Solubility and Moisture Sorption Isotherms of Whey-Protein-Based Edible Films as Influenced by Lipid and Plasticizer Incorporation

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Plasticized whey-protein and whey-protein emulsion films were produced using sorbitol and glycerol as plasticizers and butterfat and candelilla wax as lipids. Protein, plasticizer, and lipid ratios were optimized to obtain acceptable free-standing flexible films. Water solubility (20 °C, 24 h) and moisture sorption isotherms (0.18−0.90 a_w, 25 °C) of the films were determined. The experimental moisture sorption isotherm values were fitted using the Guggenheim−Anderson−DeBoer (GAB) model. Solubility and equilibrium moisture contents (EMC) of the films were influenced by plasticizer and lipid incorporation. EMCs of all films increased rapidly at $a_w \geq 0.65$. Incorporation of lipids reduced solubilities and EMCs of sorbitol- and glycerol-plasticized films. The effects of plasticizer and lipid type on GAB constants were also determined.

Keywords: *Whey protein; lipids; edible films; solubility; moisture sorption isotherm*

INTRODUCTION

Edible films can enhance food quality by acting as moisture, gas, aroma, and lipid barriers and providing protection to a food product after the primary package is opened. Renewed interest in edible films in recent years is due to concerns about the environment and a need to reduce the amount of disposable packaging, as well as consumer demand for higher-quality food products. Edible films provide food processors with a number of new and unique opportunities for product processing, handling, and development (*1*).

A number of plant and animal proteins have been investigated for the production of edible films such as corn zein, wheat gluten, soy and peanut protein, gelatin, collagen, casein, and whey proteins (*2*). Properties of these films have been reported. McHugh and Krochta (*3*), Chen (*4*), and Gennadios et al. (*2*) have reviewed the properties of milk-protein-based edible films. Milk-protein-based films have good mechanical strength and are excellent oxygen, lipid, and aroma barriers. But, like other protein films, because of their hydrophilic nature they are poor barriers to moisture (*1, 2, 4*−*6*). Their moisture barrier properties, however, can be improved by incorporation of hydrophobic materials such as lipids (*7*−*9*) to produce protein/lipid-emulsion films. However, the mechanism to predict moisture transport through protein and protein/lipid-emulsion films is complex because of their nonlinear water sorption isotherms.

The moisture sorption isotherm which is the equilibrium moisture content of a food product or a material at a given water activity (a_w) at a specified temperature represents the combined hygroscopic properties of its individual components (*10*). Any modification in the composition of the food or the material may, in turn, influence its sorption isotherm (*11*).

Hydrophilic polymers, such as proteins which contain polar groups that provide for hydrogen bonding, will absorb water from the surrounding air or from the food product they contain. Consequently, the presence of water vapor in the polymer will change the permeation of gases and vapors through that polymer (*11*). Typically, the permeation rate increases with higher water sorption because water acts as a plasticizer and increases the free volume of the polymer. Thus, experimentally derived moisture sorption isotherms are a useful way to assess the equilibrium moisture content of protein-based edible films at a given relative humidity to estimate their barrier and other characteristics.

Gennadios and Weller (*12*) reported on the isotherms for corn zein, wheat gluten, and mixed wheat gluten and soy protein films. They demonstrated that the Guggenheim−Anderson−DeBoer (GAB) isotherm model showed the best fit over the studied a_w range. The GAB model is generally the most accepted isotherm. Coupland et al. (*13*) reported on the sorption isotherms of whey-protein-based films as influenced by plasticizer amount and concluded that the equilibrium moisture content of the films was largely determined by the amount of plasticizer in the films. Hydrophobic materials such as lipids are known to decrease water sorption of foods and other materials (*10*). However, the effect of plasticizer and lipid type on moisture sorption isotherms of whey-protein edible films and on their solubility has not been reported. Thus, the intent of this research was to determine how lipid and plasticizer type may influence the moisture sorption isotherm and water solubility of whey-protein and lipid-emulsion films.

