JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Kinetics of Sucrose Crystallization in Whey Protein Films

KIRSTEN L. DANGARAN† AND JOHN M. KROCHTA*

Department of Food Science and Technology, University of California, Davis, Davis, California 95616

The kinetics of sucrose crystallization in whey protein isolate (WPI) films was studied at 25 °C in four different relative humidity environments: 23, 33, 44, and 53%. The effects of protein matrix, crystallization inhibitors, and storage environment on the rate constants of sucrose crystallization were determined using the Avrami model of crystallization. It was found that a cross-linked, denatured whey protein (WP) matrix more effectively hindered sucrose crystallization than a protein matrix of native WP. The crystallization inhibitors tested were lactose, raffinose, modified starch (Purity 69), and polyvinylpyrrolidone (Plasdone C15). Raffinose and modified starch were determined to be the more effective inhibitors of sucrose crystallization. At lower relative humidities (23, 33, and 44%), the cross-linked protein matrix played a more important role in sucrose crystallization than the inhibitors. As relative humidity increased (53%), the crystallization inhibitors were more central to controlling sucrose crystallization in WPI films.

KEYWORDS: Whey protein; sucrose; crystallization; inhibitors; kinetics; films

INTRODUCTION

Sucrose-Plasticized Whey Protein (WP) Films. Edible films made from WP and plasticized with sucrose have known beneficial properties. Sothornvit and Krochta (*1*) found that films made from β-lactoglobulin, the major fraction of WP, and plasticized with sucrose had excellent oxygen barrier properties. Whey protein isolate (WPI)/sucrose films were also determined to be highly glossy compared to WPI films plasticized with propylene glycol, glycerol, or polyethylene glycol (*2*). As sucrose concentration increased, WPI films became tougher and more durable, having moderate tensile strength and larger elongation values (*3*). The barrier, appearance, and tensile properties of WPI/sucrose films are attractive characteristics for WPI film applications. For example, all of these qualities would be desirable in a WPI/sucrose coating used to protect food quality. Specifically, high-gloss, water-based WPI/sucrose coatings have the potential to replace alcohol-based shellac glazes, which are coatings currently used by the confectionery industry. However, shellac coatings introduce volatile organic compounds into the atmosphere during processing (*4*, *5*). Finding and developing new uses of agricultural products would help to increase utilization of surplus materials such as whey, a byproduct of cheesemaking.

Although the oxygen barrier, gloss, and tensile properties of WPI/sucrose films are desirable, changes in the films over time have been qualitatively observed. The sucrose-plasticized films became brittle and hazy during storage. It was hypothesized that these negative changes were caused by the transition of sucrose from the amorphous state to crystalline state. To maintain the beneficial properties of WPI/sucrose films that initially exist, the crystallization of sucrose needs to be controlled. The food industry has studied the crystallization of sugars because of the effect on food texture and quality (*6*, *7*). The pharmaceutical industry is also interested in controlling crystallization, as it affects drugs efficacy (*8*−*10*). In both food research and pharmaceutical research, sucrose has been used as the model for crystallization studies. These studies provided insight into the crystallization transition; however, most were done in lyophilized, binary systems of sucrose plus a compound to control sucrose crystallization. WPI/sucrose films present a more complex system of sucrose interacting with a large biopolymer plus the control parameter of interest. Studying the kinetics of sucrose crystallization in WPI films not only is important to the development of applications of edible films, the system represents a more complex food model than the binary systems previously investigated.

Crystallization Kinetic Model. Crystallization models have been developed to take into account two stages of the state transformation—nucleation and crystal growth (*11*, *12*). The amorphous sucrose initially in the WPI films is in a metastable state. Kinetic approaches are more suited than thermodynamics for studying and controlling nonequilibrium systems (*13*). A frequently used kinetic model of crystallization is the Avrami equation

$$\alpha = 1 - e^{-kt^n} \quad (1)$$

where α is the degree of change in crystal mass at time t, t is time, k is the rate constant of the crystallization reaction, and n is the crystallization mechanism.

* Author to whom correspondence should be addressed [telephone (530) 752-2164; fax (530) 752-4759; e-mail jmkrochta@ucdavis.edu].
† Present address: Eastern Regional Research Center, ARS, USDA, 600 E. Mermaid Lane, Wyndmoor, PA 19038.

10.1021/jf0611670 CCC: $33.50
Published on Web 08/29/2006

By monitoring the amount of sucrose crystallization in WPI films over a period of time, the rate constant of the transformation could be calculated. The model was designed with the assumptions of constant crystal growth rate, constant density of growing nuclei, and no secondary crystallization (*14*).

Rates of crystallization can be affected by changes in molecular mobility and interactions at the crystal surface. Molecular mobility within a metastable system is directly related to the system's continued existence. Molecular mobility can be affected by molecular weight, which affects system viscosity and the glass transition temperature (T_g) (*15*). In WP films, the molecular weight of the protein polymer can be increased through thermal denaturation and chemical cross-linking. In native β-lactoglobulin, a free thiol group is hidden in the interior of the folded protein. Denaturing exposes the thiol group, and molecules of β-lactoglobulin polymerize through disulfide bonds (*16*, *17*). The end result is increased molecular weight of the protein film matrix. Molecular mobility can also be affected by film moisture content, which also affects the T_g (*15*). Storing WPI/sucrose films in different relative humidities results in different water contents in the films. Water is an excellent plasticizer of amorphous compounds. Increased moisture content in a film would lead to lower T_g, increased free volume, and increased molecular mobility. Crystal growth involves adsorption of molecules from the bulk phase onto the surface of the crystal with subsequent molecular orientation and incorporation into the crystal (*18*). Crystallization inhibitors that act at the surface of a growing crystal can impede adsorption and incorporation of bulk molecules, slowing growth rates.

