JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Compositional and Moisture Content Effects on the Biodegradability of Zein/Ethylcellulose Films

CLAUDIA A. ROMERO-BASTIDA,[†] EDUARDO FLORES-HUICOCHEA,[†] MARTHA O. MARTIN-POLO,[‡] GONZALO VELAZQUEZ,[§,||] AND J. ANTONIO TORRES*[,||]

Departamento de Desarrollo Tecnológico, Centro de Desarrollo de Productos Bióticos, Yautepec, Morelos, México, Departamento de Investigación y Posgrado, Universidad Autónoma de Querétaro, Querétaro, México, Department of Food Science and Technology, U. A. M. Reynosa-Aztlán, Universidad Autónoma de Tamaulipas, Apdo. Postal 1015, Reynosa, Tamaulipas, 88700 Mexico, and Food Science and Technology, Oregon State University, Corvallis, Oregon

The effect of moisture content and film composition on biodegradability is the focus of this study. Flexible films were first characterized for the effect on water sorption isotherms of relative humidity, temperature, zein content, and the addition of the plasticizers stearic acid, poly(ethylene glycol), or etoxylated ricine oil. Zein/ethylcellulose (EC) mixture films had a behavior between that for pure zein and EC films, which had the lowest water sorption. For films with plasticizer, the lowest water sorption at 25 °C was observed for those with stearic acid. Biodegradability of zein/EC films, evaluated using bacterial cultures selected for their zein proteolytic activity and isolated from a local solid waste landfill and a lagoon, showed no plasticizer effect even though its effect on moisture content was significant. Large differences were observed at different film zein concentration with the highest biodegradability for 100% zein. However, biodegradability did not mimic the water sorption behavior of zein/EC mixture films.

KEYWORDS: Moisture sorption isotherms; ethylcellulose; zein; plasticizer; biodegradability

INTRODUCTION

Food quality can deteriorate during storage due to losses/gains of moisture and volatile aromas; however, this can be prevented by an adequate selection of food packaging and edible coatings. Efforts to extend shelf life using modified atmosphere packaging require packaging films that are adequate moisture and gas barriers. The barrier properties of protein-, polysaccharide-, and lipid-based films have been widely investigated (*1*−*5*). However, the observation that edible coatings and biodegradable packaging used to protect foods are less damaging to the environment when discarded by end consumers has not been fully studied (*6*). The functionality of these materials can be enhanced by adding antimicrobial agents, antioxidants, nutrients, oxygen and carbon dioxide absorbers, enzymes, and other active compounds (*7*−*10*). For example, foods can spoil by surface growth of microorganisms, which can be controlled by edible coatings containing antimicrobial agents (*5*, *7*, *11*, *12*).

The absorption of water vapor by biopolymers can result in conformational changes in macromolecular structure modifying their resistance to gas and vapor permeability (*13*). Coating composition and morphology play an important role in controlling water sorption and gas transport properties. Compositional factors include addition of plasticizers; polymer properties such as saturation degree, presence of lateral chains, and polymer cross-linking; and those that involve polymer heterogeneity such as crystalline and amorphous regions (*14*). Biopolymers films have excellent oxygen and carbon dioxide barrier properties when compared with many conventional plastic packaging; however, their resistance to water vapor is limited due to their hydrophilic nature (*15*). Lipid-based films are moisture resistant, but their mechanical properties are inferior to protein- and polysaccharide-based films. Multicomponent films have been developed combining the advantages of lipid- and biopolymer-based films.

Sorption isotherms describe the equilibrium relationship at constant temperature between water activity (a_w) and water content for a given product. Most food sorption isotherms have a sigmoid curve (*16*). Sorption isotherms are extremely important because they can be used to predict food stability and to select packaging materials (*17*). The moisture sorption isotherm represents the combined hygroscopic properties of the individual components in an edible coating or film. Water sorption isotherms must be determined at different temperatures to fully study plasticizer and other composition effects on the film properties (*18*). Plasticizers are used to reduce brittleness and

* To whom correspondence should be addressed. Tel: 541/737-4757. Fax: 541/737-6174. E-mail: J_Antonio.Torres@oregonstate.edu.
[†] Centro de Desarrollo de Productos Bióticos.
[‡] Universidad Autónoma de Querétaro.
[§] Universidad Autónoma de Tamaulipas.
[||] Oregon State University.

