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# RELATIVE HUMIDITY AND TEMPERATURE EFFECTS ON TENSILE STRENGTH OF EDIBLE PROTEIN AND CELLULOSE ETHER FILMS

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**ABSTRACT.** *The effect of relative humidity and temperature on tensile strength of two types of protein-based [corn zein (CZ) and wheat gluten (WG)] and two types of cellulosic [methylcellulose (MC) and hydroxypropyl cellulose (HPC)] hydrophilic edible films was investigated. A central composite response surface design was used. Studied ranges of relative humidity and temperature were 23 to 75% and 5 to 45° C, respectively. For all four types of films, tensile strength (TS) decreased with relative humidity and increased with temperature. Ranges of mean tensile strength values among the nine different combinations of the two variables were 5.7 to 23.6 MPa, 2.7 to 21.4 MPa, 61.9 to 104.4 MPa, and 11.1 to 35.0 MPa for CZ, WG, MC, and HPC, respectively. A second-order polynomial model was fitted to the data with least squares regression. A regression model linear in relative humidity and quadratic in temperature showed a very good fit to tensile strength data of CZ ( $R^2 = 0.93$ ) and MC ( $R^2 = 0.98$ ) films. A regression equation linear with respect to both relative humidity and temperature satisfactorily fitted ( $R^2 = 0.75$ ) TS data of HPC films. A best fitted model for TS data of WG films, that included relative humidity and temperature, the square of temperature, and the cross-product of the two variables, had a poor fit ( $R^2 = 0.67$ ). Keywords. Wheat gluten, Corn zein, Methylcellulose, Hydroxypropyl cellulose.*

**E**dible films and coatings produced from renewable biological materials provide opportunities for innovative uses in food protection and preservation. Movement of moisture, gases, and solutes within food or between food and its surrounding environment can be controlled by application of edible protective layers.

Current research findings on edible film development, properties, and potential applications have been summarized in recent reviews by Kester and Fennema (1986), Guilbert (1986), Biquet (1987), and Krochta (1992). Film formation from corn, wheat, and soy proteins has been discussed in detail by Gennadios and Weller (1990 and 1991a).

Several proteins, polysaccharides, and lipid materials have been studied for development of edible films and coatings. As noted by Krochta (1992), films based on proteins and polysaccharides are very efficient oxygen and carbon dioxide barriers, whereas their resistance to water vapor transmission is limited due to their hydrophilic

nature. Lipid films are moisture resistant but their mechanical properties are inferior to those of protein and polysaccharide films. Multicomponent films have also been made in an effort to combine advantages of the individual film-forming materials.

Proteins investigated for film formation include collagen, gelatin, keratin, CZ, WG, soy protein isolate, peanut protein, casein, and whey proteins. Polysaccharide film-forming materials include cellulose derivatives, alginates, pectin, carrageenan, and starch derivatives. Substances utilized in lipid coatings include acetylated glycerides, fatty acids, and various waxes, such as beeswax, paraffin wax, carnauba wax, rice bran wax, and candelilla wax.

A number of edible films and coatings have found commercial application. Waxes are applied on fresh fruits and vegetables to replace their natural wax coating removed during washing at packing plants (Hartman and Isenberg, 1956; Kaplan, 1986). Sausage casings from collagen are manufactured and used in the place of traditional natural casings from slaughtered animals (Hood, 1987; Rust, 1987). The CZ-based solutions are commercially available for coating nutmeats and confectionery products (Alikonis, 1979; Andres, 1984). Protein-lipid films ("yuba", "soymilk skin") formed on the surface of heated soymilk have been traditionally utilized in the Far East for wrapping meats and vegetables prior to cooking (Wang, 1981; Snyder and Kwon, 1987).

Much of the information on edible films and coatings originates from the patent literature. As a consequence, quantitative data on film properties are limited. The TS is an important mechanical property of films. It expresses the maximum load developed while subjecting a film specimen to a tensile test divided by the original

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cross-sectional area of the specimen. A few studies have reported testing of edible films for TS.