On the basis of the effects of molecular weight and moisture content on molecular mobility and the effect of crystal growth inhibitors, we hypothesized that (1) a denatured, cross-linked whey protein film matrix would slow sucrose crystallization more than a native film, (2) incorporation of sucrose crystallization inhibitors—lactose, raffinose, modified starch, and polyvinylpyrrolidone (PVP)—would significantly slow crystallization, and (3) the rate constants of sucrose crystallization in WPI films would increase with increasing relative humidity.

Past studies have used a gravimetric method to study crystallization kinetics (*19*). Compared to other methods such as thermal analysis, gravimetric analysis allows study of crystallization at practical conditions that more accurately represent actual environments to which films would be exposed. Applications of WPI films or coatings would most likely involve dried foods with water activities of 0.60 or lower, such as dried cereals, confectionary products, and dried fruits. Studying sucrose crystallization in various relative humidities of <60% provides a representation of how the films would behave when in contact with dried foods.

The objectives of this study were to (1) measure the rate constants of sucrose crystallization in native and denatured WPI films, (2) measure the rate constants of sucrose crystallization in WPI films with added crystallization inhibitors, and (3) determine the effect of relative humidity on the crystallization.

METHODOLOGY

Materials. Films were made from WPI provided by Davisco Foods International (Eden Prairie, MN). They were plasticized with sucrose (Sigma-Aldrich, St. Louis, MO). The crystallization inhibitors studied were lactose (Fisher Scientific, Fair Lawn, NJ), modified starch (Purity 69, National Starch and Chemical Co., Bridgewater, NJ), polyvinylpyrrolidone (Plasdone C15, ISP Technologies, Inc., Columbus, OH), and raffinose (Acros Organics, Morris Plains, NJ).

Film Formation. Twelve film formulations were studied. They differed in state of the whey protein (native vs denatured), the presence of plasticizer (sucrose or no plasticizer), or the addition of a crystallization inhibitor to sucrose-plasticized films. **Table 1** shows film formulations and variable codes used throughout this study. Native films were made by dissolving WPI in distilled water (10% w/w solution). Denatured films were made by heat denaturing the aqueous WP solution in a 90 °C water bath for 30 min (*20*). Following denaturation, the solutions were cooled to room temperature in an ice bath. Sucrose was then added to film solutions, both native and denatured, that were being plasticized. Sucrose was added in a mass ratio of protein to plasticizer of 1:3, based on optimization in an earlier study (*3*). For those plasticized films that contained crystallization inhibitors, 10% of the sucrose was replaced with lactose, modified starch, PVP, or raffinose. The sucrose and inhibitors were stirred in the WPI solution for 30 min to allow for dissolution. To prevent air bubbles in the films, all solutions were degassed using a vacuum pump for 30−60 s. Films were cast in small, high-density polyethylene cups 5 cm in diameter (Aqualab, Decagon Devices Inc., Pullman, WA). A volume of film solution that contained 0.3 g of total solids was pipetted into each cup. Films were dried for 4 h in a controlled relative humidity (23%) environment at 25 ± 3 °C. They were then transferred to an environmental chamber equipped with a fan for air circulation. The relative humidity (RH) of the chamber was controlled with Drierite (W. A. Hammond Drierite Co., Xenia, OH) to ≤11% RH. Films were stored in the low relative humidity for 2 days.

Table 1. Whey Protein Film Formation and Variable Codes

variable code	protein state	plasticizer	inhibitor
NO	native	none	none
DO	denatured	none	none
NAT	native	sucrose	none
DEN	denatured	sucrose	none
NLAC	native	sucrose	lactose
NPUR	native	sucrose	modified starch
NPVP	native	sucrose	PVP
NRAF	native	sucrose	raffinose
DLAC	denatured	sucrose	lactose
DPUR	denatured	sucrose	modified starch
DPVP	denatured	sucrose	PVP
DRAF	denature	sucrose	raffinose

Gravimetric Analysis: Crystallization Kinetics. *Data Collection.* After conditioning, the weight of each film cast in the HDPE cups was taken with an analytical balance (Mettler-Toledo, Columbus, OH). These measurements were taken as weight of films at time zero. Samples were then transferred to environmental chambers with constant relative humidities of 23, 33, 44, or 53%. Relative humidity was controlled with saturated solutions of potassium acetate, magnesium chloride, potassium nitrate, or magnesium nitrate, respectively. All salts were purchased from Sigma-Aldrich. All chambers were equipped with fans for air circulation and kept at 25 ± 3 °C. Weights of the samples were then periodically measured and recorded.

Data Analysis: Avrami Model of Crystallization. Gravimetric data were analyzed using the Avrami model of crystallization kinetics. Because there is initially amorphous sucrose in the plasticized WPI films, there are two competing phenomena occurring when samples are first transferred from the low relative humidity environment to the higher relative humidity storage conditions. The amorphous sucrose, which is hygroscopic, will adsorb water. Therefore, the films will gain mass, and the weight of the films will increase. However, the sucrose in the films will also be crystallizing and losing water to the environment, causing a decrease in film weight. In the higher relative humidity environments, a peak in film weight was reached and then weight began to decrease. At this point, we assumed moisture adsorption had ceased, and the only phenomenon affecting film weight was crystallization of sucrose. Therefore, for determination of kinetic constants using the Avrami model, only the weight data after the peak in film weight was used. In the 23 and 33% RH samples plasticized with sucrose, no weight gain was observed, so all data points were used in the Avrami analysis.