MATERIALS AND METHODS

Materials. Whey protein isolate (WPI; ALACEN 895) was obtained from New Zealand Milk Products (North America) Inc. (Santa Rosa, CA). Glycerol, $KC_2H_3O_2$, and $NaNO_2$ were purchased from J. T. Baker Co. (Phillipsburg, NJ). Candelilla wax was purchased from Strahl and Pitssch Inc. (West Babylon, NY), and unsalted butter was purchased from Land O' Lakes, Inc. (Arden Hills, MN). Sodium azide, D-sorbitol, $LiCl·H_2O$, and $K_2CO_3·2H_2O$ were obtained from Sigma Chemi-

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cal Co. (St. Louis, MO). NaOH, NaCl, and KCl were from Mallinckrodt Speciality Chemical Co. (Paris, KY). $CaCl_2 \cdot 2H_2O$ was from Fisher Scientific Co. (Fair Lawn, NJ), and $Mg(NO_3)_2 \cdot 6H_2O$ was from EM Science (Cherry Hill, NJ).

Film Preparation. WPI contained 93.5% protein and <1% fat and lactose (data provided by New Zealand Milk Products (N. America) Inc.). Composition of WPI was confirmed using AOAC (*14*) procedures. WPI (5% w/v) and sorbitol (5.0, 4.8, or 4.2% w/v) or glycerol (3.5, 3.3, or 2.7% w/v) were mixed in distilled water and the pH was adjusted to 8 with 2 N NaOH. Solutions were heated to 90 ± 2 °C while being stirred continuously. Butterfat (BF; 0.2% w/v based on the solids content of the butter) was added to solutions containing 4.8% sorbitol or 3.3% glycerol. Candelilla wax (CW; 0.8% w/v) was incorporated into solutions containing 4.2% sorbitol and 2.7% glycerol. Lipids were added during heating process and were allowed to melt into the solutions to provide a solids content of 10% w/v for sorbitol- and 8.5% w/v for glycerol-plasticized film forming solutions. In our preliminary studies butterfat and candelilla wax were shown to be the most suitable in the production of whey-protein and lipid-emulsion edible films, and thus were chosen for these experiments. The solutions were homogenized for 2 min using a Polytron PT 10/35 homogenizer with a PTA 20 TS homogenizing head (Tekmar Co., Cincinnati, OH). The solutions were filtered through a layer of cheesecloth (to ensure complete incorporation of lipids into the film forming solutions), vacuum degassed for 30 min, and then cast on 18.5-cm circular Teflon surfaces. The films were dried at room temperature (23 ± 2 °C) and 30 ± 5% relative humidity (RH) for 18 ± 3 h, until a constant weight was reached. Dried films were peeled and stored at 23 ± 2 °C and 50 ± 5% RH until tested.

Film Thickness. Film thickness was determined using a TMI model 549M micrometer (Testing Machines Inc., Amityville, NY). Measurements were taken at five locations. Mean thickness of sorbitol-plasticized films was 140 ± 19 μm and of glycerol-plasticized films was 120 ± 15 μm.

Solubility (Total Soluble Matter) Determination. A procedure by Gontard et al. (*15*) was modified to determine the water solubility of the films. Approximately 3-g film samples were weighed to the nearest 0.0001 g and dried in a drying oven (100 ± 2 °C; 24 h) to obtain initial dry matter weight of the films. The dried films were immersed into 50 mL of deionized water containing 0.02% w/v sodium azide (to prevent microbial growth) and gently agitated (20 ± 2 °C, 24 h). The films in water were visually inspected hourly to determine the time elapsed to obtain complete solubility. At the end of 24 h, the unsolubilized films were taken out of the water and dried (100 ± 2 °C; 24 h) to determine the weight of the dry matter which was not solubilized in water. The weight of dry matter solubilized was calculated as follows and reported as percent water solubility of the films:

$$\text{water solubility (\%)} = \frac{\text{wt. of initial dry matter} - \text{wt. of dry matter not solubilized}}{\text{wt. of initial dry matter}} \times 100$$

Moisture Sorption Isotherms. All films were conditioned at 25 ± 0.1 °C in hermetically sealed glass jars containing desiccant. Moisture sorption isotherms (MSI) of the films were determined at 25 ± 0.1 °C according to standard E104 (*16*). Eight different humidity conditions (18 ± 0.5, 23 ± 0.5, 34 ± 0.5, 46 ± 0.5, 54 ± 0.5, 64 ± 0.05, 73 ± 0.05, 90 ± 0.5%) were obtained by using saturated salt solutions of $LiCl \cdot H_2O$, $KC_2H_3O_2$, $CaCl_2 \cdot 2H_2O$, $K_2CO_3 \cdot 2H_2O$, $Mg(NO_3)_2 \cdot 6H_2O$, $NaNO_2$, NaCl, and KCl, respectively. The water activity (a_w) of each of the salt solutions was calculated as % RH/100. Aluminum dishes were weighed to the nearest 0.0001 g and 3 g of film was added to each dish. The films were allowed to reach equilibrium (determined as less than 1% change in sample mass) at 25 ± 0.1 °C at the relative humidities specified above. Equilibrium moisture content (EMC) was determined by drying samples in a vacuum oven (*14*). The experimental moisture sorption isotherm values were averaged and fitted by the Guggenheim−Anderson−DeBoer (GAB) model as follows:

