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Storage Stability of a Commercial Hen Egg Yolk Powder in Dry and Intermediate-Moisture Food Matrices

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ABSTRACT: Quality loss in intermediate-moisture foods (IMF) such as high-protein nutrition bars (HPNB) in the form of hardening, nonenzymatic browning, and free amino group loss is a general concern for the manufacturers. To measure the extent of quality loss over time in terms of these negative attributes, through changing the ratio by weight between two commercial spray-dried hen egg powders, egg white (DEW) and egg yolk (DEY), the storage stability of 10 IMF systems (water activity (a_w) ~ 0.6) containing 5% glycerol, 10% shortening, 35% protein, and 50% sweetener (either maltitol or 50% high-fructose corn syrup/50% corn syrup (HFCS/CS)) were studied. Additionally, the storage stability of the DEY powder itself was investigated. Overall, during storage at different temperatures (23, 35, and 45 °C), the storage stability of DEY in dry and IMF matrices was mainly controlled by the coaction of three chemical reactions (disulfide bond interaction, Maillard reaction, and lipid oxidation). The results showed that by replacing 25% of DEW in an IMF model system with DEY, the rate of bar hardening was significantly lower than that of the models with only DEW at all temperatures due to the softening effect of the fat in DEY. Furthermore, the use of maltitol instead of HFCS/CS in all bar systems not only resulted in decreased hardness but also drastically decreased the change in the total color difference (ΔE^*). Interestingly, there was no significant loss of free amino groups in the maltitol systems at any DEW/DEY ratio.

KEYWORDS: *egg white, egg yolk, protein aggregation, water activity, storage stability, Maillard reaction, disulfide bond interaction, lipid oxidation, intermediate-moisture food, high-protein nutrition bars*

INTRODUCTION

Driven by consumer desire to follow healthier lifestyles as well as the convenient appeal and functionality of high-protein nutrition bars (HPNB), total U.S. retail sales have soared from $0.9 billion in 2006 to $1.7 billion in 2011, with an average predicted annual growth of 10.8% from 2006 to 2016.[1] HPNB is an intermediate-moisture food (IMF) having a water activity (a_w) between 0.6 and 0.85 at room temperature.[2]

One major problem for HPNB is bar hardening during storage without moisture loss.[3−6] Several mechanisms for bar hardening have been studied. These include (1) disulfide-induced protein aggregation,[7−10] (2) Maillard-induced protein polymerization,[9−12] (3) moisture migration,[13−16] and (4) phase separation.[15,17]

To control bar hardening in HPNB, several solutions are being used by the bar industry: (1) substituting protein hydrolysates for some intact proteins, which serves as a plasticizer to decrease the glass transition temperature (T_g) of the bar matrix;[10,18] (2) addition of cysteine, a food grade additive, into the bar matrix to prevent disulfide-induced aggregation;[19] and (3) using non-reducing sugar alcohols such as maltitol as a humectant to minimize Maillard-induced protein polymerization.[18] In this study, the feasibility of a potential approach for reducing bar hardening, that is, substituting some protein ingredients with protein sources containing high fat content such as hen egg yolk, was investigated. Additionally, the extent of quality loss over time in terms of color change, hardening, and free amino group loss in the protein bar model systems (a_w ~ 0.6) was analyzed.

In these bar model systems, two commercial spray-dried hen egg powders, egg white (DEW) and egg yolk (DEY), were used. The physicochemical properties of DEW have been systematically analyzed in our previous studies.[9,10,20] Briefly, although the glucose content of DEW was undetectable (Table 1),[20] it has been confirmed that DEW contains the early nonenzymatic glycosylation products, which are produced during desugarization and heat pasteurization processing.[9] These products can further undergo additional inter- and intramolecular rearrangements to produce a heterogeneous group of irreversibly bound and cross-linking moieties and eventually change the color of the products during advanced stages of the Maillard reaction.[9,10] In addition, through studying a simple DEW/water dough model system, moisture-induced aggregates were found to be produced by two chemical reactions during storage: disulfide bond interaction and the Maillard reaction.[9] As to DEY, before the DEW/DEY bar model systems were studied, several physicochemical properties of DEY powder related to storage stability such as moisture sorption isotherm, glucose content, solid fat index (SFI), protein solubility, and degree of protein aggregation, were analyzed in this study. As expected, these intrinsic characteristics of DEW and DEY powders certainly will affect the storage stability of the DEW/DEY bar model systems.

MATERIALS AND METHODS

Materials. Two spray-dried hen egg powders, dried egg whites (DEW, H227) and dried egg yolks (DEY, H483), were obtained from Deb-El Food Products, LLC (Elizabeth, NJ, USA). After receipt of both commercial powders, several sample characteristics were analyzed. The initial moisture content was determined using the Aquatest cma Karl

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Table 1. Characteristics of Two Commercial Spray-Dried Hen Egg Powders

	spray-drying conditions[a]		pasteurization[b]						
sample[c]	pressure (MPa)	inlet temp (°C)	temp (°C)	holding time	protein (% dry basis)	fat (% dry basis)[d]	glucose (% dry basis)	a_w	moisture content (% dry basis)
DEW	21.4	185	60	7 days	81	0	ND[e]	0.38	8.6
DEY	21.4	185	62	4 min	36	60	0.21	0.27	2.1

[a]Temperatures are adjusted depending on outside humidity and desired powder granulation. [b]Both egg powders were pasteurized according to the USDA regulations. DEW was pasteurized after spray-drying in a heat treatment room. DEY was pasteurized before spray-drying as liquid eggs. [c]Two spray-dried hen egg powders, dried egg whites (DEW, H227) and dried egg yolks (DEY, H483), were obtained from Deb-El Food Products, LLC (Elizabeth, NJ, USA). [d]Calculated on the basis of the data obtained from the USDA National Nutrient Database for Standard Reference.[50] [e]ND, not detected using the Glucose (HK) Assay Kit (Sigma-Aldrich Co., St. Louis, MO, USA).

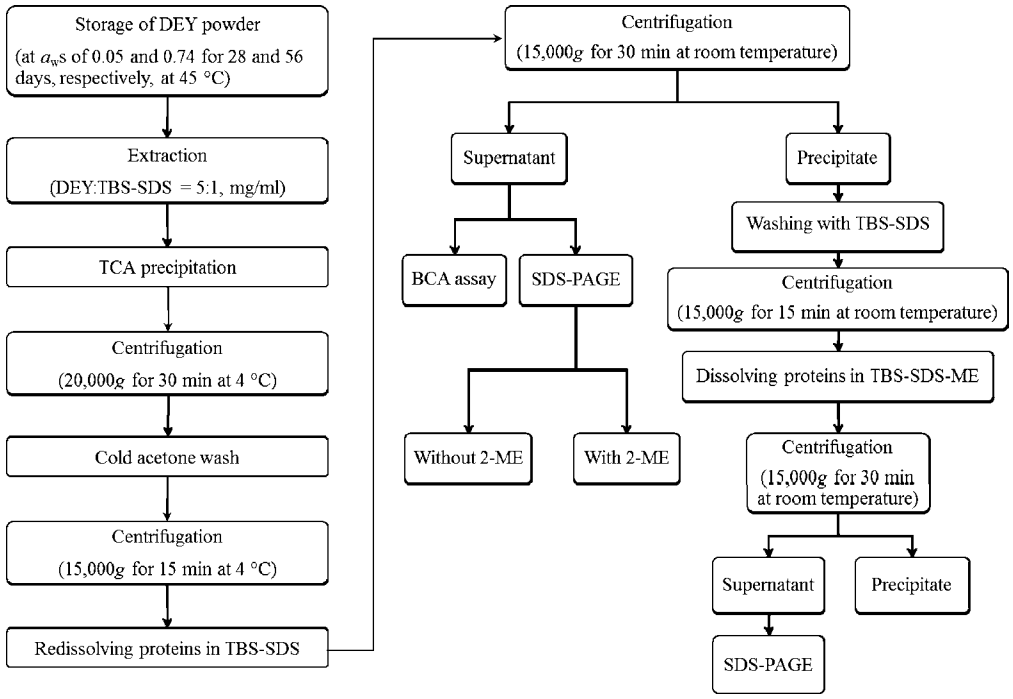

Figure 1. Schema of the analysis of buffer-soluble DEY proteins.