Data were analyzed for two pieces of kinetic information—crystallization mechanism (n value) and rate constant (k value).

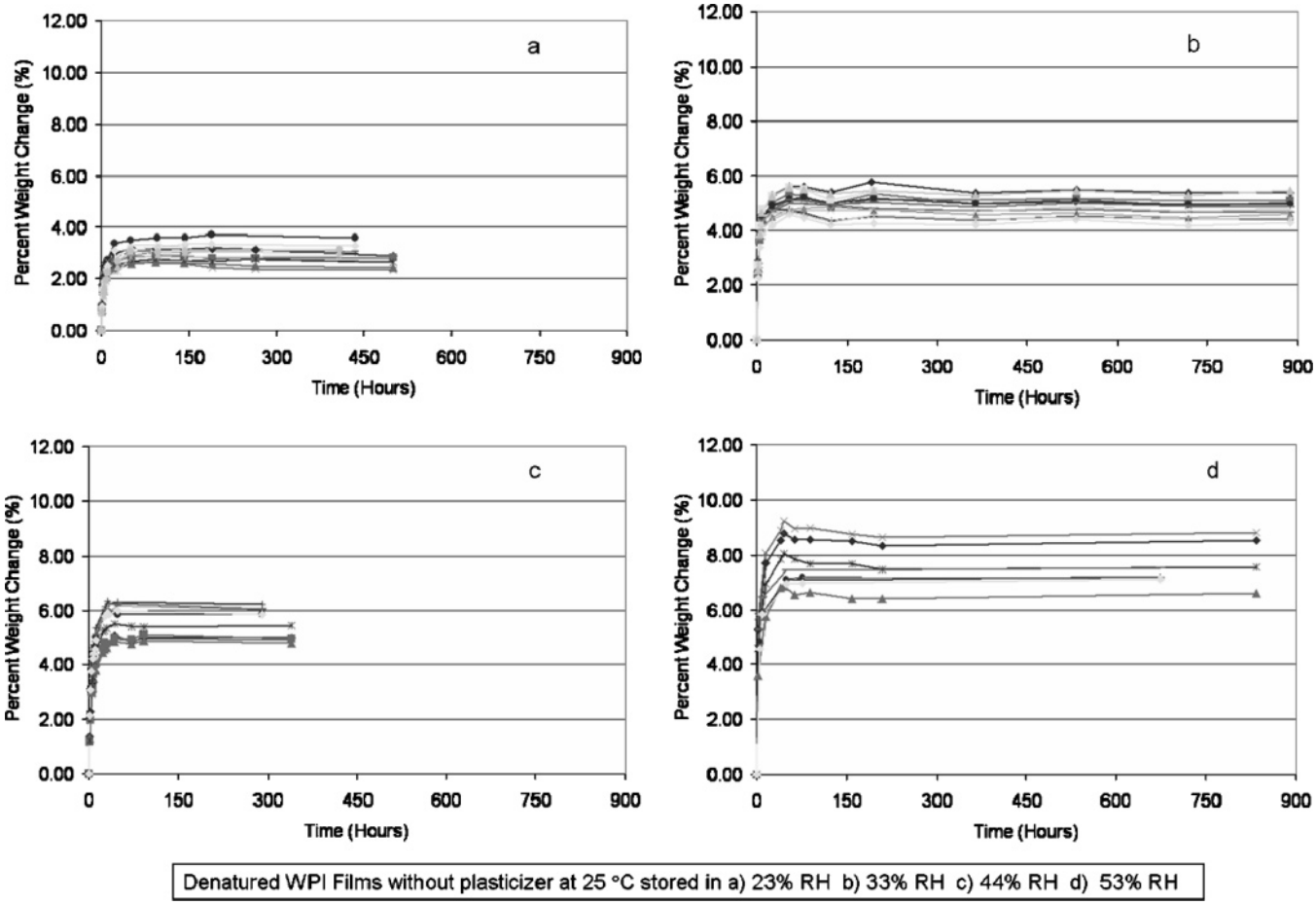

Figure 1. Weight change due to moisture absorption in denatured WPI films (no plasticizer) stored in (**a**) 23% RH, (**b**) 33% RH, (**c**), 44% RH, and (**d**) 53% RH at 25 °C. All sample replicates shown.

Mathematical manipulation of the Avrami model gives the relationship

$$\ln[-\ln(1-\alpha)] = n \ln t + \ln k \quad (2)$$

Plots of the natural log of the negative natural log$(1-\alpha)$ versus the natural log of time produced straight-line graphs. Using linear regression (Microsoft Excel, Microsoft Corp., Redmond, WA), the data were fitted to the Avrami model. The slope of the line was the n value with the intercept being the natural log of the rate constant.

Statistical Design. For each film formulation, two replicates were made. Within the replicates, four or five sample cups were cast and monitored for weight change and crystallization. For the DO and NO formulations (protein only), five sample cups were cast for each replicate for a total of 10 samples. For all other film formulations, four sample cups were cast per replicate. Statistical analysis was performed using Microsoft Excel. Within formulations, replicates were compared using the Student t test assuming equal variance. If replicates were found to be statistically similar, results for the films were pooled. When formulations were compared using single-factor ANOVA (SAS, SAS Institute Inc., Cary, NC), pooled data were used.