$$\text{EMC} = (W_m C K a_w)/[(1 - K a_w + C K a_w)]$$

where EMC = equilibrium moisture content on a dry basis; W_m = BET monolayer moisture content; C = Guggenheim constant, K = factor correcting properties of the multilayer molecules corresponding to the bulk liquid, and a_w = water activity. To estimate the specific parameters, the equation was transformed as follows:

$$a_w/\text{EMC} = \alpha(a_w)^2 + \beta(a_w) + \gamma$$

where $\alpha = K/W_m [1/C - 1]$, $\beta = 1/W_m [1 - 2/C]$, and $\gamma = 1/W_m [C \times K]$. A quadratic equation was performed on experimental values to obtain α, β, and γ.

Statistical Analysis. All experiments were replicated three times in a randomized complete block experiment. Days were blocked. A new film-forming solution and a new set of films were prepared for each replicate. Each analysis was done in duplicate. Statistical analysis was conducted using Sigma Stat 2.0 (Jandel Corp., San Rafael, CA). Treatment means were compared using the Student−Newman−Keuls comparison. Comparisons were made only within the same film. Significance of differences was defined at $p \leq 0.05$.

Table 1. Protein, Plasticizer, and Lipid Ratios of the Whey-Protein and Lipid-Emulsion Edible Films

treatment[a]	protein/ plasticizer/lipid (ratio)
1. WPI−S	1:1
2. WPI−G	1:0.7
3. WPI−S−BF	1:0.96:0.04
4. WPI−G−BF	1:0.66:0.04
5. WPI−S−CW	1:0.84:0.16
6. WPI−G−CW	1:0.54:0.16

[a] WPI, whey protein isolate; S, sorbitol; G, glycerol; BF, butterfat; CW, candelilla wax.

RESULTS AND DISCUSSION

Table 1 shows the protein, plasticizer, and lipid ratios of the whey-protein and lipid-emulsion edible films that were formed. These ratios were optimized in our lab for the production of acceptable free-standing and flexible films. At higher glycerol and butterfat concentrations the films were too soft and unpeelable from the casting surface, whereas at lower sorbitol concentrations the films were too brittle. Thus, protein and total solids contents were kept constant within the same plasticizer type. Statistical comparisons were made only within the same plasticizer type.

Water Solubility. Table 2 shows the water solubility and the elapsed time to solubilize whey-protein and lipid-emulsion edible films. Sorbitol-plasticized films were completely soluble in water, whereas glycerol-plasticized films were only partially soluble (solubilities ranging 24.7−31.6%). This difference, however, also may be due to the differences in the plasticizer concentrations rather than plasticizer type. Lower solubility of glycerol-plasticized films was somewhat of a surprise because glycerol is more hygroscopic than sorbitol (*17, 18*), and they had higher EMCs than their sorbitol-plasticized counterparts at all a_w values investigated as discussed below. Incorporation of lipids reduced ($p < 0.05$) the solubilities of the glycerol-plasticized films. Candelilla-wax-containing films were less ($p < 0.05$) soluble than butterfat-containing films. In the case of sorbitol-plasticized films, incorporation of lipids merely

Table 2. Water Solubility and Elapsed Time to Solubilize Whey-Protein and Lipid-Emulsion Edible Films (20 °C, 24 h)

treatment[a]	solubility (%)[b]	elapsed time (h)[b]
1. WPI−S	100.0 ± 0.0[a]	7.0 ± 0.0[d]
2. WPI−G	31.6 ± 0.7[b]	24.0 ± 0.0[a]
3. WPI−S−BF	100.0 ± 0.4[a]	15.0 ± 0.3[c]
4. WPI−G−BF	27.6 ± 0.5[c]	24.0 ± 0.0[a]
5. WPI−S−CW	100.0 ± 0.03[a]	17.0 ± 0.3[b]
6. WPI−G−CW	24.7 ± 0.3[d]	24.0 ± 0.0[a]