Fischer Coulometric Titrator (Photovolt Co., Minneapolis, MN, USA). The a_w was determined using the AquaLab 3TE water activity meter (Decagon Devices, Inc., Pullman, WA, USA). Protein content was determined according to the Dumas combustion method using a nitrogen determinator, TruSpec N (LECO Corp., St. Joseph, MI, USA). The specific factor for the conversion of nitrogen content to egg protein content is 6.25. The partial process information and selected initial physicochemical properties of DEW and DEY are summarized in Table 1. Both products were kept at −20 °C until used.

Other food grade ingredients for protein bar model systems were shortening (Crisco All-Vegetable Shortening, The J. M. Smucker Co., Orrville, OH, USA), glycerol (Superol Veg Glycerin USP, FCC, The Procter & Gamble Co., Cincinnati, OH, USA), HFCS (ISOSWEET 100 High Fructose Corn Syrup (HFCS), Tate & Lyle, Decatur, IL, USA), CS (STALEY 1300 Corn Syrup (CS), Tate & Lyle), and maltitol (SweetPearl P200, Roquette America, Inc., Keokuk, IA, USA).

Moisture Sorption Isotherms of DEY. To generate the moisture sorption isotherm of DEY at 25 °C, two methods built into the AquaLab Vapor Sorption Analyzer (Decagon Devices), namely, the dynamic vapor sorption (DVS) method for static isotherm and the dynamic dewpoint isotherm (DDI) method for dynamic isotherm, were used. The Guggenheim−Anderson−de Boer (GAB) isotherm model was used to describe the dry basis (db) moisture content (m) as a function of a_w.[21]

SFI of DEY. SFI, the ratio of solid fat content in a fat sample at a given temperature, is generally measured as the change in specific volume with respect to temperature.[22] The SFI of DEY was measured using a differential scanning calorimetry (DSC), DSC1 STAR^e system (Mettler-Toledo, LLC, Columbus, OH, USA). The sample was cycled through a heat−cool−reheat cycle, during which it was heated from −50 to 80 °C at a rate of 10 °C/min, cooled to −50 °C at 10 °C/min, and then reheated at 10 °C/min back to 80 °C. The melting endotherm of the fat during the second heating segment was analyzed to determine the SFI values using STAR^e software (version 11.00a, Mettler-Toledo).

Glucose Content of DEY. During storage at 23 °C (a_w values of 0.05 and 0.64) and 45 °C (a_w values of 0.05 and 0.53), the change in glucose content of DEY was measured at designated time intervals by an enzymatic method using the Glucose (HK) Assay Kit (Sigma-Aldrich Co., St. Louis, MO, USA). The change in glucose content of DEY was analyzed using an apparent first-order hyperbolic model. The initial glucose content of DEW was measured using the same method in our previous study.[20] It must be noted that only water-extractable glucose can be measured using this commercial kit.

Analysis of Buffer-Soluble DEY Proteins. After storage in darkness at 45 °C ($a_w$values 0.05 and 0.73) for 28 and 56 days, respectively, the solubility of DEY proteins in TBS-SDS [TBS containing 1% SDS (g/mL)] was determined using the method described in our previous study[9] with modifications (Figure 1). Briefly, a certain amount of powder sample (25 mg) was added into 5 mL of TBS-SDS. The mixture was mixed thoroughly using a vortex mixer (Henry Troemner LLC, Thorofare, NJ, USA) for 1 min at 3000 rpm. The suspension was then shaken on a shaker (Thermo Fisher Scientific Inc., Rockford, IL, USA) at 200 rpm for 2 h. The protein in the suspension was precipitated using a trichloroacetic acid (TCA) method. Briefly, the

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Table 2. Sample Information of Different DEW/DEY Bar Model Systems

ingredient (%, g/g)	X0W100Y	X25W75Y	X50W50Y	X75W25Y	X100W0Y
DEW (W)	0	8.75	17.5	26.25	35
DEY (Y)	35	26.25	17.5	8.75	0
sweetener (X[a])	50	50	50	50	50
shortening	10	10	10	10	10
glycerol	5	5	5	5	5
total	100	100	100	100	100

[a]X indicates that the protein bar model contained either H (HFCS/CS: 50% HFCS and 50% CS, g/g) or M (maltitol) as the sweetener.

volume of the protein extract was adjusted to a final concentration of 20% (mL/mL) TCA using the 100% (g/mL) TCA stock solution. The mixture was then incubated on ice for 30 min. After centrifugation at 20000*g* for 30 min at 4 °C, the supernatant was discarded. To remove the TCA, the pellet was washed three times with 1 mL of cold acetone. For each wash, the mixture was mixed thoroughly using a vortex mixer (Henry Troemner) for 1 min at 3000 rpm. After centrifugation at 15000*g* for 15 min at 4 °C, the liquid portion was removed carefully. After three washes, the precipitate was held in a fume hood for 10 min for acetone evaporation and then mixed with 5 mL of TBS-SDS. After thorough mixing using a vortex mixer (Henry Troemner) for 1 min at 3000 rpm, the mixture was then shaken on a shaker (Thermo Fisher Scientific) at 200 rpm overnight. After centrifugation at 15000*g* for 30 min, the protein concentration in the supernatant was determined using the Pierce BCA (bicinchoninic acid) Protein Assay Kit (Thermo Fisher Scientific). Bovine serum albumin (BSA) was used as the protein standard (working range 20−2000 μg/mL). The relative protein solubility (RPS, %) compared with that of the original DEY at day 0 was calculated.

Simultaneously, the precipitate, that is, TBS-SDS buffer-insoluble proteins, was washed three times with 1 mL of TBS-SDS. For each wash, the mixture was mixed thoroughly using a vortex mixer (Henry Troemner) for 1 min at 3000 rpm. After centrifugation at 15000*g* for 15 min, the liquid portion was removed carefully. After three washes, the precipitate was then mixed with 3 mL of TBS-SDS containing 5% 2-mercaptoethanol (mL/mL, TBS-SDS-ME). The mixture was heated in a water bath (95 °C) for 5 min. After centrifugation at 15000*g* for 30 min, the supernatant was carefully collected for SDS-PAGE analysis. The control, the original DEY stored at −20 °C, was prepared using the same procedure. All steps were carried out at room temperature unless otherwise specified. The buffer-soluble proteins from both the supernatant and the precipitate were analyzed using SDS-PAGE according to the method of Laemmli.[23] The relevant procedure was mentioned in our previous study.[9]

Preparation of the DEW/DEY Bar Model Systems. To study the effect of temperature and humectant on DEW/DEY bar model systems, 10 formulations of DEW/DEY bar model systems were prepared (Table 2). Briefly, three ingredients (sweetener, shortening, and glycerol) were mixed at low speed for 1 min and then at medium-low speed for 1 min in a mixer (KitchenAid, St. Joseph, MI, USA) using the flat beater attachment. The DEW and/or DEY powders were then slowly added in portions with the mixer on low speed to prevent spattering. The resulting dough was further mixed at low speed for 1.5 min followed by a hand kneading for 2 min. The finished dough was then sealed in a plastic bag (Thermo Fisher Scientific) and kept at 4 °C for 1 day for moisture equilibration. The batch size prepared for each formula was about 1 kg.