DSC: T_g Measurment. Measurements of the glass transition temperatures of the films were made using a Perkin-Elmer DSC. Thermal changes in WPI/sucrose films were monitored in the temperature range from −40 to 150 °C at a scan rate of 20 °C min^{-1}. Indium was used as a standard. The information was used to gain insight into the crystallization kinetics of the films.

RESULTS AND DISCUSSION

WPI Films with No Plasticizer. Figure 1 shows the moisture adsorption results for all replicates of the denatured (DO) WPI films without a plasticizer stored in 23, 33, 44, and 53% RH at 25 °C. Data for native (NO) WPI films without plasticizer (not shown) showed similar moisture adsorption trends. For all replicate samples, there was an initial gain in weight due to moisture uptake. Weight change in both the NO and DO samples plateaued, and there was no observed weight loss during the storage period. On the basis of these results, it was concluded that any weight loss observed in WPI/sucrose films was attributed to transition in state of the plasticizer, not the whey protein. The denatured WP films absorbed less water than the native films. In 23, 33, 44, and 53% RH, the average percent weight gains for the DO samples were 2.94, 4.47, 5.52, and 7.56%, respectively. For the NO samples in the same relative humidity environments, average percent weight gains were 4.34, 5.99, 7.14, and 9.55%, respectively. Denatured whey protein likely absorbed less water because of fewer exposed hydration sites. After unfolding, protein−protein interactions increased through increased van der Waals interactions of exposed hydrophobic amino acids and covalent bonding of free thiol groups. The denatured matrix was tighter with fewer available sites for water to bind to.

Matrix Effect on Sucrose Crystallization: Native versus Denatured WPI Films. Figure 2 shows weight loss data for native and denatured WPI films plasticized with sucrose stored in 23, 33, 44, and 53% RH at 25 °C. For each formulation, average weight change data for all samples from both replicates are shown. Statistical analysis found replicates were not significantly different within the NAT and DEN film formulations.

In the higher relative humidity environments, 44 and 53%, there was an observed initial increase in weight. This increase was greater in the DEN films in both RH environments. On

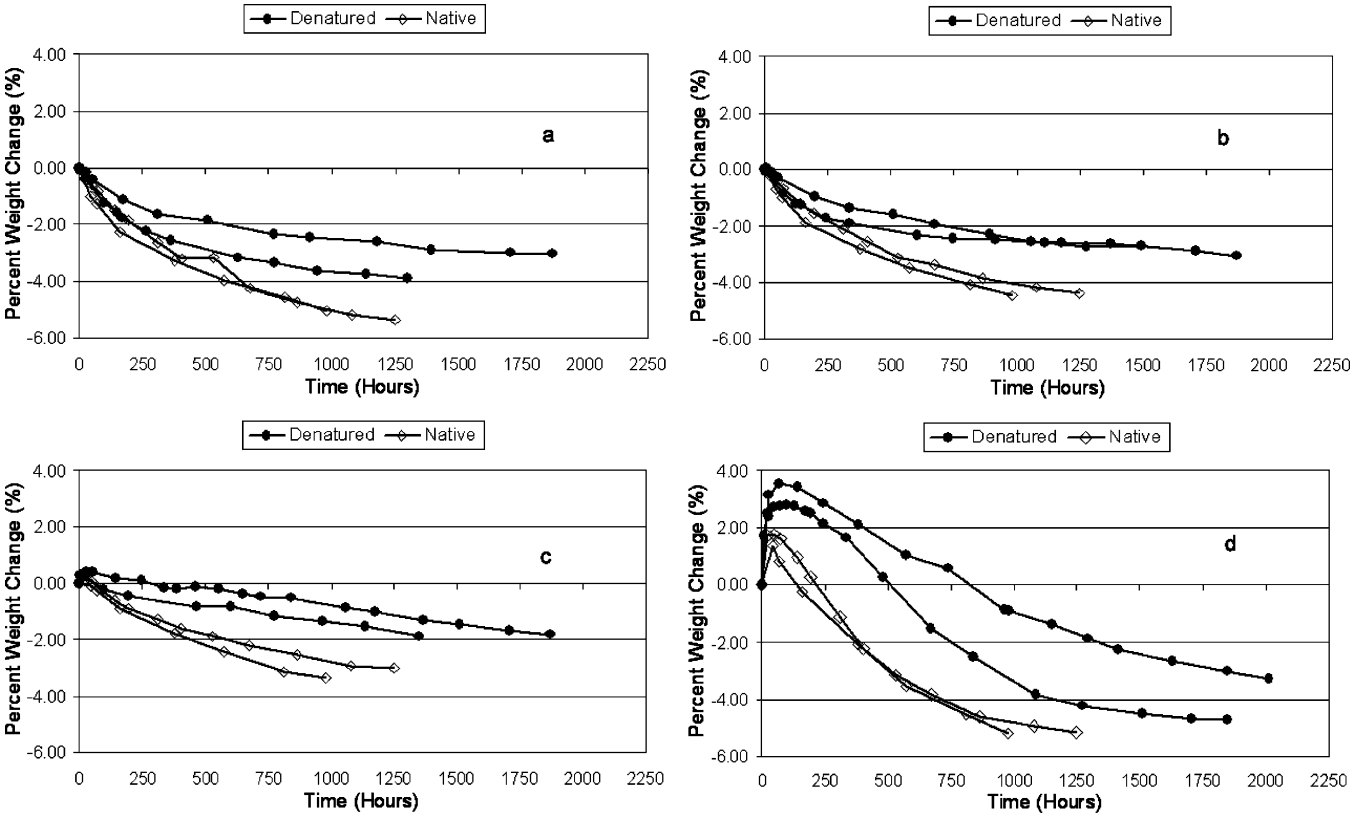

Figure 2. Change of film weight due to moisture adsorption and crystallization in denatured and native WPI films plasticized with sucrose stored in (**a**) 23% RH, (**b**) 33% RH, (**c**), 44% RH, and (**d**) 53% RH at 25 °C.