[a] WPI, whey protein isolate; S, sorbitol; G, glycerol; BF, butterfat; CW, candelilla wax. Protein/plasticizer/lipid ratios: 1 = 1:1; 2 = 1:0.7; 3 = 1:0.96:0.04; 4 = 1:0.66:0.04; 5 = 1:0.84:0.16; 6 = 1:0.54:0.16. [b] Means with different superscripts (a−d) are significantly different ($p < 0.05$). Comparisons are made only within the same plasticizer type ($n = 3$ for all treatments).

delayed their solubility, with candelilla-wax-containing films taking longer to dissolve ($p < 0.05$) than films with butterfat. However, it should be noted that plasticizer concentrations were lower in the lipid-containing films. Candelilla-wax-incorporated films contained less plasticizer than butterfat-containing films did. Water solubility is an indication of the film's hydrophilicity. Butterfat, being a more polar lipid than candelilla wax, probably may partially account for the higher solubilities of the butterfat-containing films compared to those of films with candelilla wax. In addition, butterfat contains other compounds, such as alcohols, esters, phenolics, and ketones, which may contribute to its polarity (*19*). Candelilla wax consists primarily of hydrocarbons and wax esters and is classified as a nonpolar lipid (*20*). Reduction of total soluble matter due to incorporation of fatty acids (lauric, myristic, palmitic, or oleic acids) into soy-protein films was reported by Gennadios et al. (*21*). They reported a 50% reduction in solubility of the soy-protein films with incorporation of 30% oleic acid. Their studies also showed that the soy-protein films with lower solubilities had better structural integrity as evidenced by greater tensile strength and elongation. Incorporation of milk-fat fractions into soy-protein films had minimal effect on water solubility (*21*).

Moisture Sorption Isotherms. The GAB water sorption isotherm model is generally the most accepted model for foods. It has been shown to fit moisture sorption data well for many food products (*22*), as well as for edible films (*12*, *13*, *23*) over a wide range of a_w values. Therefore, the GAB model was selected for this study also. Experimental data and moisture sorption isotherm curves predicted by the GAB model of whey-protein and lipid-emulsion edible films plasticized with sorbitol and glycerol tested at 25 °C are shown in Figures 1 and 2, respectively. Initially for all films, the increase in EMC was slow with increasing a_w. At $a_w \geq 0.65$ the EMC of films increased rapidly. Although not statistically compared, glycerol-plasticized films had higher EMCs than those of their sorbitol-plasticized counterparts at all a_w investigated. This is probably attributable to the higher hygroscopicity of glycerol than that of sorbitol (*17*, *18*), although a higher concentration of sorbitol was used in all sorbitol-plasticized films. Higher plasticizer contents have been reported to increase EMC of protein-based films (*12*, *13*). Our study indicates that EMC appears to be a function of the hygroscopicity of the plasticizer more so than its concentration. In the case of both plasticizer types (glycerol and sorbitol), addition of butterfat or candelilla wax lowered ($p < 0.05$) the EMCs at all a_w investigated.

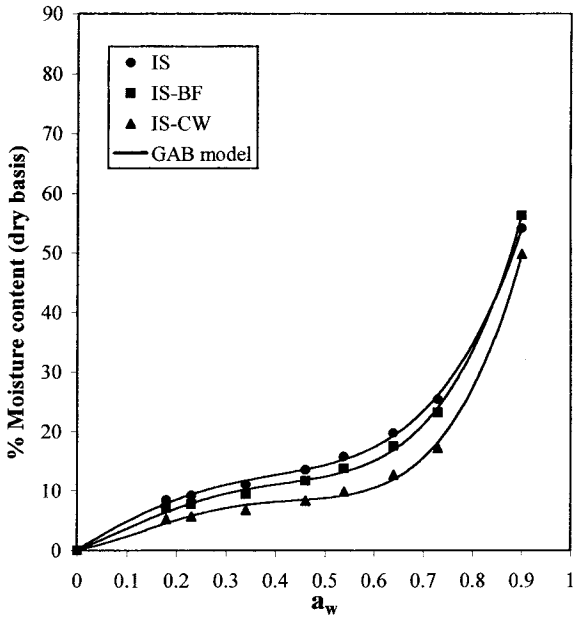

Figure 1. Moisture sorption isotherm of whey-protein and lipid-emulsion edible films plasticized with sorbitol tested at 25 °C. I, whey protein isolate; S, sorbitol; BF, butterfat; CW, candelilla wax. Protein/plasticizer/lipid ratios: IS = 1:1; IS−BF = 1:0.96:0.04; IS−CW = 1:0.84:0.16.