Before packing, the moisture-equilibrated dough was kept at room temperature for at least 2 h. The resultant dough (∼9 g) was weighed and pressed into a plastic disposable sample cup (Decagon Devices) and then immediately covered with the lid (Decagon Devices), sealed with Parafilm, and placed into glass canning jars. The packaged samples were stored at three temperatures (23, 35, and 45 °C) for up to 260 days. The samples were removed at designated time intervals and cooled at room temperature for at least 2 h before being analyzed immediately or frozen at −45 °C for further analysis.

Physicochemical Changes in the Bar Model Systems. During storage, several physicochemical properties of the bar model systems were analyzed. First, the a_w of the samples was determined using the AquaLab 3TE Water Activity Meter (Decagon Devices). Second, the total color difference (ΔE^* value) of the samples was analyzed using the Minolta Chroma Meter CR-200 (Minolta Camera Co., Osaka, Japan). ΔE^* is a single dynamic parameter that takes into account the differences between the three color coordinates (L^*, a^*, and b^*) of the sample as compared to the same sample at zero time at room temperature (eq 1). Third, the degree of hardness of the samples was analyzed using the TA.XT*Plus* Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA) fitted with a flat-ended cylinder stainless steel probe (3 mm diameter). All relevant methods are described in detail in our previous studies.[20,24] Additionally, reaction kinetics related to two dynamic parameters, ΔE^* and hardness, were analyzed using an apparent first-order hyperbolic model and an apparent zero-order model, respectively, which are described in our previous studies.[9,24] The activation energy (E_A) related to quality changes in different bar model systems was also calculated using the Arrhenius equation.[25]

$$\Delta E^* = \sqrt{(L^*_{\text{sample}} - L^*_{\text{standard}})^2 + (a^*_{\text{sample}} - a^*_{\text{standard}})^2 + (b^*_{\text{sample}} - b^*_{\text{standard}})^2} \quad (1)$$

Analysis of Remaining Free Amino Groups in the Bar Model Systems. During storage at 35 and 45 °C, the effect of temperature and humectant on the loss of free amino groups in the bar model systems was measured using the *o*-phthalaldehyde (OPA) spectrophotometric method[26] with modifications. Briefly, a small amount of HPNB sample was mixed with 5 mL of 2% (g/mL) SDS and then stirred on a magnetic stir plate at 500 rpm for 30 min. The amount of protein bar sample added was based on the amount needed to yield ∼10 μg/μL protein in the suspension. The suspension was incubated overnight at room temperature. The OPA reagent was freshly prepared according to the procedure of Goodno et al.[27] For spectrophotometric determination of the free amino groups, each sample (100 μL) was added to 4 mL of OPA reagent in a glass test tube. After brief vortexing, the solution was incubated for 2 min at room temperature followed by another brief vortex. The resulting sample (1.5 mL) was added into a disposable cuvette (BRAND GmbH & Co. KG, Wertheim, Germany) and then was measured at 340 nm in a spectrophotometer (Biochrom Ltd., Cambridge, U.K.). The absorbance was plotted against a standard curve prepared from a zero day sample of the bar.

Statistical Analysis. Each sample condition was tested at least in duplicate at each time. For reaction kinetics analysis, a 95% confidence interval was used to find the best-fit value of the parameters. To compare the change in the buffer-soluble DEY proteins during storage, one-way ANOVA with Dunnett's multiple-comparisons test was performed. The Pearson correlation between color change and hardness change in the same bar model system was measured. $P < 0.05$ was considered to be statistically significant. The software GraphPad Prism for Windows (version 6.02, GraphPad Software, La Jolla, CA, USA) was used to analyze the data.

RESULTS AND DISCUSSION

Moisture Sorption Isotherms of DEY. Two moisture sorption isotherms of DEY at 25 °C, static and dynamic, were generated using the GAB model (Figure 2). There was no

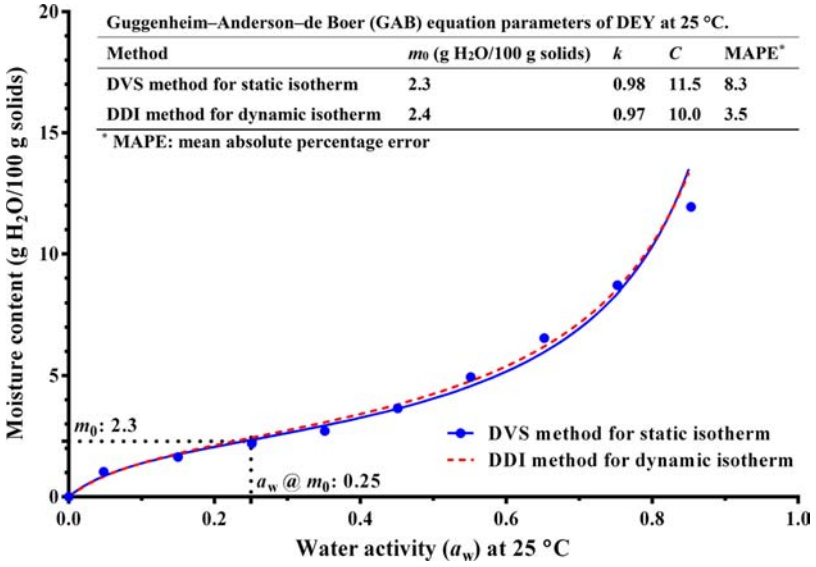

Figure 2. Moisture sorption isotherms of a commercial spray-dried hen egg yolk powder (DEY) generated from the GAB model. The dotted lines indicate the GAB m_0 and the relevant a_w of DEY.

