



Storage stability of hen egg white powders in three protein/water dough model systems

Qinchun Rao^a, Jeancarlo R. Rocca-Smith^b, Theodore P. Labuza^{a,*}

^a Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108, United States

^b Department of Food Science, University of Udine, Via Sondrio 2, I-33100 Udine, Italy

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ABSTRACT

In recent years, due to the specific health benefits associated with bioactive peptides and the reduction of protein allergenicity by enzymatic hydrolysis, the utilisation of protein hydrolysates in the intermediate-moisture food (IMF) market, such as high protein nutrition bars (HPNB), has significantly increased. Currently, no reported study is related to the storage stability of dried hen egg white (DEW) and its hydrolysates (HEW) in an IMF matrix. Therefore, three DEW/HEW dough model systems (100%HEW + 0%-DEW, 75%HEW + 25%DEW and 50%HEW + 50%DEW) were established using two commercial spray-dried egg white powders to study the effect of temperature and fraction of HEW on these IMF models (water activity (a_w): ~0.8). During storage at three different temperatures (23, 35 and 45 °C) for 70 days, the selected physicochemical properties of the dough systems were compared. Overall, kinetic analysis showed an apparent zero-order model fit for the change in the colour (L^*), fluorescence intensity (FI) and hardness, as a function of time, for different dough model systems. As expected, the L^* , FI and hardness increased as a function of time mainly due to the Maillard reaction. The amount of free amino groups decreased, with an increase in rate of loss, as temperature increased in the 100%HEW + 0%DEW model. When DEW was substituted for some HEW, the regeneration of the free amino groups after loss was observed as a function of time. Furthermore, when the percentage of HEW was decreased, the incidence of mouldy samples occurred sooner, which indicates that HEW has some antimicrobial ability, especially in the 100%HEW + 0%DEW system where mould growth did not occur.

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1. Introduction

Intermediate-moisture food (IMF) products are products with a moderate moisture content (10–40%, wet basis, wb) and a moderate water activity (a_w : 0.55–0.90) created to be shelf-stable without refrigeration (Karel & Heidelbaugh, 1973; Pavey & Schack, 1969; Taoukis, Elmesline, & Labuza, 1988). Most of the commercial high protein nutrition bars (HPNB, a_w : 0.50–0.65) fit into the IMF category. HPNB is a growing segment in the sports nutrition, muscle building, health supplement, and weight reduction markets which are estimated to grow to about \$3 billion in 2016 in the U.S. (Mintel, 2012b).

One major problem related to HPNB is that they generally become harder (aggregation) during storage without moisture loss, making the product unacceptable to consumers (Ahmed, 2004; Berry, 2011; Hazen, 2010; Hutchinson, 2009; Wade, 2005). One of the mechanisms is moisture-induced protein/peptide aggregation which can occur chemically (covalent interactions such as disulfide bond formation/exchange and the Maillard reaction)

and/or physically (non-covalent interactions such as hydrophobic interactions), causing changes in the structure and texture of the food matrix which degrade eating quality (Liu, Zhou, Tran, & Labuza, 2009; Rao, Rocca-Smith, & Labuza, 2012; Zhou, Liu, & Labuza, 2008a; Zhou, Liu, & Labuza, 2008b; Zhu & Labuza, 2010). Moisture-induced Maillard browning would occur during storage if reducing sugars are present in any ingredients or added directly. Compared with bar hardening, the quality changes related to the Maillard reaction in HPNB are seldom noticed by consumers. The major reason is that these undesirable changes are usually masked intentionally or accidentally by other added ingredients in HPNB, such as a chocolate coating or caramel colour added.

It is well-known that the hardness of a food matrix is closely related to its glass transition temperature (T_g) (Slade & Levine, 1991). One of the impact factors of T_g is the molecular size of proteins in the food matrix (Chuy & Labuza, 1994). Due to the smaller molecular weight distribution compared with their original intact proteins, protein hydrolysates are easier to form a liquid bridge between two particles, which can effectively decrease the T_g of their powder matrix below room temperature and lead to stickiness during storage at high a_w level (Masuda, Gotoh, Higashitani, & Matsusaka, 2006; Netto, Desobry, & Labuza, 1998; Rao & Labuza,

* Corresponding author. Tel.: +1 612 624 9701; fax: +1 612 625 5272.

E-mail address: tplabuza@umn.edu (T.P. Labuza).