10.1021/jf0350414 CCC: $27.50
Published on Web 03/17/2004

modify the mechanical properties of the film. In general, plasticizers are low molecular weight components that work as spacers among polymer chains decreasing intermolecular forces, increasing flexibility and extensibility, and lowering the glass transition temperature (T_g) of amorphous or partially crystalline polymeric films. Compounds typically added for this purpose are glycerol, sorbitol, propylene glycol, and poly(ethylene glycol) (*19*). In hydrophilic materials, water interacts with the biopolymer acting also as a plasticizer. Addition of plasticizers usually increases permeability of gases and water vapor due to an increase in the free volume between chains (*4*, *20*, *21*).

The objectives of this work were to determine the effect of film composition including plasticizer type on the water sorption isotherms of zein and ethylcellulose (EC) films and to relate the effect of these parameters to film biodegradability by local bacterial strains selected for their ability to biodegrade zein.

MATERIALS AND METHODS

Materials. Zein and EC were purchased from Sigma Chemical Co. (St. Louis, MO). The plasticizers poly(ethylene glycol), stearic acid, and etoxylated ricine oil (Emulgin) were purchased from a local chemical distributor (Drogueria Cosmopolita, Mexico, DF). Ethanol and salts used to maintain desiccators with constant relative humidity for the determination of sorption isotherms were purchased from J. T. Baker Inc. (Phillipsburg, NJ).

Film Preparation. A 2.5 g amount of EC, zein, or zein/EC blends was dissolved in 50 mL of ethanol and kept at 75 °C for 20 min while stirring (*22*, *23*). To prepare films containing plasticizers, propylene glycol, stearic acid, and Emulgin were added as 25, 12.5, and 12.5% v/w based on the film forming polymer, respectively. Stearic acid and Emulgin were first melted and mixed with 3% w/w Tween 80. Films were cast on glass plates (20 cm × 20 cm) using a thin-layer chromatography spreader set at 0.75 mm (*24*). Coated plates were then air-dried in a 75 °C oven for 15 min. After they were dried and cooled, films were removed from the plates, placed in plastic bags, and stored at room temperature in desiccators over silica gel and used within 72 h.

Determination of Moisture Adsorption Isotherms. Moisture sorption studies at 4, 25, or 35 °C followed the static microclimate method (*25*) with some equipment modifications. Airtight glass jars (1 L) containing saturated solutions of lithium chloride, potassium acetate, magnesium chloride, potassium carbonate, sodium bromide, strontium chloride, potassium chloride, and barium chloride with a_w values as a function of temperature ranging from 0.11 to 0.90 (*25*, *29*) were used to keep film samples for 7 days in a thermostatically controlled water bath. Equilibrated samples were weighed with 0.0001 g precision and then placed in an oven at 105 °C for 5 h to determine their dry weight.

Mathematical Models. The Brunauer−Emmet−Teller (BET, eq 1) and the Guggenheim−Anderson−de Boer (GAB, eq 2) models were used to calculate parameters describing sorption isotherms.

$$\frac{a_w}{(1-a_w)X} = \frac{(C-1)a_w}{X_m C} + \frac{1}{X_m C} \quad (1)$$

$$X = \frac{X_m C k a_w}{(1-ka_w)(1-ka_w+Cka_w)} \quad (2)$$

where a_w = water activity (no units), X = water content at a specific water activity a_w (g water/g dry matter), X_m = water content of the monolayer (g water/g dry matter), C = constant (kJ/mol), and k = constant (no units).

BET and GAB parameters were estimated by fitting the corresponding equation to the experimental data using nonlinear regression. The linear form of the BET model was used to obtain initial X_m and C values to solve its nonlinear form. For the GAB equation, the polynomial form was used to obtain starting values for the estimation with the nonlinear form (Statistica ver. 4.0, Stat Software). The relative deviation modulus (DM) used to quantify the fitting of the model to the experimental data was calculated as follows:

$$\mathrm{DM} = \frac{100}{n}\sum_{i=1}^{n}\frac{v_i - v_p}{v_i} \quad (3)$$

where v_i and v_p are the observed and the predicted values, respectively. A very good fit corresponds to DM < 5, a good fit to 5≤DM≤10, and a poor fit to DM > 10 (*16*).