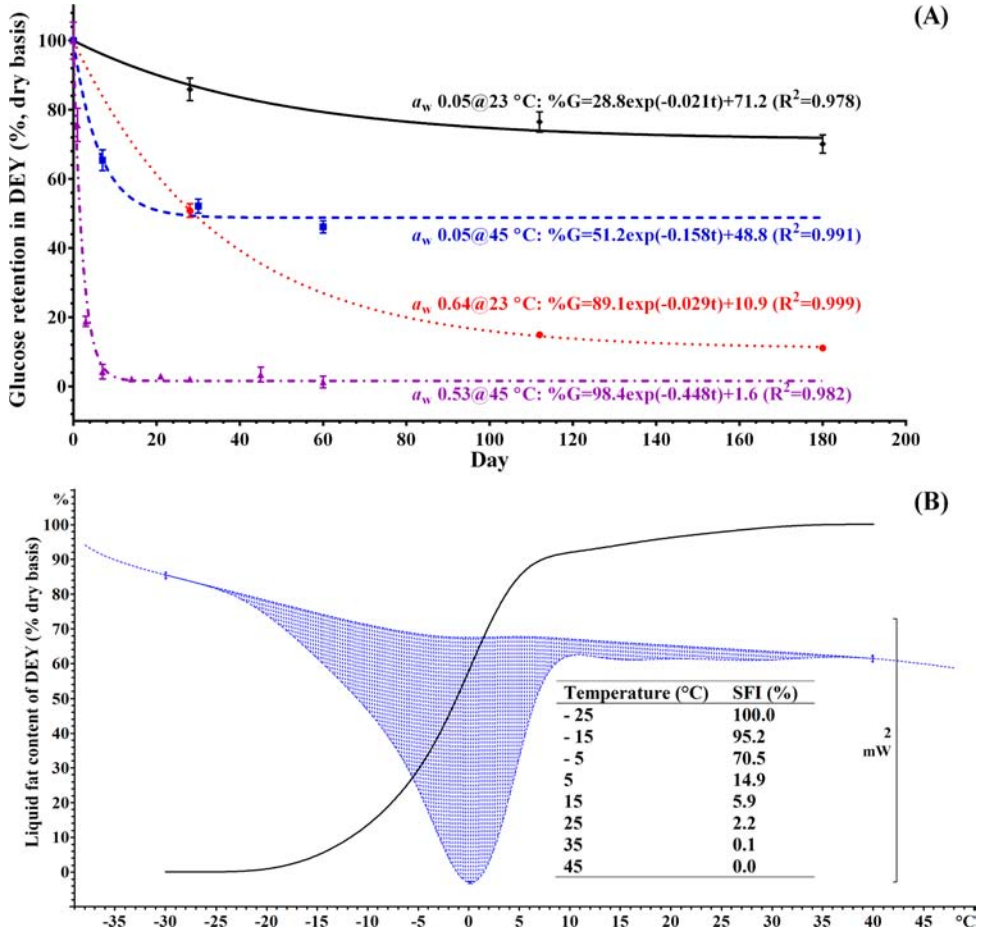

Figure 3. (A) Glucose retention in DEY as a function of storage time. (B) liquid fat content in the initial DEY as a function of temperature. The blue dotted area indicates the total area of fat melting. SFI, solid fat index.

significant difference ($P > 0.05$) between these two isotherms (Figure 2). DEY shows the typical type II isotherm (sigmoidal curve) for proteins. The MAPE values of both methods are small (Figure 2), indicating that the GAB model has an excellent fit to the measured data.[28] To maximize its shelf life, the optimal moisture content of DEY should be at its GAB monolayer moisture value (m_0, ~2.3 g H_2O/100 g solids), at which its a_w is about 0.25 at 25 °C (Figure 2). Actually, the initial moisture content of DEY is very close to its m_0 (Table 1). A type II moisture sorption isotherm of DEW at 23 °C using the GAB

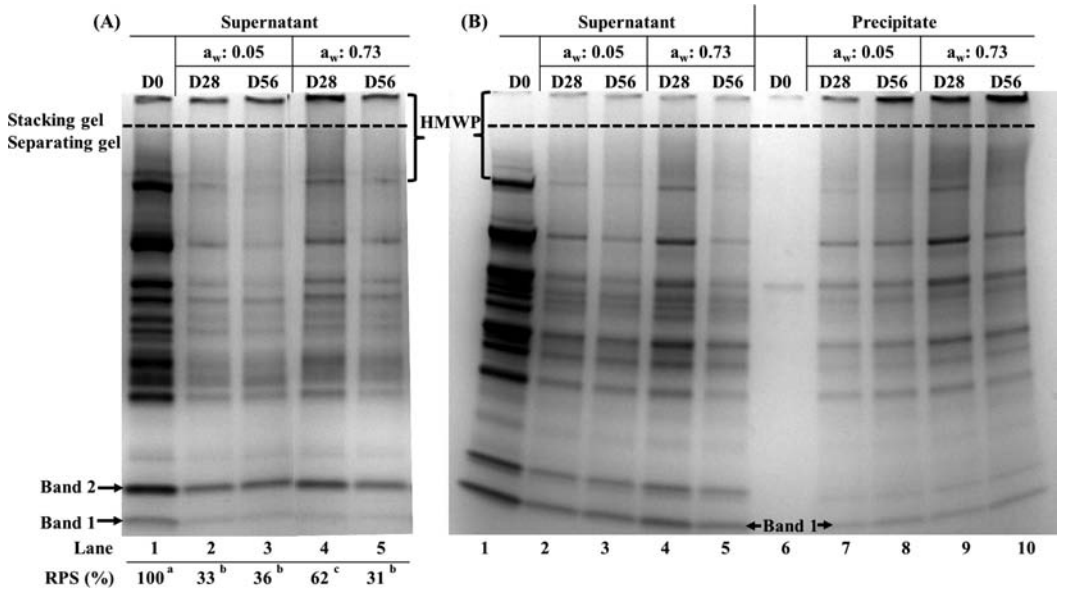

Figure 4. SDS-PAGE of the buffer-soluble proteins in DEY powder stored at 45 °C in the absence (A) and presence (B) of 2-mercaptoethanol. The relative protein solubility (RPS, %) of the TBS-SDS buffer-soluble proteins compared with that of DEY at day 0 is labeled in (A). Values with different letters (a–c) are significantly different ($P < 0.05$). Band 1 indicated in (A) and (B) contains apovitellenin-I (9 kDa), and band 2 indicated in (A) contains its homodimer formed with a disulfide bond. HMWP, high molecular weight proteins (>200 kDa); D0, at day 0; D28, at day 28; D56, at day 56. The dashed line is the boundary of the stacking gel and the separating gel.

model has been published in our previous study.[20] It should be noted that DEY holds less water at any a_w compared with DEW. This is due to the fact that DEY has about 60% of the solids as lipid, which holds little water (Table 1).

Effect of Moisture on the Change in Glucose Content of DEY during Storage. The initial water-extractable glucose content of DEY is 0.21% (db, Table 1). During storage at different $a_w$values and temperatures, the amount of glucose in DEY decreased as a function of time and followed first-order kinetics (Figure 3A). This reduction is the result of two factors. One is physical. As seen in Table 1, the total fat content of DEW is negligible, whereas it is about 60% (db) in DEY. About 64% of the fats in DEY are unsaturated fatty acids such as oleic acid and linoleic acid.[29] Its SFI is 2.2 and 0% at 25 and 45 °C, respectively (Figure 3B), indicating that at least 97% of the fats in DEY are in their liquid form during storage at or above room temperature and can act as a solvent and mobilizing agent for glucose.[30] Therefore, the ratio between the remaining water-extractable glucose and the lipid-dissolved glucose should decrease over storage time until an equilibrium is reached. As expected, the lipid-dissolved glucose cannot be easily extracted with water. The other reason is chemical. It is well-known that glucose as a reducing sugar can react with amino groups such as lysine to produce various cross-linked products during storage, that is, the Maillard reaction (nonenzymatic browning, NEB).[31] This chemical reaction can further decrease the amount of water-extractable glucose in DEY during storage.