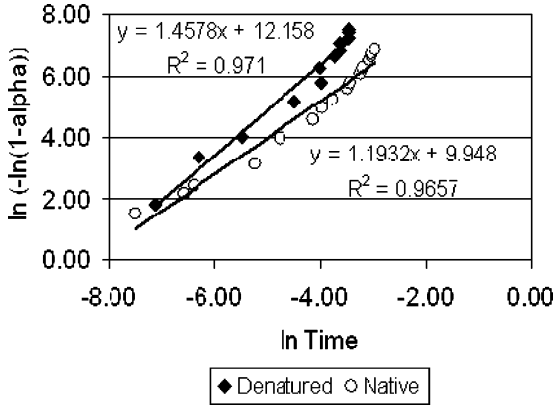

Figure 3. Avrami analysis of moisture adsorption/desorption data for native and denatured WPI films plasticized with sucrose stored at 23% RH and 25 °C.

the basis of the moisture isotherms of β-lactoglobulin, amorphous sucrose, and crystalline sucrose (*21*), this weight gain was expected when films were transferred from low relative humidity (11%) to the higher relative humidity. However, the moisture absorption was greater for the DEN sample than for the NAT samples. On the basis of the results from the DO and NO films without plasticizer, the opposite was expected. With the inclusion of sucrose in both the amorphous and crystalline state, the maximum weight gain depends on the percentage of each in the films. Because the DEN films had greater moisture absorption, it was concluded that a larger percentage of amorphous sucrose was present in these films compared to the NAT. The cross-linked, denatured protein matrix empirically appeared to be more effective at slowing the change of amorphous sucrose to crystalline sucrose. These findings were verified with the mathematical analysis.

Table 2. *n* Values from Avrami Analysis of Data for Sucrose Crystallization in Native WP, Denatured WP, and Denatured WP with Inhibitor Film Matrices Stored in Various Relative Humidity (RH) Environments at 25 °C

	matrix effect		inhibitor effect			
RH (%)	native	denatured	lactose	mod starch	PVP	raffinose
23	1.25	1.58	1.60	1.67	1.59	1.71
33	1.41	1.63	1.92	1.57	2.04	2.06
44	1.02	1.34	1.59	1.36	1.58	1.55
53	1.17	0.69	0.58	0.82	0.56	0.65

Avrami analysis of data for a NAT film and a DEN film, both stored in 23% RH, is depicted in **Figure 3**. The regression equations and R^2 values for the fits are also given. For conciseness, other data sets are not shown. However, weight change data for all plasticized film samples were able to be modeled with the Avrami equation and had similar fits as those shown in **Figure 3**. The calculated *n* and *k* values for the NAT and DEN films stored in 23, 33, 44, and 53% RH are given in **Tables 2** and **3**, respectively.

The crystallization mechanism can be indicated by *n* values. *n* values are affected by the dimensionality of crystal growth (one-, two-, or three-dimensional) and by the rate-limiting growth mechanism (diffusion controlled or phase-boundary controlled) (*22*, *23*). The relationship of the Avrami exponent to these effects is given by

$$n = (P/S) + Q$$

where *n* is the Avrami exponent, *P* is the indicator of dimensionality ($P = 1, 2, 3$), *S* is the indicator of rate-limiting growth ($S = 1$ for phase-boundary controlled, $S = 2$ for diffusion-controlled crystal growth), and *Q* is a constant ($Q = 0$ or 1).

Table 3. Rate Constants[a] for Sucrose Crystallization in Native WP, Denatured WP, and Denatured WP with Inhibitor Film Matrices Stored in Various Relative Humidity (RH) Environments at 25 °C

	matrix effect[b]		inhibitor effect[b]			
RH (%)	native	denatured	lactose	mod starch	PVP	raffinose
23	49.27a	4.98b	3.99b	3.31b	4.54b	3.02b
33	31.77a	5.11b	2.02b	3.95b	0.71b	0.77b
44	63.71a	9.35b	4.49b	5.11b	1.57b	2.66b
53	111.02a	113.50a	96.89a	25.04c	89.45ab	30.66bc

[a] Rate constants $\times 10^{-6}$ h^{-1}. [b] Letters within rows indicate values that are significantly different at the 95% level. Comparisons are made within rows only.

The results of this study produced n values that were fractional. It was reasoned that for sucrose crystallization in WPI film matrices, crystallization was diffusion controlled.

Statistical analysis of rate constants shown in **Table 3** found significant differences ($p < 0.001$) between the crystal growth kinetics of native and denatured WPI films in 23, 33, and 44% RH. Cross-linking the whey proteins through thermal denaturation decreased the rate constant by an order of magnitude. As relative humidity increased to 53%, the effect of cross-linking was lost and the difference between rate constants for the two films was insignificant, with values of 111.02 ($\times 10^{-6}$ h^{-1}) and 113.50 ($\times 10^{-6}$ h^{-1}) for the NAT and DEN films, respectively.