Film Biodegradability. In preliminary studies, soil samples from a local solid waste landfill and a lagoon were inoculated on nutrient−salts agar (Difco, Becton, Dickinson and Co., Franklin Lakes, NJ) and incubated aerobically at 30 °C for up to a week (*26*). In this first screening, 120 colonies capable of aerobic growth on this minimal nutrients medium were identified. Further strain selection was done by observing every 24 h and up to a week the halo formed on Petri dishes in the same minimal media but with 2% (dry basis) zein added. The halo reflected the zein proteolytic activity of a particular isolate. The 46 bacterial strains, identified in this second screening as having zein proteolytic activity, were characterized as Gram+, nonsporulating, mesophilic bacilli that grew on simple culture media. Of these, 15 strains were further identified as having proteolytic activity for zein in film form. This third screening was done by placing rectangular film samples (1.5 cm × 5 cm) on the media surface and measuring biodegradation by assigning a score of 0 for no change and 4 when degradation produced near total translucence (over 90%) of the film sample. Score values of 1, 2, and 3 corresponded to less than 25, 25−50, and 51−90% change in sample translucence, respectively (*26*). Film samples on uninoculated medium were used as controls.

Testing conditions for the determination of zein film biodegradability were optimized using two strains from the local solid waste landfill and two from the lagoon having the highest biodegradability activity. The variables included growing media form (solid or liquid), added glucose concentration (0, 0.05, and 0.1%), and incubation time before adding the zein film piece (0, 48, and 96 h). The liquid media test consisted of film pieces in test tubes containing 9 mL of minimal media without agar. On the basis of these studies, conditions for film biodegradability evaluation were selected as follows: three film pieces (1.5 cm × 5 cm) on the surface of nutrient−salts agar with 0.05% glucose, incubation at 30 °C for 48 h, separate Petri dishes for each bacterial strain, and three film pieces (1.5 cm × 5 cm) on uninoculated dishes as controls.

Zein/EC films were formed from solutions with no pH adjustment (pH 7 ± 0.1) or adjusted to pH 4.4 and 9.4. Biodegradability of rectangular film samples placed on the media surface was measured by visual changes in film opacity assumed to be caused by microbial activity of the bacterial strains selected. This change was measured daily during 21 days using the 0−4 scoring scale previously described (*26*). The sum of the average daily score (DS) for the two bacterial strains from each of the two sources (solid waste landfill and lagoon) was then normalized and reported as a biodegradability percentage index (BPI) defined as follows:

$$\mathrm{BPI}_j = \frac{\sum_{i=1}^{j}\mathrm{DS}_i}{4j} \times 100 \quad j = 1, ..., 21 \text{ days} \quad (4)$$

where DS = average daily score based on readings for each of the four strains.

RESULTS AND DISCUSSION

Experimental data for moisture sorption at 25 °C for zein, EC, and equal zein/EC mixture films without plasticizer are shown in **Figure 1**. Because the BET model is applicable only in the low a_w range, the GAB model was used to generate curves for the entire isotherm (**Figures 1−3**). As shown by DM values, experimental data for blended film were the only ones that gave a poor fit to the GAB model (**Table 1**). The lowest water sorption values were observed for EC films, which is charac-

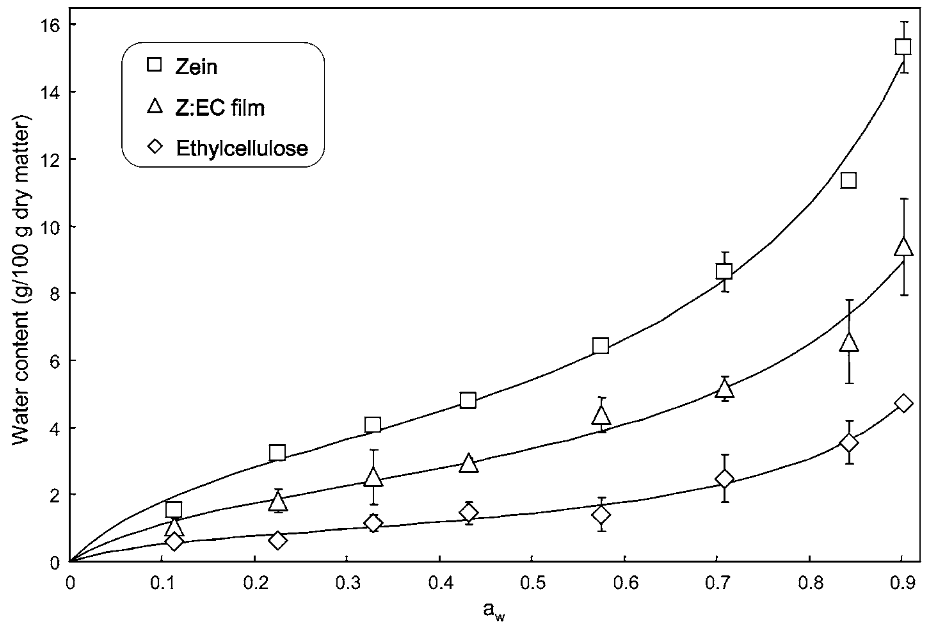

Figure 1. Water sorption isotherms at 25 °C for zein, EC, and equal zein/EC mixture films without plasticizer. Parameters for the GAB equations fitted to the experimental data are provided in **Table 2**.