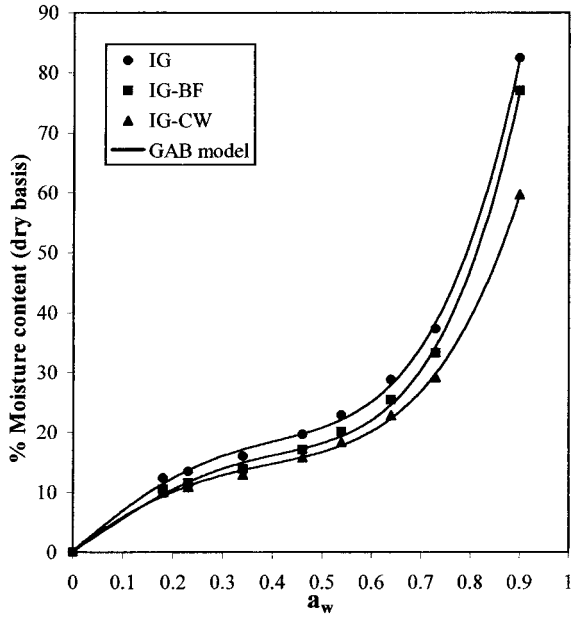

Figure 2. Moisture sorption isotherm of whey-protein and lipid-emulsion edible films plasticized with glycerol tested at 25 °C. I, whey protein isolate; G, glycerol; BF, butterfat; CW, candelilla wax. Protein/plasticizer/lipid ratios: IG = 1:0.7; IG−BF = 1:0.66:0.04; IG−CW = 1:0.54:0.16

However, it should be noted that the plasticizer amount in the films was reduced because of incorporation of lipid. Butterfat-containing films had higher ($p < 0.05$) EMCs than films with candelilla wax at all a_w investigated. The higher polarity of butterfat compared to that of candelilla wax (*20*) probably accounts for the differences observed.

The GAB constants obtained by fitting the GAB equation to the experimental isotherms of the films ($R^2 = 0.95$) are presented in Table 3. W_m was higher for glycerol-plasticized films compared to that of their sorbitol-plasticized counterparts. For both glycerol- and sorbitol-plasticized films, W_m decreased with incorpora-

Table 3. Parameters Fitted to Guggenheim−Anderson−DeBoer Equation for Whey-Protein and Lipid-Emulsion Edible Films

treatment[a]	W_m[b]	C[c]	K[d]
1. WPI−S	7.88	21.92	0.95
2. WPI−G	11.26	23.73	0.96
3. WPI−S−BF	6.77	23.83	0.98
4. WPI−G−BF	9.83	25.68	0.97
5. WPI−S−CW	4.54	24.01	1.01
6. WPI−G−CW	9.24	28.39	0.99

[a] WPI, whey protein isolate; S, sorbitol; G, glycerol; BF, butterfat; CW, candelilla wax. Protein/plasticizer/lipid ratios: 1 = 1:1; 2 = 1:0.7; 3 = 1:0.96:0.04; 4 = 1:0.66:0.04; 5 = 1:0.84:0.16; 6 = 1:0.54:0.16. [b] W_m = monolayer moisture content, g H_2O/100 g film. [c] C = Guggenheim constant. [d] K = factor correcting properties of the multilayer molecules corresponding to the bulk liquid.

tion of butterfat and candelilla wax. This reduction, however, was more pronounced with candelilla wax than with butterfat. Both C and K were higher for glycerol-plasticized films compared to those for sorbitol films. The C and K parameters increased upon incorporation of butterfat and candelilla wax. Coupland et al. (*13*) reported higher W_m and lower C with increased hydrophilicity of whey-protein-isolate films due to increased glycerol concentration. Although their results are consistent with ours as they relate to the hydrophilicity of the film components and the films, we did not investigate the effect of plasticizer concentration on our films.

In conclusion, solubility and EMC of whey-protein-isolate and lipid-emulsion films may be altered by plasticizer and lipid incorporation. Information on the solubility and moisture sorption isotherms of whey-protein/lipid-emulsion edible films should be useful in predicting their barrier properties and tensile characteristics. This information should be useful in determining their specific application.

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