According to the food stability map,[32] when the a_w is smaller than that of its m_0, that is, at an a_w of 0.05, the Maillard reaction is stopped due to the lack of moisture as a reaction medium. Thus, lipids act as the solvent phase and dominate the decrease of glucose content of DEY over storage time (Figure 3A). When the a_w is greater than that of its m_0, the moisture is adsorbed into the dry system and functions as a solvent for the dissolution of reactants, resulting in a faster reaction rate for the Maillard reaction.[33] Therefore, at the a_w values of 0.64 at 23 °C and 0.53 at 45 °C, the decrease of glucose content of DEY was the coaction of both the physical and the chemical reasons (Figure 3A). As an example, compared with that at the a_w of 0.05, the water-extractable glucose in DEY decreased about 59% for the system at a_w of 0.64 after 180 days of storage at 23 °C (Figure 3A). Similar results have been found in a model system consisting of glucose (10%, g/g), monosodium glutamate (MSG, 11.8%, g/g), and lipids (8%, g/g) during storage.[30] Overall, the molecular mobility of food components not only depends on the physical state of the food but also is influenced by water. In addition, water can affect the reaction's sensitivity to temperature. From Figure 3A, the loss rate of the water-extractable glucose in DEY increased when the storage a_w and/or temperature increased.

Storage Stability of DEY Proteins. To study the effect of moisture on the storage stability of DEY proteins, an accelerated storage study at 45 °C was conducted at two a_w values (0.05 and 0.73). After 28 and 56 days of storage, the profiles of the TBS-SDS buffer-soluble proteins (supernatant) were analyzed using SDS-PAGE (lanes 2–5, Figure 4A,B). The TBS-SDS buffer-insoluble proteins (precipitate) were further extracted with the same buffer containing 2-mercaptoethanol (TBS-SDS-ME). The profiles of the TBS-SDS-ME buffer-soluble proteins were also analyzed using SDS-PAGE (lanes 7–10, Figure 4B). It is well-known that 2-ME is a strong reductant which can cleave the inter- and intramolecular disulfide bonds in the aggregates (soluble and insoluble).[9] In the presence of both SDS and 2-ME, both noncovalent (hydrophobic) and disulfide bond interactions can be destroyed.

For the control, the original DEY at day 0, its TBS-SDS buffer-soluble protein patterns (lane 1, Figure 4A,B) are similar to those in other studies.[34,35] Compared with that of hen egg white, the protein composition of hen egg yolk is more complex.[9,35] It has been reported that 119 proteins from hen egg yolk had been identified.[36] For more detailed egg yolk protein information such as protein name and its molecular weight, the readers can refer to several relevant studies.[34−36] Additionally, no visible TBS-SDS

Table 3. Reaction Rate Constant (k) of the Changes in Color and Hardness in Different DEW/DEY Dough Systems and the Pearson Correlation Coefficients (r) between Color Change and Hardness Change in the Same Dough System

		color (ΔE^* value),[a] $k \times 10^2$ (day^{-1})			hardness,[a] $k \times 10^2$ (day^{-1})			Pearson correlation coefficients (r, $P < 0.05$)		
bar system	water activity ($a_w \pm$ STD)[b]	23 °C	35 °C	45 °C	23 °C	35 °C	45 °C	23 °C	35 °C	45 °C
M0W100Y	0.624 ± 0.016	N/A[c]	6.8ax	6.0ax	0.2ax	0.7ay	1.1ay	0.425	0.774	0.961
M25W75Y	0.626 ± 0.015	N/A	4.7ax	11.7ax	0.3ax	1.1by	1.5ay	0.539	0.744	0.906
M50W50Y	0.612 ± 0.011	N/A	2.5ax	9.4ax	0.6bx	1.8by	1.8axy	0.411	0.846	0.889
M75W25Y	0.609 ± 0.008	N/A	4.1ax	13.6ax	1.3bx	2.2bx	3.3ax	0.746	0.784	0.940
M100W0Y	0.593 ± 0.013	N/A	2.2ax	19.8ay	2.6bx	6.3cx	7.0ax	0.815	0.890	0.863
H0W100Y	0.668 ± 0.010	1.8ax	3.8ay	9.3az	0.1ax	0.7ay	1.3az	0.938	0.743	0.801
H25W75Y	0.661 ± 0.011	2.4ax	3.0ax	8.6ay	0.1ax	1.6by	3.0bz	0.856	0.636	0.650
H50W50Y	0.655 ± 0.012	2.6ax	2.4ax	6.9by	0.1ax	3.2cy	6.2cz	0.739	0.635	0.648
H75W25Y	0.646 ± 0.013	1.7ax	3.1ay	6.3bz	0.2bx	7.5dy	14.9dz	0.810	0.629	0.658
H100W0Y	0.636 ± 0.018	1.8ax	5.6by	10.5az	0.8cx	14.3ey	30.6ez	0.767	0.480	0.580

[a]For the same sweetener system, values in the same column with different letters (a−e) are significantly different ($P < 0.05$). For the same dynamic parameter, values in the same row with different letters (x−z) are significantly different ($P < 0.05$). [b]a_w is the average of the a_w values measured at designated time intervals ($n = 37$) during storage at three temperatures. [c]N/A, not available.

buffer-insoluble precipitate was observed. However, after extraction with the TBS-SDS-ME buffer, a weak protein band (~78 kDa) was found in its TBS-SDS buffer-insoluble precipitate (lane 6, Figure 4B). This protein may be ovotransferrin from egg white, which has 15 disulfide groups,[37] and/or apovitellin.[35] This assumption needs to be further confirmed using other methods such as immunoblotting.

In the gel portion containing the high molecular weight proteins (HMWP, >200 kDa), especially in the stacking gel, the band color intensity of all supernatant samples in the absence of 2-ME (lanes 1−5, Figure 4A) decreased dramatically after the addition of 2-ME to the same sample (lanes 1−5, Figure 4B). Compared with the change in the control (lane 1, Figure 4A,B), it is clearly shown that the TBS-SDS buffer-soluble disulfide-induced protein aggregates were formed during storage at both a_w values. On the other hand, compared with the pattern of the precipitate from the control in the presence of 2-ME (lane 6, Figure 4B), it is confirmed that many different proteins in DEY were involved to form the TBS-SDS buffer-insoluble disulfide-induced protein aggregates during storage at both a_w values (lanes 7−10, Figure 4B). One typical example is apovitellenin-I (9 kDa, primary accession number P02659), which is one of the low-density lipoproteins (LDL) in hen egg yolk and has a disulfide-linked homodimer.[38] In the absence of 2-ME, for different storage samples (lanes 2−5, Figure 4A), the color intensity of the band containing the homodimer of apovitellenin-I (band 2) is much greater than that of the band containing its monomer (band 1). However, when its intermolecular disulfide bond was destroyed by 2-ME, the color intensity of band 1 (lanes 2−5, Figure 4B) increases dramatically. Additionally, band 1 was observed in the precipitates of all storage samples (lanes 7−10, Figure 4B), indicating that apovitellenin-I was involved in the formation of the TBS-SDS buffer-insoluble disulfide-induced aggregates during storage.

As previously discussed, when the moisture content of DEY is greater than its m_0, the amino groups can react with glucose to produce the Maillard cross-linked products during storage. From Figure 3A, after 7 days of storage at 45 °C, the water-extractable glucose in DEY decreased about 61% at an a_w of 0.53 compared with that at an a_w of 0.05.