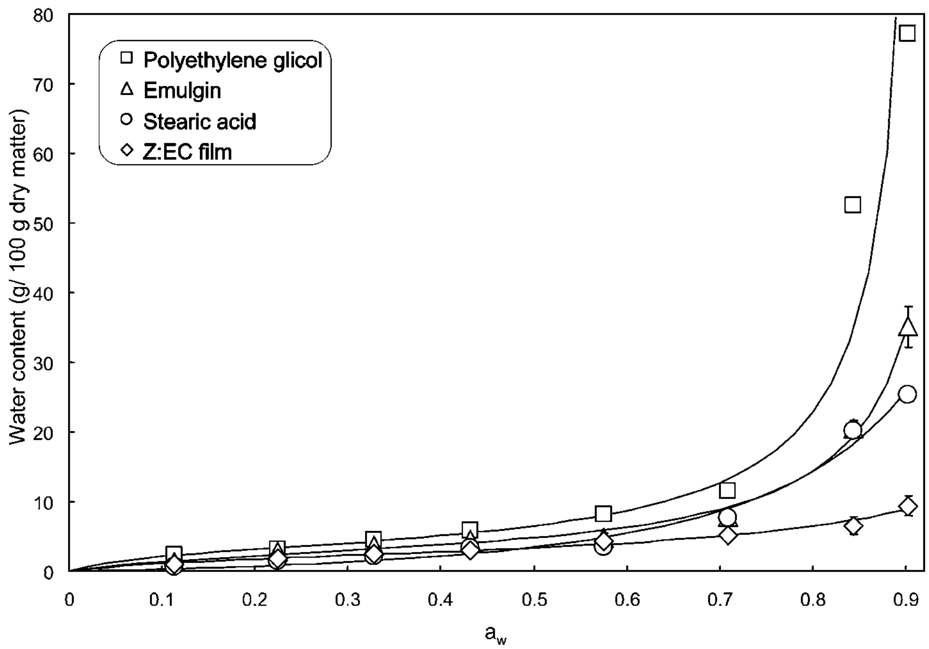

Figure 2. Effect of plasticizer on the water sorption isotherms at 25 °C for equal zein/EC mixture films. Parameters for the GAB equations fitted to the experimental data are provided in **Table 2**.

Table 1. GAB Equation Parameters for the Water Sorption Isotherms at 25 °C for Films without Plasticizer[a]

film composition	X_m (g/100 g dry matter)	*C* (kJ/mol)	*K*	DM	R^2
zein	3.57	9.05	0.851	8.38	0.992
equal zein/ EC mixture	2.24	9.14	0.840	12.33	0.979
EC	0.87	11.26	0.909	6.19	0.988

[a] X_m = monolayer value, *C* and *k* = GAB model constants, and DM = relative deviation modulus.

teristic of films that are not hydrophilic. The moisture content for zein films at high a_w was three times higher than the values for pure EC films. Mixture films had a behavior between that for pure zein and EC films. The moisture content values observed in this work (**Figure 1**) agree with those reported in one study (*15*) but are lower than values reported by others (*4*). A comparison with values reported for pure EC films (*27*) showed that the moisture values obtained in this study were comparable.

Monolayer moisture (X_m) and heat of sorption (*C*) values calculated for water activity values up to 0.57 when using the BET equation (**Table 2**) and using all experimental data when using the GAB equation (**Table 1**) are shown in decreasing order of water vapor affinity. In addition, the GAB model provided information on the number of water layers as reflected in the parameter *k*. The monolayer value (X_m) for films without plasticizer at 25 °C obtained from the GAB equation was higher than those calculated using the BET equation (**Table 1**). In both