TS values have been determined for CZ films (Takenaka et al., 1967; Aydt et al., 1991; Park et al., 1992a), wheat protein films (Wall and Beckwith, 1969; Watanabe and Okamoto, 1976; Gennadios et al., 1990; Aydt et al., 1991; Gennadios et al., 1991; Park et al., 1992a), soy protein films (Wu and Bates, 1973; Okamoto, 1978; Brandenburg et al., 1992), peanut protein films (Aboagye and Stanley, 1985), and commercial sausage casings from collagen (Hood, 1987). The MC and HPC cellulose films were evaluated for TS by Park et al. (1992b). TS values of MC films were also reported by Takenaka et al. (1967).

In some of the above studies, testing was performed after conditioning the materials at specific relative humidity (RH) and temperature (T) conditions. For example, conditioning prior to testing for two days at 50% RH and 25° C was practiced by Gennadios et al. (1990 and 1991), Park et al. (1992a and 1992b), and Brandenburg et al. (1992). In other cases no information on pretesting conditioning of specimens was provided (Wall and Beckwith, 1969; Okamoto, 1978; Hood, 1987).

Films based on protein and cellulose ethers are moisture sensitive due to the hydrophilicity of these film-forming materials. Properties of such films are dependent on their moisture content. Both RH and T are two environmental factors that determine the amount of water absorbed by hydrophilic films and, consequently, affect film properties.

The present study was undertaken in order to investigate and quantify the effect of RH and T on TS of two protein films (CZ and WG) and two cellulose ether films (MC and HPC) by using response surface experimentation.

## MATERIALS AND METHODS

### EXPERIMENTAL DESIGN

A response surface experiment was designed to identify the relationship between two environmental variables (RH and T) and a film mechanical property (TS). The specific experimental design adopted in this study was a central composite response surface design (Box and Draper, 1987). RH and T were independent variables (factors), while TS was a dependent variable (response). Coded and natural values for the levels of the independent variables are shown in table 1. Variables were coded using the following transformation:

$$X_C = (X_N - X_B)/J \quad (1)$$

where

$X_C$  = the coded value of the factor

$X_N$  = the natural value of the factor

$X_B$  = the natural value of the basic level (center point)

J = the variation interval

Variation intervals were set at 12.5% for RH and 10° C for T.

Nine level combinations were included in the constructed design (table 2). The center point (0,0 or 50% RH and 25° C) was replicated five times. Replicated points at the design center offer an estimation of the experimental error.

Table 1. Levels of independent variables in the experimental design

Code	Relative Humidity ( $X_1$ ) (%)	Temperature ( $X_2$ ) (° C)
-2	25	5
-1	38	15
0	50	25
1	62	35
2	75	45

Responses  $Y_i$  for each experimental point were means of 10 replicates. The following second order polynomial model consisting of two linear, one interaction, and two quadratic terms was fitted with regression to the obtained responses:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \quad (2)$$

where b values are the regression coefficients and  $X_1$  and  $X_2$  are the coded independent variables.

All statistical calculations were performed using the SAS/STAT™ statistical package (SAS Institute, Inc., 1988).

### REAGENTS

The CZ protein (F-4000) was donated by Freeman Industries, Tuckahoe, New York. Vital WG protein (DO-PEP) was donated by ADM Arkady, Olathe, Kansas. Both MC (viscosity 25 cp) and HPC (molecular weight 1,000,000) were obtained from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin. Glycerol (USP grade), potassium carbonate, and potassium acetate were purchased from Baxter Diagnostics, Inc., McGaw Park, Illinois. Magnesium chloride was bought from Fisher

Table 2. Level combinations of independent variables included in the experimental design

Design Point	Level of $X_1$ *		Level of $X_2$ *	
	Coded	Natural	Coded	Natural
1	-1†	38‡	-1	15
2	-1	38	1	35
3	1	62	-1	15
4	1	62	1	35
5	2	75	0	25
6	-2‡	25‡	0	25
7	0	50	2	45
8	0§	50§	-2	5
9	0	50	0	25

\* Variables  $X_1$  and  $X_2$  are relative humidity (%) and temperature (° C), respectively.

† An actual value of -1.36 (33%) was obtained in the lab and used in the calculations.

‡ An actual value of -2.16 (23%) was obtained in the lab and used in the calculations.

§ An actual value of -0.16 (48%) was obtained in the lab and used in the calculations.

|| This design point (center point) was repeated five times.

Scientific, Pittsburgh, Pennsylvania. Ammonium hydroxide (5 N) and 95% ethanol were obtained from Aldrich Chemical Company, Inc.