Relative Humidity Effect on Sucrose Crystallization. On the basis of moisture isotherms of whey protein and amorphous and crystalline sucrose, the water content of the films should increase with increasing relative humidity. Increased water content leads to increased free volume and decreased glass transition temperature of the material. DSC thermoscans of NAT and DEN film samples found T_g to be less than the storage temperature (25 °C) for 33, 44, and 53% RH. The measured T_g values for the DEN samples were 18, 7, and −5 °C for these storage conditions, respectively. The comparable NAT samples had T_g values of 4, −3, and −5 °C, respectively.

In past studies of binary systems of water and sucrose, rate constants for the transformation of amorphous sucrose to crystalline sucrose increased with the relative humidity of the storage conditions (*24*). As expected, there was an increase in rate constants for the NAT and DEN samples as storage relative humidity increased from 33 to 53% (**Table 3**). The increase in free volume by the plasticizing water was likely the predominant driving force for crystallization, leading to an increased rate constant.

There was no observable increase in rate constants of the WPI films stored in 33% RH compared 23% RH (**Table 3**). The gravimetric method for analyzing crystallization kinetics had the benefits of being easily accessible, inexpensive, and done in practical, applicable experimental conditions. However, complex structural changes cannot be explained with weight measurements. Methodology such as solid-state NMR could be used to verify state changes happening in films. Despite the disadvantages, the gravimetric method was still able to test the main hypothesis of this experiment. In storage conditions with <53% RH, the cross-linked, denatured WPI film matrix was a more effective control of sucrose crystallization.

Inhibitor Effect on Crystallization Kinetics. Figure 4 shows weight changes of denatured whey protein/sucrose films in the presence of sucrose crystallization inhibitors in the 23, 33, 44, and 53% RH storage environments. For clarity, only the averaged weight change for one replicate is shown for each variable. Weight changes were the average of the four samples cast per replicate. Statistical analysis determined that there were no significant differences between the replicates for all variables. **Tables 2** and **3** show the average n and k values for the denatured films with inhibitors. In all storage conditions, the control films (denatured WPI/sucrose films without inhibitor) had the largest rate constants. However, the efficacies of the inhibitors did not become statistically significant ($p < 0.001$) until the relative humidity increased to 53%. The rate constants for the control films and those inhibited with lactose, modified starch, PVP, and raffinose were 113.50, 96.89, 25.04, 89.45, and 30.66 ($\times 10^{-6}$ h^{-1}), respectively. The raffinose and starch molecules were the most successful at slowing sucrose crystallization in WPI films.

For denatured WPI/sucrose films stored in 23% RH, there was no significant difference in weight loss kinetics between the control film and the films with inhibitors. No crystal formation was visible during the entire storage period, although there was measurable weight loss. As the relative humidity increased, the effect of inhibitors became more observable. In 44% RH, there was a definite difference seen between rates of weight loss after 800 h of storage. It should be noted that at that time point, crystallization became visible on a macroscopic scale in the control, but no crystals were seen in the films with inhibitors for the entire storage time. In 53% RH, lactose and PVP were the least effective inhibitors and were insignificantly different from each other. Crystallization was visible in the control films after 200 h of storage. In the lactose- and PVP-inhibited films, visible crystallization was delayed until 400 h. Observable crystallization was delayed in the modified starch-inhibited and raffinose-inhibited films until at least 1500 h of storage. It should be noted that the differences in weight loss in the films may not only be because of differences in sucrose crystallization. Addition of inhibitors can change the equilibrium relative humidity (ERH) of the sucrose solution in the films. If the ERH is increased because of the addition of the inhibitors, less moisture would be lost from the film to reach equilibrium.

Past studies have investigated sucrose crystallization inhibitors in binary systems. Raffinose has been found to be an excellent inhibitor because of its mode of inhibition. Inhibitors interact with the surface of sucrose crystals. In the cases of lactose, starch, and PVP, these inhibitors adsorb onto the face of the growing crystal. Before new sucrose molecules can be incorporated into the crystal from the bulk solution, the inhibitor must first desorb and migrate away from crystal face. This slows crystal growth, but does not prevent it. Raffinose is a more effective inhibitor because it is physically incorporated into the growing sucrose crystal. A trisaccharide, raffinose can be thought of as a sucrose molecule with an extra galactose. When incorporated in the sucrose crystal, the galactose molecule creates a steric hindrance on that face of the sucrose crystal, preventing more growth on that side of the sucrose crystal. In addition to affecting bulk migration and creating steric hindrances, it is possible that the inhibitors slowed sucrose crystallization in the WPI films by changing the critical supersaturation point of sucrose. As reported in Hartel and Shastry (*25*), adding invert sugar to a sucrose solution increased the saturation concentration of the solution. A similar result could have occurred in these WPI/sucrose films.

At lower relative humidities, the matrix effect was found to be more important to slowing crystallization than incorporation of inhibitors. There was a significant difference between native and denatured WPI films plasticized with sucrose in 23, 33, and 44% RH; however, there was no difference among denatured films with and without inhibitors. As the relative