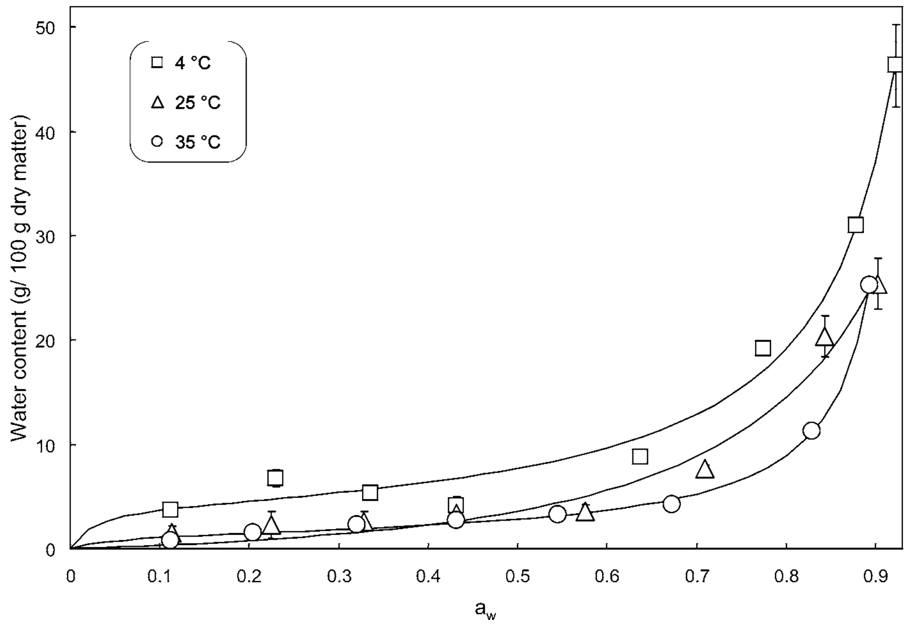

Figure 3. Effect of temperature on the water sorption isotherms of equal zein/ethyl cellulose mixture films containing stearic acid. Curves drawn using GAB equations fitted to the experimental data.

Table 2. BET Equation Parameters for the Water Sorption Isotherms at 25 °C for Films without Plasticizer[a]

film composition	X_m (g/100 g dry matter)	C (kJ/mol)	R^2
zein	2.75	16.41	0.975
equal zein/EC mixture	1.71	17.37	0.962
EC	0.71	21.07	0.914

[a] X_m = monolayer value and C = BET equation constant.

Table 3. HLB Values for the Plasticizers Used to Elaborate Films

plasticizer	HLB value
stearic acid	14.2
poly(ethylene glycol)	15.7
emulgin (etoxylated ricine oil)	14.4

Table 4. Effect of Temperature on BET Sorption Isotherms Parameters for Equal Zein/EC Mixture Films Containing Stearic Acid[a]

temperature (°C)	X_m (g/100 g dry matter)	C (kJ/mol)	R^2
4	3.63	23.35	0.909
25	1.99	16.51	0.900
35	1.58	10.87	0.948

[a] X_m = monolayer value and C = BET equation constant.

models, the X_m values for equal zein/EC mixture film, 1.71 (**Table 2**) and 2.22 g water/100 g dry matter (**Table 1**) calculated from the BET and GAB equations, respectively, were close to the midpoint of the X_m values for the pure zein and EC films, 1.73 and 2.22 calculated from the BET and GAB X_m values, respectively. The trend for higher X_m values obtained from the GAB model has been observed for starch (*28*), fish flour, and cornmeal (*29*). A similar behavior has been observed for methylcellulose but not for EC at the same temperature (*27*).

The GAB model constant *k* varies from 0.56 to nearly 1 for a large number of food constituents with values for proteins falling in the 0.8−0.9 range (*30*). In this study, a *k* value of 0.851 was found for zein and 0.84 and 0.909 for equal zein/EC mixture and EC films, respectively. The value for the equal zein/EC mixture films was closer to the value of the pure zein. The parameter *C* as calculated by the GAB (**Table 1**) and BET (**Table 2**) models was higher for the EC films while the value for the equal zein/EC mixture films was again closer to the value for the pure zein films.

Plasticizers used to improve the mechanical properties of films can also affect their moisture uptake. **Figure 2** shows sorption isotherms at 25 °C for equal zein/EC mixture films containing the plasticizers with the hydrophilicity values reported in **Table 3**. Films with poly(ethylene glycol) gave the highest water absorption reflecting the higher hydrophile−lipophile balance (HLB) value for this plasticizer (**Table 3**). Significantly lower but similar values were obtained for etoxylated ricine oil (Emulgin) and stearic acid. The latter was therefore selected for further studies on the effect of temperature (**Figure 3**). **Table 4** shows the expected decrease in the BET monolayer value with temperature, which was not observed with the GAB model. This deviation from expected behavior is frequently observed in nonlinear regressions, especially where functions having three or more parameters are involved. This has been attributed to the observation that in the calculation of model parameters unique solutions are not always guaranteed (*31*).