#### PREPARATION OF FILM-FORMING SOLUTIONS

The CZ film-forming solutions were prepared by dissolving 54 g of protein into 325 mL of 95% ethanol. Glycerol (18 mL), which is necessary for film plasticization, was subsequently added to the solutions.

Film-forming solutions for WG films were prepared by mixing 75 g of protein, 360 mL of 95% ethanol, and 228 mL of distilled water. Glycerol (21 mL) and 5 N ammonium hydroxide (72 mL) were subsequently added and the solutions were homogenized (Virtishear®, The Virtis Company, Inc., Cardiner, NY) at 6,000 rpm for 5 min and filtered through cheese cloth. For the two cellulosic films 9 g of MC or HPC were dissolved into 200 mL of 95% ethanol and 100 mL of distilled water. Both types of solutions were homogenized at 6,000 rpm for 5 min.

Prior to pouring, all four types of solutions were conditioned for 15 min in a waterbath (Versa-Bath® S, Model 236, Fischer Scientific, Pittsburgh, PA) maintained at 80° C.

#### POURING AND DRYING OF FILM-FORMING SOLUTIONS

Film-forming solutions were poured and evenly spread on leveled glass plates (24 cm × 24 cm). The amount of liquid poured on the glass surface was 80 mL for CZ and WG films and 200 mL for MC and HPC films. Solutions on the plates were allowed to settle for 15 min and subsequently they were placed for 30 min in a drying oven (Isotemp®, model 338F, Fisher Scientific, Pittsburgh, PA) set at 55° C. At the end of the 30-min drying period, plates were taken out of the oven, films were peeled off, and specimens for TS testing were cut.

#### THICKNESS

Film thickness was determined to the nearest 2.54 µm (0.1 mil) with a hand-held micrometer (B.C. Ames Co., Waltham, MA). Thickness values reported for each film type represent a mean of 130 measurements.

#### FILM CONDITIONING

For all experimental points, film specimens were stored under the specified RH and T conditions for two days. An environmental chamber (model 317332, Hotpack, Corp., Philadelphia, PA) was used to achieve required conditions for all design points except Nos. 1, 6, and 8 (table 2). These three points did not fall within the operation range of the chamber.

Portable desiccator cabinets containing appropriate saturated salt solutions were used for design points 1, 6, and 8. Magnesium chloride, potassium acetate, and potassium carbonate saturated salt solutions were used for points 1, 6, and 8, respectively. The RH values obtained by using magnesium chloride, potassium acetate, and potassium carbonate were 33%, 23%, and 48%, respectively (Rockland, 1960). These values slightly deviated from respective targeted values of 38%, 25%, and 50%. Adjusted RH coded values of -1.36 (point 1), -2.16 (point 6), and -0.16 (point 8) were used in the calculations

in place of theoretical values -1, -2, and 0, respectively, to account for these deviations.

The desiccators were kept in an environmental chamber set at the desired temperatures and 50% RH. For point 6, desiccators were placed in the same chamber mentioned earlier, whereas a second chamber (model 790-001, Lab-Line Instruments, Inc., Melrose Park, IL) was used for points 1 and 8.

#### MEASUREMENT OF TS

An Instron Universal Testing Instrument (model 4201, Instron Engineering Corp., Canton, MA) was used to determine film TS. Initial grip separation and crosshead speed were set at 50 mm and 500 mm/min, respectively. Calculations were performed as described by Gennadios et al. (1990 and 1991). Tested film specimens were rectangular strips 100-mm long and 25.4-mm wide as suggested in ASTM Standard Method D 882-88 (ASTM, 1989).

## RESULTS AND DISCUSSION

### FILM THICKNESS AND ELONGATION

Mean thicknesses of prepared films were 141, 106, 57, and 54 µm for CZ, WG, MC, and HPC films, respectively. The range of thickness measurements was within 40% of mean values for each of the four types of films.

Elongation at break values were also recorded for film specimens subjected to tensile test. While film thickness is accounted for in the calculations of TS, this is not the case with elongation. As a result, comparisons in terms of elongation are meaningful only for films of practically equal thickness. Due to thickness variability of the four types of films in this study, elongation values were not statistically analyzed in a manner similar to the TS values. At the center experimental point (50% RH, 25° C) elongation at break values had ranges of 3 to 7%, 100 to 280%, 8 to 25%, and 10 to 50% for CZ, WG, MC, and HPC films, respectively.