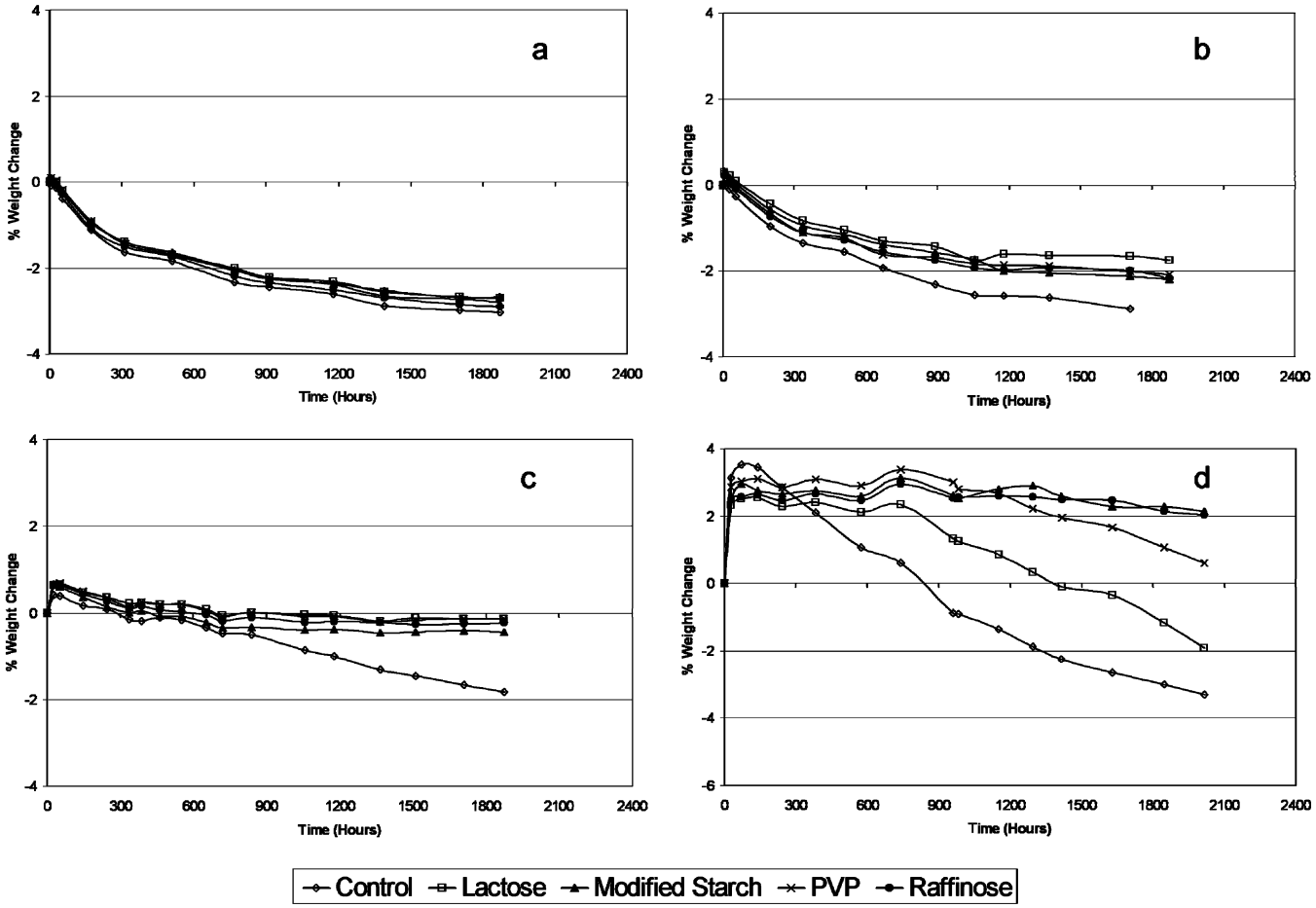

Figure 4. Change of film weight due to moisture adsorption and crystallization in denatured WPI film plasticized with sucrose, with and without inhibitors, stored in (**a**) 23% RH, (**b**) 33% RH, (**c**), 44% RH, and (**d**) 53% RH at 25 °C.

Table 4. Comparison of Rate Constants of Sucrose Crystallization in Various Biopolymer Systems Stored in 53% Relative Humidity

system	sucrose/ polymer ratio (dry basis)	rate order (*n* value)	rate constant (h^{-1})
100% sucrose[a] (freeze-dried)		1	1.1×10^{-1}
sucrose/guar gum[a]	76.9:23.1	1	9.7×10^{-3}
sucrose/CMC[a]	76.9:23.1	1	5.0×10^{-3}
sucrose/starch/CMC[a]	43.5:43.5:13	1	5.7×10^{-4}
sucrose/native WPI[b]	66.7:33.1	1.17	1.1×10^{-4}
sucrose/denatured WPI[b]	66.7:33.1	0.69	1.1×10^{-4}
sucrose/denatured WPI + raffinose[b]	67.5:25:7.5	0.65	3.1×10^{-5}

[a] Inglesias and Chirife (*26*). [b] This study.

humidity increased to 53%, the inhibitors became more important to controlling crystallization than the protein matrix.

A past study of delayed crystallization of amorphous sucrose in a freeze-dried model system by Iglesias and Chirife (*26*) looked at sucrose crystallization kinetics as affected by carbohydrate polymers. Sample preparation was different from that for the WPI films, but a similar technique of monitoring changes in moisture adsorption over time was used. For native and denatured WPI films stored in 53% RH, rate constants for sucrose crystallization were similar to those of freeze-dried sucrose combined with starch and carboxymethylcellulose (CMC). **Table 4** gives a comparison of rate constants of various amorphous sucrose−biopolymer systems.

Sucrose crystallization in WPI films has deleterious effects on the desirable properties of low oxygen permeability, high gloss, and good durability. However, the transition of amorphous to crystalline sucrose can be slowed by cross-linking the WP matrix through thermal denaturation. The sucrose crystallization rate constant in denatured films was significantly smaller than in native films when measured in 23, 33, and 44% RH. Sucrose crystallization can be further slowed by the addition of an inhibitor. Raffinose and modified starch were found to be the most effective inhibitors of sucrose crystallization in WPI films, especially in higher relative humidity conditions. Controlling sucrose crystallization in WP films will improve the applications of the WPI/sucrose system as a possible coating for dry foods.