A statistical analysis of film biodegradability data showed no effect of the plasticizer and pH of the film forming solution (data not shown) even though the plasticizer effect on moisture content was significant (**Figure 2**). The data presented in **Figures 4** and **5** correspond to biodegradability tests for zein/EC mixture films containing stearic acid. Significant differences in film biodegradability were observed for films containing different zein concentrations. BPI values as a function of incubation time with the lagoon (**Figure 4a**) and local solid waste landfill (**Figure 4b**) bacilli strains selected for this study showed a high biodegradability for 100 and 75% zein films and a much lower level at 50% zein. The biodegradability of samples with 25% zein differed little from the low values observed for pure EC films, which showed no biodegradation signs for 4−5 days and only minimum biodegradation after the 21 day testing time used in this study. This biodegradability behavior did not

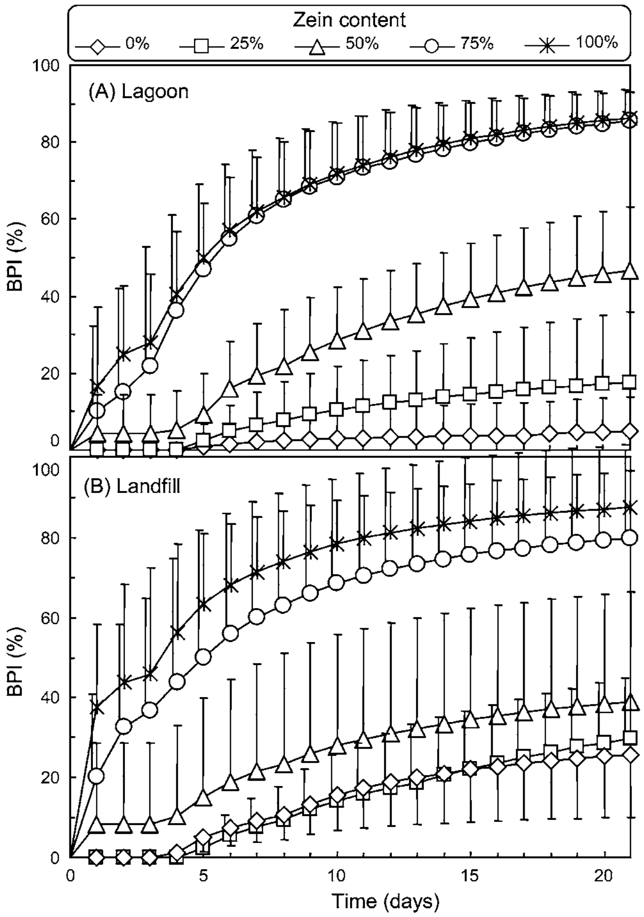

Figure 4. Effect of time on the BPI of zein/EC mixture films using bacilli isolated from a local lagoon or landfill and an incubation temperature of 30 °C for 48 h.

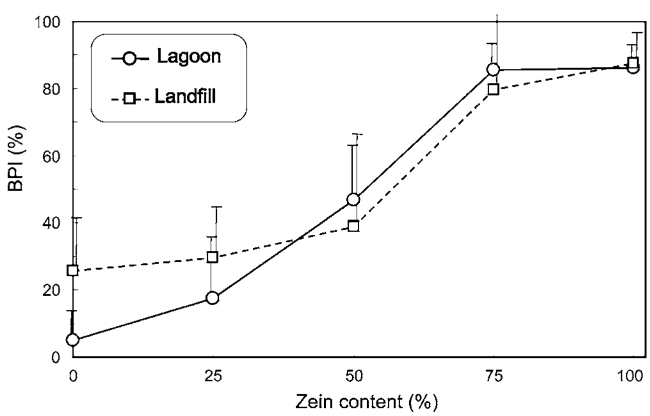

Figure 5. Effect of protein concentration on the BPI of zein/EC mixture films using bacilli isolated from a local lagoon or landfill and an incubation temperature of 30 °C for 48 h.

mimic the water sorption behavior of the zein/EC mixture films. As shown in **Figure 1**, the moisture content of equal mixture films was approximately the midpoint of the pure film components for the entire a_w range covered in this study. The same was true when the water sorption behavior was analyzed in terms of X_m values as previously noted. The values for the parameters C (BET and GAB models) and k (GAB model) for the equal zein/EC mixture films were very close to the value for the pure zein films while the biodegradability did not follow this behavior. These results suggest that the water sorption isotherms do not explain the biodegradability behavior of zein/EC mixture films.