Besides disulfide bond interaction and the Maillard reaction, lipid oxidation can also affect the storage stability of DEY due to its high unsaturated fat content (38%, g/g). The effect of moisture on lipid oxidation has been studied extensively.[2,39,40] In general, as a_w increases from the dry state, the reaction rate of lipid oxidation decreases to a minimum just above the m_0. Additional moisture adsorption above the m_0 promotes an increase in reactivity. Two types of lipid oxidation reactions, namely, radical reactions and reactions involving the secondary products, can cause both rancidity and free radical attack on proteins and amino acids.[41] This protein damage caused by oxidized lipids includes (1) formation of various fluorescent chromophores and aggregates (lipid−protein adducts and protein−protein cross-links), (2) protein scission, and (3) amino acid damage.[42] Additionally, both lipid oxidation and the Maillard reaction pathways (1) can produce common intermediate products such as carbonyl compounds, which results in increased browning, and (2) may have common polymerization mechanisms that lead to hardening.[43]

In summary, on the basis of the characteristics of three chemical reactions, when the moisture content of DEY is smaller than its m_0, such as that at an a_w of 0.05, two reactions, that is, disulfide bond interaction and lipid oxidation, can affect its storage stability. Otherwise, this list extends to the Maillard reaction such as those at the a_w values of 0.53 and 0.74. Due to the coaction of these three chemical reactions and noncovalent interactions such as hydrophobic interactions, different types and amounts of aggregates (soluble and insoluble) formed in DEY powder under different storage conditions. As expected (Figure 4A), the relative protein solubility of all storage samples (lanes 2−5) decreased significantly ($P < 0.05$) compared with that of the original DEY (lane 1). It must be noted that the TBS-SDS-ME buffer-insoluble proteins was visually observed in each storage sample. These precipitates are most likely produced by the Maillard reaction and/or lipid oxidation during storage and need further study. To further study the lipid oxidation-induced aggregates formed during storage, the major lipoprotein in hen egg yolk, namely, LDL, has been extracted from hen egg yolk and is currently under investigation in our laboratory.

Color Change in the DEW/DEY Bar Model Systems. From column 2 in Table 3, for the DEW/DEY bar model systems containing the same sweetener, the a_w decreased slightly with increasing ratio of DEW/DEY in the formulation. According to Raoult's law, the a_w of the aqueous ideal solution depends only on the total moles of solute molecules such as water-soluble proteins, sugars, and sugar alcohols. The larger the total moles of

the solute, the greater its a_w-lowering effect per unit of weight dissolved.[44] In the DEW/DEY bar model systems, when the percentage of DEW increased from 0 to 100%, the total moles of the water-soluble proteins actually increased because the total protein content of DEW is about 2.3 times higher than that of DEY (Table 1). Similar results were also observed in several dough systems containing DEW and its hydrolysates.[10]

In general, the color change in the DEW/DEY bar model systems is mainly due to the coaction of two reactions during storage: the Maillard reaction and lipid oxidation. Zamora and Hidalgo[43] have published an excellent review on the interactions in the NEB produced as a consequence of both reactions. For the Maillard reaction, one of the reactants, glucose (reducing sugar), has been found in two ingredients: the egg powders and the sweeteners. It must be noted that maltitol used as a nonreducing sweetener in this study still contains about 0.02% (g/g) reducing sugars. As an example, the color of two bar model systems, H50W50Y and M50W50Y, darkened during storage at different temperatures (Figure 5). Due to a much greater amount of reducing sugars in H50W50Y, after 98 days of storage at 45 °C, its L^* value decreased about 73%, whereas it was reduced by only 45% in M50W50Y (Figure 5C). For lipid oxidation, lipid comes from two ingredients: DEY and shortening. The shortening used in this study contains 18% (g/g) fat, of which 71% is unsaturated fat. However, the relationship between the Maillard reaction and lipid oxidation during color change in these bar model systems still needs to be further studied.

During storage, the total color difference (ΔE^*) increased in all systems until an equilibrium was reached, following an apparent first-order hyperbolic model (Table 3 and Figure 5). However, the change in ΔE^* in the HFCS/CS system was greater than that in the maltitol system during storage at different temperatures (Figure 5). For the same HFCS/CS system, the reaction rate constant (k) of ΔE^* increased with increasing storage temperature as expected (Table 3). For the maltitol systems, except for M100W0Y, there was no significant difference for the k of ΔE^* with both increasing storage temperature and increasing ratio of DEW/DEY (Table 3). At 23 °C, the change in ΔE^* in the maltitol systems was not obvious (Table 3 and Figure 5A).

Hardness Assessment of the DEW/DEY Bar Model Systems. According to our previous studies related to DEW and DEY powders, hardening in bar model systems can be induced by hydrophobic interaction and three chemical reactions during storage, that is, disulfide bond interaction, Maillard reaction, and lipid oxidation. During storage at three temperatures (23, 35, and 45 °C), the hardness changes in different DEW/DEY bar model systems are shown in Figure 6. The change in hardness could be expressed using an apparent zero-order model over the storage time at different temperatures. As expected, the effect of storage temperature increased as a function of time in all HFCS/CS bar model systems. For the same sweetener system, the k of bar hardening increased with both increasing storage temperature and increasing ratio of DEW/DEY (Table 3). As found in our previous studies,[18] at a hardness value of 12 N, there is significant bar hardening, which is recognized as objectionable by consumers. In the maltitol bar systems, except for M100W0Y, the hardness of the other four systems was below 12 N during storage at the three temperatures (Figure 6A–C). The hardness of M100W0Y was above 12 N after about 10 days at 45 °C (Figure 6C). However, replacing 25% of DEW with DEY, it would take about 221 days for M75W25Y to reach 12 N at 45 °C according to its zero-order prediction. On the other hand, the addition of DEY effectively lowered the initial hardness of the model system. For example, replacing 25% of DEW with DEY, the initial hardness of both M75W25Y and H75W25Y at 23 °C decreased about 24 and 56%, respectively, compared with that of the same sweetener system containing 100% DEW. Furthermore, the addition of DEY effectively lowered the k for hardness at the same temperature (Table 3). These results clearly show the function of DEY in the bar models as a system softener because DEY contains 38% (g/g) of unsaturated fat. The more unsaturated fat, the less hardening. Additionally, for the same bar model system stored at the same temperature, there was a

