵ_b) is a quantitative representation of the film's ability to stretch (*36*). Elastic modulus (E) represents the intrinsic stiffness of the film (*37*).

Table 1. Tensile Strength (σ_{max}), Elongation (ϵ_b), and Modulus of Elasticity (E) of Wheat Gluten Films under Different Drying Conditions

drying temp (°C)	drying rel humidity (%)	σ_{max}[b] (MPa)	ϵ_b[c] (%)	E[d] × 100 (MPa)
20	35	4.5 ± 0.4	254 ± 39	75 ± 15
50	35	6.3 ± 0.3	218 ± 25	97 ± 10
80	35	8.2 ± 0.5	260 ± 39	87 ± 12
20	70	5.8 ± 0.8	109 ± 30	110 ± 14
50	70	4.4 ± 0.3	328 ± 19	40 ± 6.0
80	70	3.3 ± 0.2	269 ± 30	48 ± 7.0

[a] Means ± standard deviations ($n = 2$). [b–d] Means ± standard deviations ($n \geq 6$).

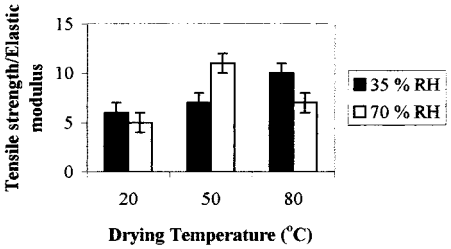

Figure 1. Hypothetical coating strength (tensile strength over modulus of elasticity) of plastisized wheat gluten films as affected by drying conditions.

Mechanical resistance of wheat gluten films depends on the type and density of various bonds such as disulfide, hydrophobic, and hydrogen bonding in the protein network. It is the common suggestion that high temperatures induce cross-linkages within the wheat gluten structure by formation of intermolecular covalent bonds, which results in an increase of mechanical strength (*19*, *20*, *21*, *24*).

Tensile properties of films prepared under different drying conditions are given in **Table 1**. Presented values were obtained from the mean of at least six samples. The effects of drying temperature and relative humidity on tensile properties of films were significant except for the effect of relative humidity on elongation ($p < 0.05$). σ_{max} values increased with increasing drying temperature at 35% RH but decreased with increasing temperature at 70% RH. σ_{max} of the films dried at 80 °C and 35% RH was higher than that of the films dried at the same temperature but at 70% RH. The lowest ϵ_b value was found for a film dried at 20 °C and 70% RH, whereas the E value of this film showed the highest value. This film was less stretchable than all of the other samples. No significant differences were observed in ϵ_b between films dried at 80 °C at 35 and 70% RH. However, an apparent decrease in ϵ_b was observed for the films dried at 20 °C and 70% RH.

Film coatings have great prospects for food and nonfood applications. Joshi et al. (*33*) proposed that HCS is good indicator for a film's hypothetical performance as a coating. Alcantara et al. (*25*) proposed that successful film coatings depend on the ability to resist certain mechanical forces after application onto food. HCS results are shown in **Figure 1**. Statistical analysis revealed that drying temperature—but not relative humidity—affected HCS significantly ($p < 0.0001$). The highest HCS value was observed for the films dried at 50 °C. The larger value would suggest the more desired coating for mechanical protection (*38*).

The thickness of the films decreased significantly with increasing drying temperature; however, the effect of relative humidity on film thickness was statistically insignificant ($p < 0.0001$) (**Figure 2**). Alcantara et al. (*25*) found that film thickness decreased with increasing drying temperature, and they indicated that thinner films would hypothetically be weaker. Thinner but not weaker films were obtained by increasing temperature in our study.

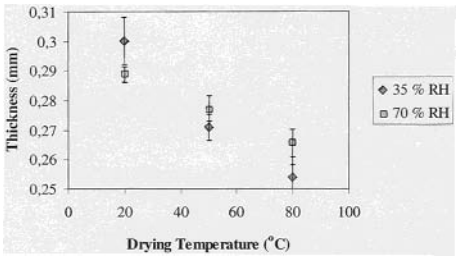

Figure 2. Thickness of wheat gluten films as affected by drying conditions.

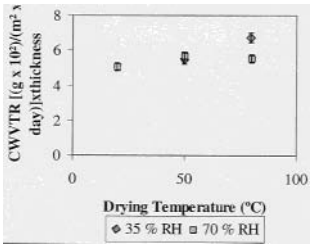

Figure 3. Corrected water vapor transmission rate (CWVTR) of the films as affected by drying conditions.

Corrected Water Vapor Transmission Rate. Although the main focus of the study was to investigate the effects of drying conditions on mechanical properties, their effects on the water vapor transmission rate were also determined by using two sets of data. Therefore, statistical analysis was not performed. CWVTR values of films dried at several conditions are shown in **Figure 3**. As observed, small differences were obtained. The CWVTR of films dried at 80 °C and 35% RH showed a slightly higher value than the rest. However, those films became wrinkled at the end of the test.

Solubility of Film. Film solubility is an important property for the film's application. Potential applications may require water insolubility to enhance product integrity and water resistance. However, in some cases such as food coating, film solubility in water before consumption of the product might be beneficial (*30*). The high solubility of wheat gluten in aqueous solution has been cited as the reason for the better film formation at basic pH and, thus, for improved mechanical properties (*13*). The maximum solubility for wheat gluten in aqueous solution was observed at pH 11 (*22*). Therefore, wheat gluten films were produced at pH 11. Films did not lose their integrity in aqueous solution with or without denaturant after 24 h. This confirmed that the protein polymer network was highly stable and only small molecules (small peptide, monomers, and nonprotein material) were soluble (*39*).