BPI values at day 21 were plotted as a function of zein content showing again the high biodegradability of 75 or 100% zein films and the consistent behavior in biodegradation for the two bacilli sources used in this work (**Figure 5**). The low and similar biodegradability of 0, 25, and 50% zein films suggests that zein is not accessible to microorganisms when it is not the major film constituent.

CONCLUSIONS

Plasticizers improve the mechanical properties, and in the case of edible films, they have the additional functionality of increasing their moisture barrier. This study showed that for EC/zein films, stearic acid appears to be the most appropriate plasticizer as it had the lowest moisture adsorption effect, particularly at the high a_w found in fresh food applications. The moisture content over the entire a_w range was approximately equal to the average of the moisture content for the pure component films. The zein content effect on the biodegradability of zein/EC films was not the average of the two component films. Films containing 0, 25, and 50% zein had low biodegradability while 75 and 100% zein films had similar high biodegradability. It was interesting to note that a_w, moisture content, and zein concentration of the composite films did not explain biodegradability and it appears that EC in composite films limits access to zein by the four biodegradability test microorganisms selected on the basis of their zein proteolytic activity. The lack of a relationship between microbial biodegradation and film composition, particularly zein content, was unexpected.

ACKNOWLEDGMENT

Special thanks to E. Coleman for his contribution to the preparation of this manuscript.

LITERATURE CITED

(1) Perez-Gago, M. B.; Krochta, J. M. Lipid particle size effect on water vapor permeability and mechanical properties of whey protein-beeswax emulsion films. *J. Agric. Food Chem.* **2001**, *49*, 996−1002.
(2) Rakotonirainy, A. M.; Padua, G. W. Effects of lamination and coating with drying oils on tensile and barrier properties of zein films. *J. Agric. Food Chem.* **2001**, *49*, 2860−2863.
(3) Torres, J. A.; Mireles, C.; Savant, V. D. Two food applications of biopolymers: edible coatings controlling microbial surface spoilage and chitosan use to recover proteins from aqueous processing wastes. In *Biopolymers: Utilizing Nature's Advanced Materials*; American Chemical Society Symposium Series No. 723; Imam, S. H., Greene, R. V., Zaidi, B. R., Eds.; American Chemical Society Publications: Washington, DC, 1998; pp 248−282.
(4) Lai, H. M.; Padua, G. W. Water vapor barrier properties of zein films plasticized with oleic acid. *Cereal Chem.* **1998**, *75*, 194−199.
(5) Torres, J. A. Edible coatings and films from proteins. In *Protein Functionality in Food Systems*; Hettiaraccchy, N., Ziegler, G., Eds.; IFT/Marcel Dekker: New York, 1994; pp 467−507.
(6) Krochta, J. M.; De Mulder-Johnston, C. Edible films and biodegradable films: Challenges and Opportunities. *Food Technol.* **1997**, *51*, 61−74.
(7) Torres, J. A.; Bouzas, J. O.; Karel, M. Microbial stabilization of intermediate moisture food surfaces. III. Effectiveness of surface preservative concentration and surface pH control on microbial stability of an intermediate moisture cheese analogue. *J. Food Process. Preserv.* **1985**, *9*, 107−119.