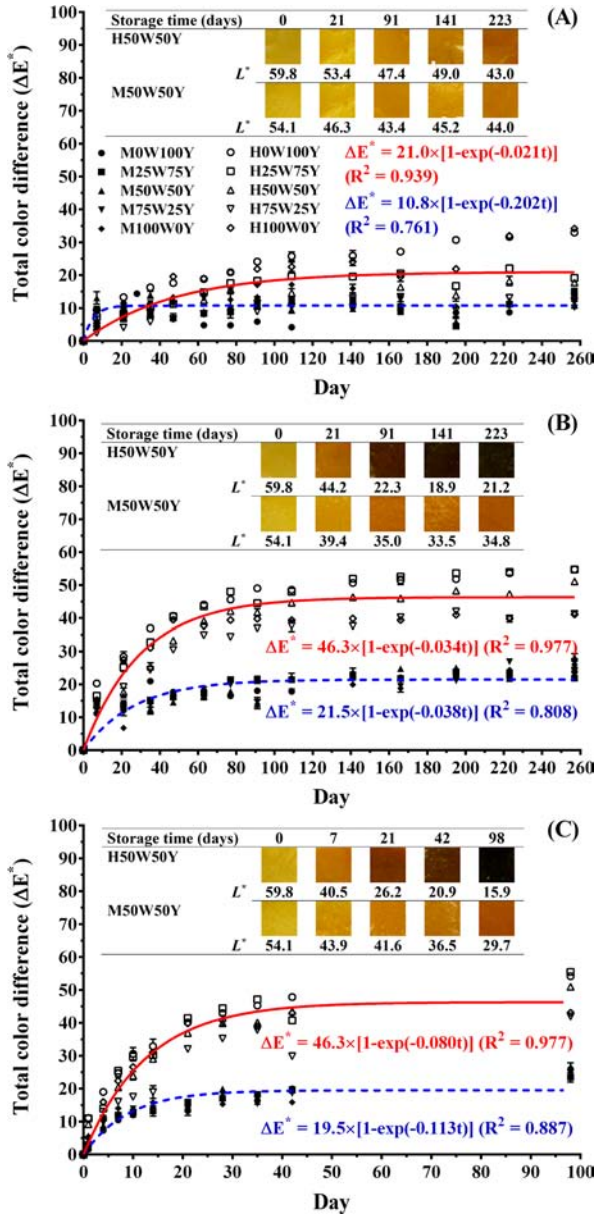

Figure 5. Effect of storage temperature on the total color difference (ΔE^*) of different DEW/DEY bar model systems: (A) 23 °C; (B) 35 °C; (C) 45 °C. As an example, the color change (L^* value) of two model systems, H50W50Y and M50W50Y, is compared during storage at different temperatures. Two trend lines are generated from the average data of five HFCS/CS systems (solid red) and five maltitol systems (dashed blue), respectively, using an apparent first-order hyperbolic model.

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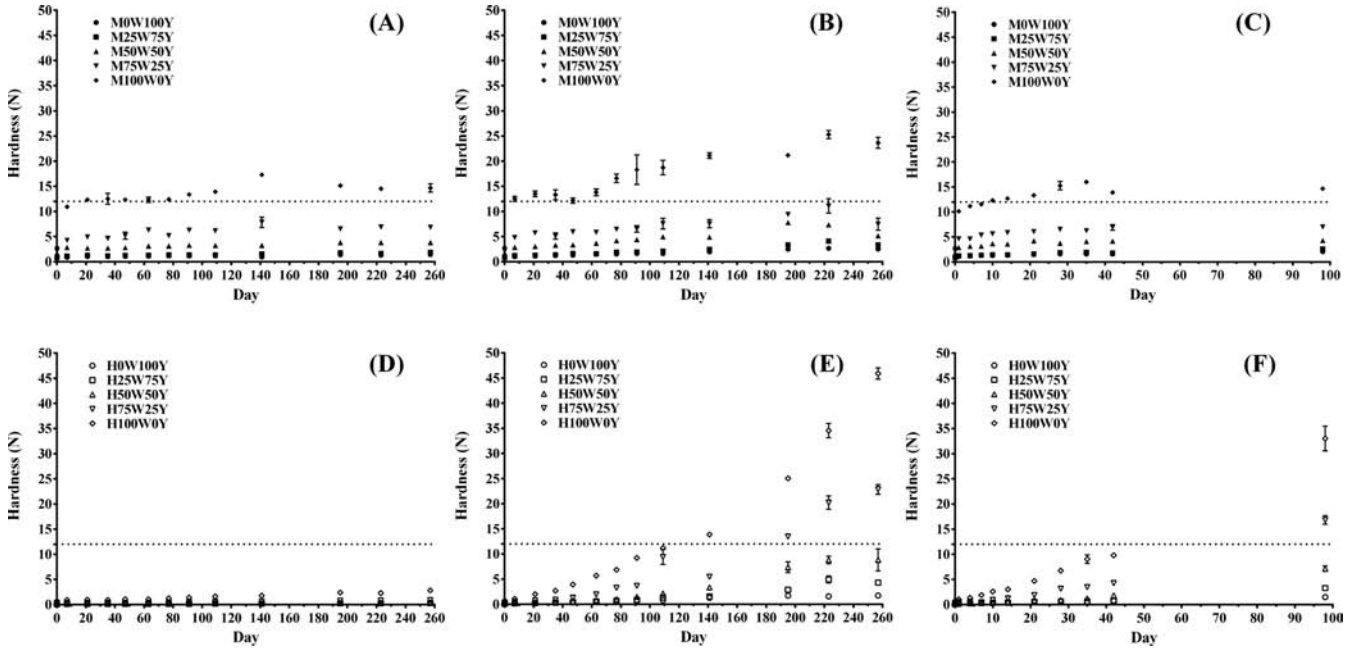

Figure 6. Effect of storage temperature on the hardness of different DEW/DEY bar model systems: (A, D) 23 °C; (B, E) 35 °C; (C, F) 45 °C. The dotted line indicates 12 N.

Table 4. Influence of Accelerated Shelf Life Testing (ASLT) Conditions on Shelf Life Prediction

	color (ΔE^* value)				hardness			
bar system	E_A (kJ/mol)	Q_{10}	Q_A (from 45 to 23 °C)	months at 23 °C equivalent to 3 months at 45 °C	E_A (kJ/mol)	Q_{10}	Q_A (from 45 to 23 °C)	months at 23 °C equivalent to 3 months at 45 °C
M0W100Y	N/A[a]				47.7	1.91	3.93	12
M25W75Y	N/A				44.0	1.81	3.53	11
M50W50Y	N/A				28.2	1.46	2.24	7
M75W25Y	N/A				32.1	1.54	2.51	8
M100W0Y	N/A				28.4	1.47	2.26	7
H0W100Y	66.5	2.46	6.73	20	66.9	2.47	6.80	20
H25W75Y	64.8	2.40	6.40	19	68.1	2.51	7.04	21
H50W50Y	51.5	2.01	4.38	13	71.1	2.62	7.66	23
H75W25Y	50.7	1.99	4.28	13	72.4	2.66	7.96	24
H100W0Y	56.9	2.16	5.10	15	75.8	2.79	8.78	26

[a]N/A, not available.

significant Pearson correlation ($P < 0.05$) between the ΔE^* value and hardness (Table 3).

Shelf Life Prediction of the DEW/DEY Bar Model Systems. In general, stability testing is a time-consuming process. To achieve shelf life prediction in a shorter length of time, accelerated shelf life testing (ASLT) can be used. During ASLT, the Arrhenius model can be used to describe how much faster a reaction will go if the product is held at some other temperature, including high abuse temperatures.[45] According to the Arrhenius relation, the activation energy (E_A) related to the changes of the two parameters (color and hardness) of the bar model systems was calculated (Table 4). The E_A is a measure of the temperature sensitivity of the reaction. As shown by Labuza and Kamman,[25] the mathematical relationship between E_A and the Q_{10} (how much faster the rate change is for a 10 °C increase in temperature) is

$$\ln Q_{10} = \frac{1000E_A}{R} \times \frac{10}{T(T+10)} \quad (2)$$

where E_A is the activation energy in kJ/mol, R is the gas constant [8.3145 J/(mol K)], and T is the temperature in Kelvin (°C + 273).