### EFFECT OF RELATIVE HUMIDITY

Mean TS values of the four types of films at each combination of independent variables are shown in table 3. Three-dimensional plots of TS with respect to RH and T including predicted data points generated by the SAS statistical package are shown in figures 1, 2, 3, and 4. As the figures show, a decrease in TS was evidenced when RH increased for all four types of films. This was concluded to result from increasing film moisture content with RH. As shown in table 4, substantial increases in equilibrium moisture content with increases in RH have been documented for hydrophilic CZ, WG, MC, and HPC films.

Absorbed moisture has a plasticizing effect on hydrophilic films, such as films from proteins and cellulose ethers (Ashley, 1985). Consequently, tensile strength and barrier properties of such films are negatively affected by moisture uptake.

### EFFECT OF TEMPERATURE

The effect of T was opposite to that of RH. That is, TS values increased with temperature for all four types of films (figs. 1, 2, 3, and 4). This behavior is also concluded to be related to film moisture content. In general, at

**Table 3. The TS values of CZ, WG, MC, and HPC films conditioned for two days at various sets of RH and T**

Design Point	RH (%)	T (°C)	Tensile Strength (MPa)			
			CZ*	WG*	MC*	HPC*
1	33	15	12.5±1.3 de	3.8±0.6 e	70.2±3.3 f	23.7±2.3 c
2	38	35	17.1±1.2 e	21.4±2.8 a	96.7±4.1 b	35.0±1.7 a
3	62	15	7.9±0.8 g	2.7±0.3 f	66.0±3.9 g	11.1±0.8 f
4	62	35	12.2±0.9 e	8.5±1.1 c	92.2±6.1 c	29.4±3.0 b
5	75	25	5.7±0.5 h	2.7±0.6 f	72.3±2.6 f	17.6±1.6 e
6	23	25	19.5±2.0 b	5.2±0.7 d	84.5±3.3 d	24.4±1.2 c
7	50	45	23.6±1.1 a	12.1±1.0 b	104.4±3.4 a	30.1±1.5 b
8	48	5	10.6±1.1 f	3.3±0.3 ef	61.9±2.1 h	16.3±1.7 e
9	50	25	13.3±1.6 de	3.8±0.3 e	80.4±4.3 de	19.8±1.9 d
10	50	25	13.6±1.5 d	3.9±0.5 e	77.6±4.3 e	20.8±1.7 d
11	50	25	12.8±1.3 de	3.9±0.3 e	79.2±4.6 e	20.7±1.6 d
12	50	25	12.8±1.1 de	3.8±0.3 e	76.3±4.3 e	19.5±2.0 d
13	50	25	13.5±1.7 de	3.8±0.4 e	80.4±4.5 de	20.0±1.5 d

\* The TS values are means of 10 replicates plus/minus one standard deviation. Any two means followed by the same small case letter are not significantly different at  $\alpha = 0.05$  level of significance according to Duncan's New Multiple-Range Test.

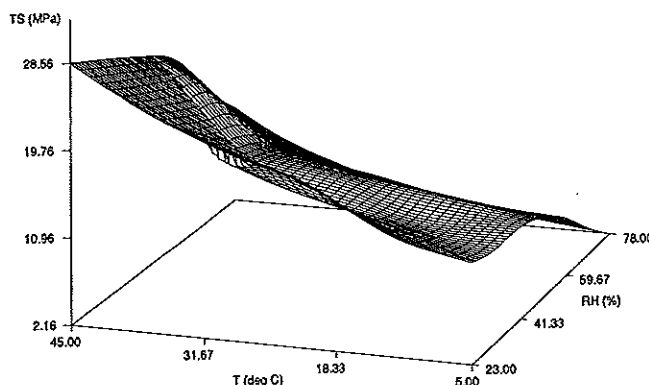
constant relative humidity the amount of water absorbed by food materials decreases with an increase in temperature (Labuza, 1968; Labuza, 1984). Therefore, more water was bound at lower temperatures increasing film plasticizing and causing weakening.

For instance, equilibrium moisture content of WG protein at 42% RH was measured as, approximately, 0.088, 0.086, and 0.082 g moisture/g dry solids at 20.2°, 30.1°, and 40.8° C, respectively (Bushuk and Winkler, 1957). The MC and HPC films had equilibrium moisture contents of 0.08 and 0.06 g moisture/g dry solids at 21° C, whereas these values increased to 0.13 and 0.08 g moisture/g dry solids, respectively, at 5° C (Park, 1991).