(8) Labuza, T. P.; Breene, W. M. Applications of "active packaging" for improvement of shelf life and nutritional quality of fresh and extended shelf life foods. *J. Food Process. Preserv.* **1989**, *13*, 1−69.
(9) Labuza, T. P. An introduction to active packaging for foods. *Food Technol.* **1996**, *50*, 68−71.
(10) Petersen, K.; Nielsen, P. V.; Bertelsen, G.; Lawther, M.; Olsen, M. B.; Nilsson, N. H.; Mortensen, G. Potential of biobased materials for food packaging. *Trends Food Sci. Technol.* **1999**, *10*, 52−68.
(11) Vojdani, F.; Torres, J. A. Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose multilayer films. *J. Food Process. Preserv.* **1989**, *13*, 417−430.
(12) Vojdani, F.; Torres, J. A. Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose films: effect of fatty acids. *J. Food Sci.* **1990**, *55*, 841−846.
(13) Watt, I. C. The theory of water sorption by biological materials. In *Physical Properties of Foods*; Jowitt, R., Escher, F., Hallstrom, B., Meffert, H. F., Spiess, W. E. L., Eds.; Applied Science Publishers: London, U.K., 1983; pp 27−41.
(14) Gennadios, A.; Weller, C. L. Moisture adsorption by grain protein films. *Trans. ASAE* **1994**, *37*, 535−539.
(15) Gennadios, A.; Park, H. J.; Weller, C. L. Relative humidity and temperature effects on tensile strength of edible protein and cellulose ether films. *Trans. ASAE* **1993**, *36*, 1867−1872.
(16) Lomauro, C. J.; Bakshi, A. S.; Labuza, T. P. Evaluation of food moisture sorption isotherm equations. Part I: fruit, vegetable and meat products. *Lebensm.-Wiss. Technol.* **1995**, *18*, 111−117.
(17) Ayranci, E.; Ayranci, G.; Dogantan, Z. Moisture Sorption Isotherms of dried Apricot, fig and raisin at 20 °C and 36 °C. *J. Food Sci.* **1990**, *55*, 1591−1593.
(18) Kim, S. J.; Ustunol, Z. Solubility and moisture sorption isotherms of whey-protein based edible films as influenced by lipid and plasticizer incorporation. *J. Agric. Food Chem.* **2001**, *49*, 4388−4391.
(19) Cuq, B.; Gontard, N.; Guilbert, S. Edible films and coatings as active layers. In *Active Food Packaging*; Rooney, M. L., Ed.; Blackie Academic & Professional: London, U.K., 1995; pp 111−142.
(20) Debeaufort, F.; Voiley, A. Methylcellulose-based edible films and coatings: 2. Mechanical and thermal properties as a function of plasticizer content. *J. Agric. Food Chem.* **1997**, *45*, 685−689.
(21) Chang, Y. P.; Cheah, P. B.; Seow, C. C. Plasticizing-antiplasticizing effects of water on physical properties of tapioca starch films in the glassy state. *J. Food Sci.* **2000**, *65*, 445−451.
(22) Romero Bastida, C. A. Desarrollo y Evaluación de una película biodegradable a base de zeina y etilcelulosa: Efecto de la hidratación sobre la eficiencia de la misma. Master Thesis. Universidad Autónoma de Querétaro, Mexico, 1994.
(23) Romero-Bastida, C.; Martin-Polo, M. O.; Velazquez de la Cruz, G. Permeation and moisture sorption behavior of zein-ethylcellulose based films. Paper 12e-8. IFT Annual Meeting, Anaheim, CA, 1995.
(24) Kamper, S. L.; Fennema, O. Use of an edible film to maintain water vapor gradients in foods. *J. Food Sci.* **1985**, *50*, 382−384.
(25) Wolf, W.; Spiess, W. E. L.; Jung, G. Standardization of isotherm measurements (Cost-project 90 and 90 bis). In *Properties of Water in Foods in Relation to Quality and Stability*; Simatos, D., Multon, J. L., Eds.; Martinum Nijhoff Publishers: Dordrecht, Netherlands, 1985; pp 661−679.
(26) ASTM. Standard practice for determining resistance of plastics to bacteria. Standard Designation: G G22-76. *Annual Book of American Standard Testing Methods*; ASTM: Philadelphia, 1988.
(27) Velazquez de la Cruz, G.; Torres, J. A.; Martin-Polo, M. O. Temperature effect on the moisture sorption isotherms for methylcellulose and ethylcellulose films. *J. Food Eng.* **2001**, *48*, 91−94.
(28) Van Den Berg, C. Development of B. E. T.-like models for sorption of water on foods, theory and relevance. In *Properties of Water in Foods*; Simatos, D., Multon, J. L., Eds.; Martinum Nijhoff Publishers: Dordrecht, Netherlands, 1985; pp 119−131.
(29) Labuza, T. P.; Kaanane, A.; Chen, J. Y. Effect of temperature on the moisture sorption isotherms and water activity shift of two dehydrated foods. *J. Food Sci.* **1985**, *50*, 385−391.
(30) Chirife, J.; Timmermann, E. O.; Iglesias, H. A.; Boquet, R. Some features of the parameter k of the GAB equation as applied to sorption isotherms of selected food materials. *J. Food Eng.* **1992**, *15*, 75−82.
(31) Schuchmann, H.; Roy, I.; Peleg, M. Empirical models for moisture sorption isotherms at very high water activities. *J. Food Sci.* **1990**, *55*, 759−762.

Received for review September 11, 2003. Revised manuscript received February 9, 2004. Accepted February 13, 2004. The financial support from CONACyT-Mexico is gratefully acknowledged.

JF0350414