The effect of drying temperature—but not relative humidity—on protein solubility in water of the films was significant ($p < 0.0001$). The solubility in water of protein dried at 80 °C was considerably lower than that of the films dried at other conditions (**Figure 4A**). This can be an indication of the presence of cross-links formed during film formation at high temperature. To determine the bond type, covalent or noncovalent, 1.5 w/v SDS was added as denaturant to the aqueous solution (*22*). Protein solubility of the films increased with the addition of denaturant, except films dried at 50 °C (**Figure 4B**). This might indicate that a protein network occurred from covalent bonds formed mainly at that drying condition.

To determine the effect of drying conditions on film morphology, SEM analysis was performed; pictures of two

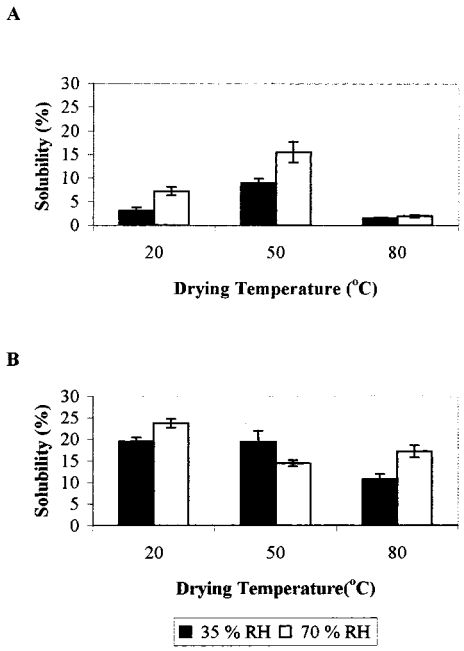

Figure 4. (A) Solubility of films in aqueous solution; (B) solubility of films in denaturant (1.5% SDS w/v) added aqueous solution as affected by drying conditions.

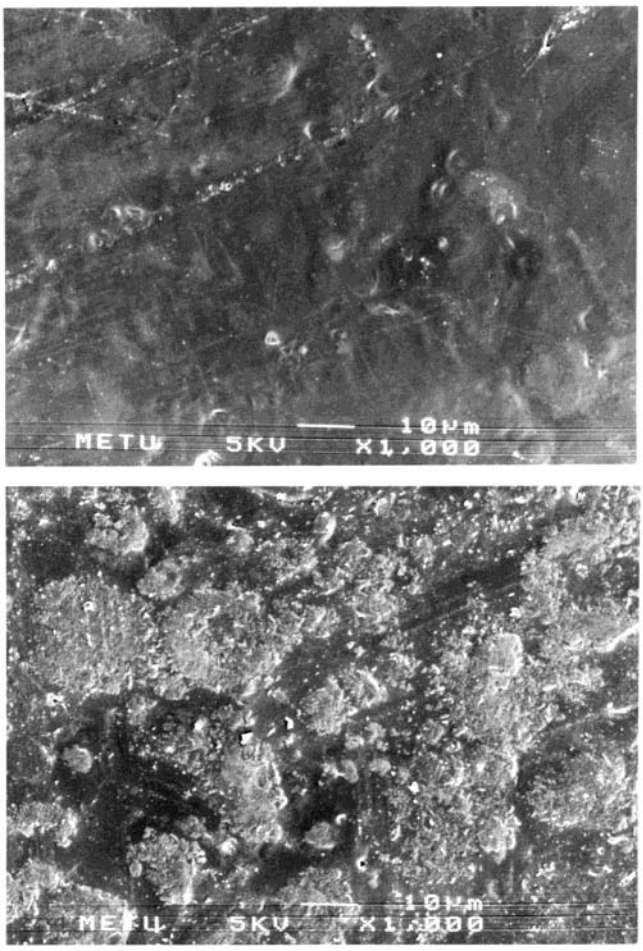

Figure 5. SEM images of surface of the films: (A) film dried at 50 °C and 70% RH; (B) film dried at 80 °C and 35% RH.

films, dried at 50 °C and 70% RH and at 80 °C and 35% RH, respectively, are given in **Figure 5**. Microstructures of the surface of films dried at 50 °C and 70% RH were smoother than the films dried at 80 °C and 35% RH. It is well-known that heat induces the formation of a covalent network of the protein and gives strength to the film. The tensile strength of films dried at 80 °C and 35% RH had the highest value, whereas the protein solubility of this film was lower than that of the other films in the denaturant solution. Films with high tensile strength values might result in high amount of covalent bonds during drying of film at 80 °C and 35% RH compared to other drying conditions.

The results of the presented study lead us to conclude that both drying temperature and relative humidity affected the mechanical and physical properties and film morphology of the films. However, the effect of drying temperature is predominant.

ABBREVIATIONS USED

% RH, percent relative humidity; σ_{max}, tensile strength; ϵ_b, strain at break value; E, modulus of elasticity; HCS, hypothetical coating strength; WVTR, water vapor transmission rate; SDS, sodium dodecyl sulfate; ∅, radius; S−S, disulfide bond; SH, sulfhydryl bond; SEM, scanning electron microscopy.

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