LITERATURE CITED

(1) Sothornvit, R.; Krochta, J. M. Plasticizer effect on oxygen permeability of β-lactoglobulin films. *J. Agric. Food. Chem.* **2000**, *48*, 6298−6302.

(2) Lee, S. Y.; Dangaran, K. L.; Krochta, J. M. Gloss stability of whey protein/plasticizer coating formulations on chocolate surface. *J. Food Sci.* **2002**, *67* (3), 1121−1125.

(3) Dangaran, K. L.; Krochta, J. M. Aqueous whey protein coatings for panned products. *Manuf. Confect.* **2003**, *83* (1), 61−65.

(4) Minifie, B. W. *Chocolate, Cocoa and Confectionery: Science and Technology*, 2nd ed.; AVI Publishing: Westport, CT, 1982; p 735.

(5) Beckett, S. T. *The Science of Chocolate*; The Royal Society of Chemistry: Cambridge, U.K., 2000; p 175.

(6) Hartel, R. W. Controlling sugar crystallization in food products. *Food Technol.* **1993**, Nov, 99−107.

(7) Roos, Y.; Karel, M. Plasticizing effect of water on thermal behavior and crystallization of amorphous food models. *J. Food Sci.* **1991**, *56* (1), 38−43.

(8) Lipp, R. Selection and use of crystallization inhibitors for matrix-type transdermal drug-delivery systems containing sex steroids. *J. Pharm. Pharmacol.* **1998**, *50*, 1343−1349.
(9) Ma, X.; Taw, J.; Chiang, C.-M. Control of drug crystallization in transdermal matrix system. *Int. J. Pharm.* **1996**, *142*, 115−119.
(10) Hancock, B. C.; Zografi, G. Characteristics and significance of the amorphous state in pharmaceutrical systems. *J. Pharm. Sci.* **1997**, *88* (1), 1−12.
(11) Roos, Y. *Phase Transition in Foods*; Academic Press: San Diego, CA, 1995; p 360.
(12) Hartel, R. W. *Crystallization in Foods*; Aspen Publishers: Gaithersburg, MD, 2001; p 325.
(13) Fennema, O. Water and ice. In *Food Chemistry*, 3rd ed.; Fennema, O., Ed.; Dekker: New York, 1996; pp 17−94.
(14) Verhoyen, O.; Dupret, F.; Legras, R. Isothermal and non-isothermal kinetics of polyethylene terephthalate: mathematical modeling and experimental measurement. *Polym. Eng. Sci.* **1998**, *38* (9), 1594−1610.
(15) Slade, L.; Levine, H. Beyond water activity: recent advances based on alternative approach to the assessment of food quality and safety. *Crit. Rev. Food Sci. Nutr.* **1991**, *30* (2−3), 115−360.
(16) Otte, J.; Zakora, M.; Qvist, K. B. Involvement of disulfide bonds in bovine β-lactoglobulin B gels set thermally at various pH. *J. Food Sci.* **2000**, *65* (3), 384−389.
(17) Manderson, G. A.; Creamer, L. K.; Hardman, M. J. Effect of heat treatment on the circular dichroism spectra of bovine β-lactoglobulin A, B, and C. *J Agric. Food Chem.* **1999**, *47*, 4557−4567.
(18) Mathlouthi, M.; Reiser, P. *Sucrose, Properties and Applications*; Kluwer Academic: New York, 1994; p 320.
(19) Schmitt, E. A.; Law, D.; Zhang, G. G. Z. Nucleation and crystallization kinetics of hydrated amorphous lactose above the glass transition temperature. *J. Pharm. Sci.* **1999**, *88* (3), 291−296.
(20) McHugh, T. H.; Krochta, J. M. Plasticized whey protein edible films: water vapor permeability properties. *J. Agric. Food. Chem.* **1994**, *59* (2), 416−419.
(21) Iglesias, H. A.; Chirife, J. *Handbook of Food Isotherms: Water Sorption Parameters for Food and Food Components*; Academic Press: New York, 1982; p 347.
(22) Thomas, J. J.; Jenning, H. M. Effects of D_2O and mixing on the early hydration kinetics of tricalcium silicate. *Chem. Mater.* **1999**, *11*, 1907−1914.
(23) Starink, M. J. Kinetics equations for diffusion-controlled prcip-tation reactions. *J. Mater. Sci.* **1997**, *32*, 4061−4070.
(24) Makower, B.; Dye, W. B. Equilibrium moisture content and crystallization of amorphous sucrose and glucose. *J. Agric. Food Chem.* **1956**, *4*, 72−81.
(25) Hartel, R. W.; Shastry, A. V. Sugar crystallization in food products. *Crit. Rev. Food Sci. Nutr.* **1991**, *30* (1), 49−112.
(26) Iglesias, H. A.; Chirife, J. Delayed crystallization of amorphous sucrose in humidified freeze dried model systems. *J. Food Technol.* **1978**, *13*, 137−144.

Received for review April 25, 2006. Revised manuscript received July 6, 2006. Accepted July 28, 2006. We are grateful for the support provided for this research by the California Milk Advisory Board and Dairy Management, Inc., through the California Dairy Research Foundation and the California Dairy Foods Research Center.

JF0611670