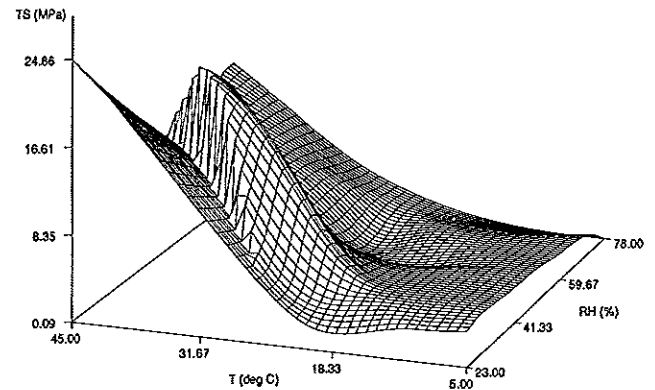
#### MODEL FITTING TO CZ FILMS

Mean TS values of CZ films ranged from 5.7 to 23.6 MPa among the 13 different experimental points (table 3). Fitting of the second order polynomial model showed that two terms, the square of RH and the cross-product RH·T, were insignificant ( $P < 0.05$ ).

A reduced regression model fitted after omitting these two terms had a very good fit ( $R^2 = 0.93$ ). The F-value



**Figure 1—Plot of TS values of CZ films with respect to conditioning RH and T.**



**Figure 2—Plot of TS values of wheat gluten films with respect to conditioning RH and T.**

(41.9) calculated for the regression was significant ( $P < 0.01$ ) indicating that an important amount of the variation can be explained by the regression model.

Therefore, TS of CZ films exhibited a linear dependence on RH and a quadratic dependence on T. The best fitted prediction equation had the form:

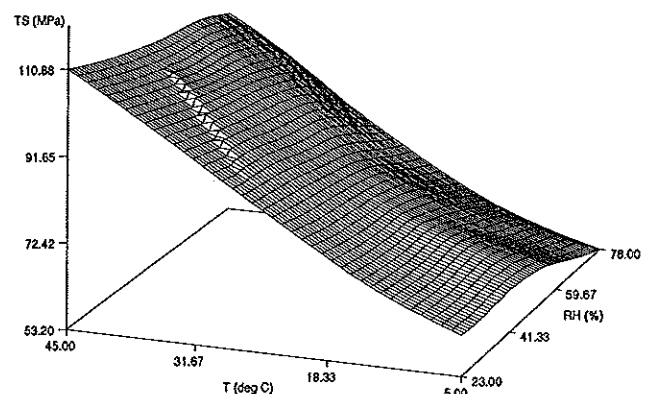
$$TS = 12.468 - 2.888 \cdot RH + 3.071 \cdot T + 0.952 \cdot T^2 \quad (3)$$

#### MODEL FITTING TO WG FILMS

WG films had TS values between 2.7 and 21.4 MPa (table 3). Fit of the second order model was poor with an insignificant ( $P < 0.1$ ) F-value for regression. An improvement was noticed (regression F-value significant at  $P < 0.05$ ) by dropping the term  $RH^2$  from the model. The best fitted regression equation ( $R^2 = 0.67$ ) was:

$$TS = 5.091 - 1.605 \cdot RH + 3.299 \cdot T + 1.103 \cdot T^2 - 2.568 \cdot RH \cdot T \quad (4)$$

T in the above model was significant at  $P < 0.05$ , whereas RH,  $T^2$ , and  $RH \cdot T$  were significant at  $P < 0.2$ . Probably a power model is necessary to describe adequately TS dependence on RH and T for WG films.