For any temperature difference (ΔT), the change in rate Q_A is

$$Q_A = Q_{10}^{(\frac{\Delta T}{10})(T+10)/(T+\Delta T)} \quad (3)$$

Thus, having data for shelf life at two abuse temperatures such as 35 and 45 °C, one can predict the storage time for the same quality change at room temperature such as 23 °C. Table 4 shows the results of these calculations for each bar model system. Thus, the change in a quality parameter after 3 months at 45 °C for each bar model is equivalent to 7−26 months for hardness and 13−20 months for color change at 23 °C (Table 4). Note that the variation between treatments cannot be determined statistically as only three temperatures were used except for the maltitol systems at 23 °C, at which no useful data could be found for color as little change occurred. It must be noted that ASLT has several limitations due to the increase of temperature. As temperature rises, (1) the a_w, pH, and reactant solubility are different compared with ambient conditions and (2) phase changes may

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result in a structurally different product and accelerate certain reactions.[45] Therefore, great care must be exercised when using ASLT so that significant under- or overestimation of product shelf life can be avoided.

Change in Remaining Free Amino Groups in the DEW/DEY Bar Model Systems. From Figure 7A, after 35 days of storage at different temperatures, there was no significant difference ($P > 0.05$) for the remaining free amino groups in the maltitol systems containing 100% DEW. However, it decreased about 40, 75, and 80% in the HFCS/CS systems containing 100% DEW at 23, 35, and 45 °C, respectively, after 35 days of storage (Figure 7A), indicating that the effect of storage temperature on the HFCS/CS system was much greater than that on the maltitol system. In addition, after 84 days of storage at 23 °C, the remaining free amino groups in the HFCS/CS system decreased with increasing ratio of DEW/DEY (Figure 7B). Interestingly, there was no significant loss of free amino group in the maltitol systems at any DEW/DEY ratio during storage at three temperatures (data not shown).

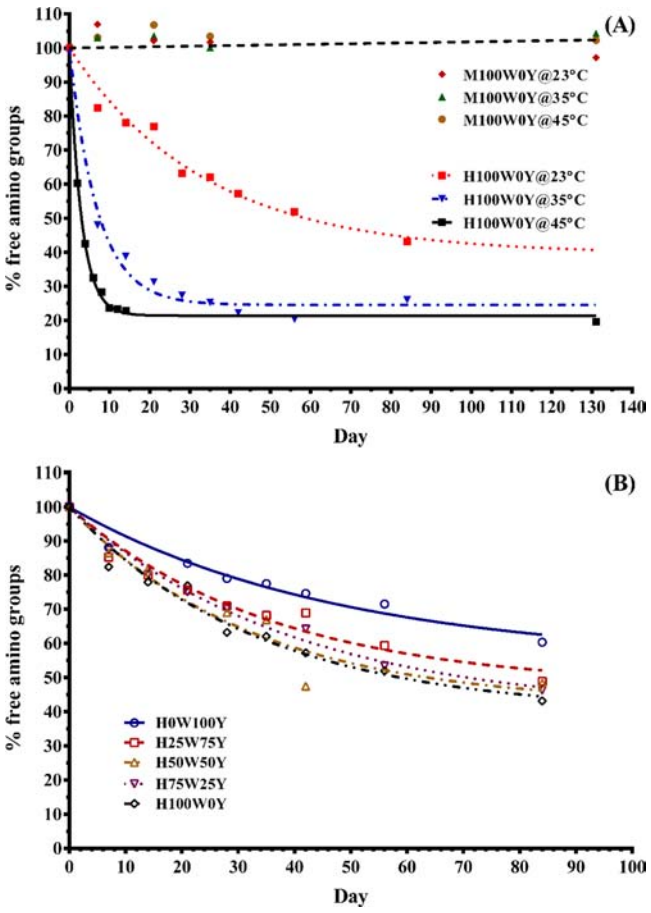

Figure 7. (A) Effect of storage temperature on the remaining free amino groups of the X100W0Y bar model system containing different sweeteners (X), that is, maltitol (M) and HFCS/CS (H). (B) Effect of ratio of DEW/DEY in the formulation on the remaining free amino groups of the HFCS/CS bar model systems during storage at 23 °C. Different lines in (A) and (B) indicate the trend of the bar model systems during storage.

In general, the coaction of two chemical reactions, the Maillard reaction and lipid oxidation, can affect the amount of free amino groups in the IMF system during storage. From our previous study,[10] the Maillard reaction could not only reduce but also regenerate the free amino groups in a DEW/water dough system. During the early stage of the Maillard reaction, a decrease in free amino groups was expected, as a result of the condensation with carbonyl groups to produce Amadori compounds. During the intermediate stage (or advanced stage) of the reaction, Amadori rearrangement products could regenerate free amino groups through fission, deamination, and/or dehydration reactions.[31,46−48] On the other hand, lipid oxidation-induced protein degradation can cleave −N−C− links of the polypeptide chains, resulting in an increase in free amino groups.[49] In addition, lipid hydroperoxides can damage amino acid residues especially histidine, cysteine, methionine, lysine, and tyrosine.[41]

In summary, through studying the physicochemical changes of DEY in dry and IMF matrices during storage at different temperatures, our results clearly showed that the effect of two chemical reactions (disulfide bond interaction and the Maillard reaction) on storage stability of the model systems. Currently, the direct effect of lipid oxidation on the storage stability of DEY in dry and IMF matrices is unavailable. Thus, its degree of influence and the relationship with the other two reactions during storage need to be further studied. In addition, from the results of the DEW/DEY bar model systems, (1) by replacing 25% of DEW in a protein bar system with DEY, the rate of bar hardening was significantly lower than that of the bars with only DEW at all temperatures due to the fat in DEY; (2) the use of maltitol (nonreducing sugar alcohol) instead of HFCS/CS (reducing sugars) in all bar systems resulted in decreased hardness; (3) the use of maltitol drastically decreased the change in color; and (4) supporting this is the fact that there was no significant loss of free amino groups in the maltitol systems at any DEW/DEY ratio. On the basis of our findings, there are several suggestions for the egg industry. (1) The optimal moisture for maximum shelf life is at or below the m_0 values, which are 6.2 g water/100 g solids for DEW and 2.3 g water/100 g solids for DEY. Thus, for DEW, the working range for in-plant drying operations should be between about 5.5 and 6.5 g water/100 g solids. For optimum stability, DEY should be dried to no less than 2 or more than 3 g water/100 g solids to minimize lipid oxidation. (2) The moisture sorption isotherms of dry egg powders can be used to select the optimum packaging material needed to maintain the powder below an a_w for a given desired shelf life at a selected external temperature and humidity of storage. (3) Further fermentation to completely remove glucose in DEY should be considered. (4) Becuase egg yolk has a high content of unsaturated fat, lipid oxidation can occur in DEY during distribution and storage and result in color changes, off-flavor, and lipid−protein complexes. Prevention approaches include adding antioxidants and metal chelators or improving packaging such as using vacuum or a nitrogen environment. (5) To minimize the reducing sugar content in HPNB, the use of sugar substitutes that do not have residual reducing sugars is important. It is recommended that HFCS/CS sweeteners can be replaced by maltitol syrup to maintain product quality and texture throughout the shelf life of a protein bar. (6) Due to the high fat content, substituting DEY helps soften bar texture and lower the rate of hardening over time. In bars with HFCS/CS, DEY will not prevent other deleterious changes associated with the Maillard reaction. (7) ASLT can be a useful approach for estimating the shelf life of a new product.

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