**Figure 3—Plot of TS values of MC films with respect to conditioning RH and T.**

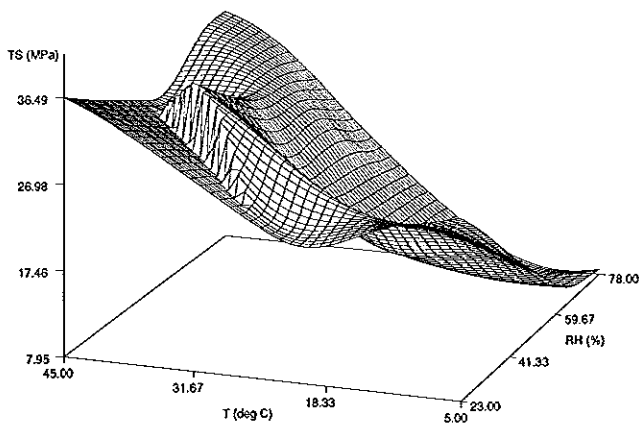


Figure 4—Plot of TS values of HPC films with respect to conditioning RH and T.

### MODEL FITTING TO MC FILMS

Mean TS values for MC films ranged from 61.9 to 104.4 MPa among the nine level combinations (table 3). Analysis of variance for the fitted original model showed that the interaction term (RH·T) and the square of RH were insignificant ( $P < 0.1$ ).

A reduced model fitted after dropping these two terms had an excellent fit to the data ( $R^2 = 0.98$ , regression F-value significant at  $P < 0.01$ ). Both RH and T were significant at  $P < 0.01$ , while  $T^2$  was significant at  $P < 0.05$ . The best fitted model, linear with respect to RH and quadratic with respect to T, was as follows:

$$TS = 79.003 - 2.566 \cdot RH + 11.607 \cdot T + 1.100 \cdot T^2 \quad (5)$$

### MODEL FITTING TO HPC FILMS

HPC films had mean TS values between 11.1 and 35.0 MPa at the different level combinations studied (table 3). These values were substantially lower than TS values of MC films. The high level of hydroxypropyl substitution in HPC causes internal plasticizing effects and results in films weaker than MC films (Krumel and Lindsay, 1976).

Both quadratic terms, as well as the interaction term in the original second order model were insignificant ( $P < 0.1$ ). A reduced model linear in both T ( $P < 0.01$ ) and RH ( $P < 0.05$ ) gave a reasonably good fit ( $R^2 = 0.75$ , regression F-value significant at  $P < 0.01$ ). This best fitted equation had the form:

$$TS = 22.063 - 2.601 \cdot RH + 4.926 \cdot T \quad (6)$$

Table 4. Equilibrium moisture content of CZ, WG, MC, and HPC films at different RH values

RH (%)	Moisture Content (g H <sub>2</sub> O/g dry solids)			
	CZ Films*	WG Films*	MC Films†	HPC Films†
33	0.02	0.06	0.05	0.04
53	0.06	0.12	0.08	0.06
75	0.15	0.28	0.15	0.12

\* From Gennadios and Weller (1991b) at 25° C.

† From Park (1991) at 21° C.

### FILM HYDROPHILICITY

Using as a criterion the ratio of the lowest TS value over the highest TS value among the nine different environmental combinations tested, we concluded that variations in RH and T affect WG films more than they affect CZ films (the ratios were 0.13 and 0.24 for WG and CZ films, respectively).

This may be explained by the more hydrophobic nature of CZ protein than that of WG. Belitz et al. (1986) calculated an average CZ hydrophobicity of 1,263 cal/mol, while average hydrophobicities of wheat gliadin and wheat glutenin, the two major fractions of WG, were 1,047 and 955 cal/mol, respectively.

Similar lowest/highest TS value ratios for MC and HPC films were 0.59 and 0.32, respectively, suggesting that MC films are more sensitive than HPC films to variations of RH and T. This was expected considering the greater relative hydrophilicity of MC in comparison with HPC (Krumel and Lindsay, 1976).

### IMPLICATIONS

Envisioned packaging applications for hydrophilic protein and cellulose ether films include use of such films in multilayer packages in the place of currently used moisture-sensitive polymeric materials; employment of protein and cellulosic coatings as carriers of antioxidants and other food additives; and development of edible protective coatings for use on food items that require films of high water vapor permeability (e.g., meat pies; high-moisture, low-sugar cakes).

A thorough knowledge of film properties is needed for tailoring protein and cellulose ether films to such applications. It was evidenced in the present study that relative humidity and temperature, factors that affect film moisture absorption, substantially influenced film TS, an important mechanical property. Consequently, property testing of hydrophilic protein and cellulosic films at the anticipated conditions of use is essential for proper prediction of film performance. Empirical models, similar to the models developed in the present study, can allow for estimates of film properties at specific relative humidity and temperature conditions.

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