2012; Rao, Rocca-Smith, Schoenfuss, & Labuza, 2012). Therefore, one of the solutions for the reduction of bar hardening is to use protein hydrolysates as a humectant to decrease the T_g of the bar matrix (Taoukis & Richardson, 2007). Furthermore, the consumers can not only get the specific health benefits associated with the bioactive peptides in protein hydrolysates, but also reduce the risk of the adverse protein allergenicity (Kitts & Weiler, 2003).

Recently, through changing the moisture content, i.e., a_w (from 0.05 to 0.85), the storage stabilities of two commercial hen egg powders, spray-dried egg white powder (DEW) and its hydrolysates (HEW), were studied, respectively (Rao & Labuza, 2012; Rao et al., 2012). Overall, the physicochemical differences between the two powders during storage were closely related to their inherent characteristics. Firstly, as mentioned above, compared with DEW, the average molecular weight of HEW is smaller (≤ 10 kDa) due to enzymatic hydrolysis. Therefore, at the same a_w , the T_g of HEW is much lower than that of DEW, which makes HEW subject to the results of increased molecular mobility and reaction rates. Secondly, after desugarization during product processing, both powders still contain a small amount of reducing sugar such as glucose and/or its residuals such as carbonyl groups which can react with amino compounds and result in non-enzymatic browning (Maillard reaction) (Rao & Labuza, 2012; Rao et al., 2012; Rao et al., 2012). The major difference is the existing form of reducing sugar in both powders (Rao et al., 2012). In DEW, because of the early stage Maillard reaction during dry-heat pasteurisation (60 °C for 7 days), the remaining carbonyl groups are covalently bound to the proteins (glycosylation). However, in HEW, mainly because the reaction time for glycosylation is too short during pasteurisation (60 °C for 3.5 min), non-covalently bound glucose (0.07%, g/g) can be easily detected using some commercial kits which simply extract glucose with distilled and deionized water (Rao & Labuza, 2012).

Additionally, through studying a DEW/water dough model system (a_w : 0.95), moisture-induced aggregates were produced by two chemical reactions during storage: disulfide interaction and the Maillard reaction (Rao et al., 2012). To further study the effect of temperature and fraction of HEW on DEW/water model system, three simple DEW/HEW dough model systems (100%HEW + 0%DEW, 75%HEW + 25%DEW and 50%HEW + 50%DEW) were established in this study. During storage at three different temperatures (23, 35 and 45 °C) for 70 days, the selected physicochemical changes of the dough systems were compared.

2. Materials and methods

2.1. Materials

Two spray-dried hen egg white powders, Dried Egg Whites (DEW, H227) and Hydrolyzed Egg White (HEW, EP-1 #400) were obtained from Deb-El Food Products, LLC (Elizabeth, NJ, USA) and Henningsen Foods, Inc. (Omaha, NE, USA), respectively. Both products were kept at -20 °C until used. The partial process information and selected physicochemical properties of both products were summarized in our previous studies (Rao & Labuza, 2012; Rao et al., 2012).

2.2. Preparation of DEW/HEW dough model systems

To study the effect of temperature and fraction of HEW on DEW/water model system, three different formulations (100%HEW + 0%DEW, 75%HEW + 25%DEW and 50%HEW + 50%DEW) of DEW/HEW dough model systems were prepared. Briefly, DEW and HEW were mixed well using a powder mixer (IKA Works, Inc., Wilmington, USA) to prepare protein powder blends containing 50, 75, and

100% (g/g) of HEW, respectively. A certain amount of distilled and deionized water was then added into each powder blend to obtain about 25% moisture (wb) in the dough. After mixed until a uniform dough texture was achieved, the dough was then sealed in a plastic bag (Thermo Fisher Scientific Inc., Rockford, IL, USA) and kept at 4 °C for 2 days for moisture equilibration. Before packaging, the moisture-equilibrated dough was kept at room temperature for 2 h. The resultant dough (~ 10 g) was weighed and pressed into a plastic disposable sample cup (Decagon Devices, Inc., Pullman, WA, USA), and then immediately covered with the lid (Decagon Devices). All finished samples were placed in moisture barrier pouches (IMPAK Corporation, Los Angeles, CA, USA), then heat-sealed for storage. The water vapour transmission rate of the pouch is 0.009 g/m²/24 h. The packaged samples were stored at three different temperatures (23, 35 and 45 °C) for 70 days. The samples were removed at designated time intervals and cooled at room temperature for at least 2 h before being analysed immediately or frozen at -45 °C for later analysis. The initial sample information of three DEW/HEW dough model systems is summarized in Table 1.

2.3. Physicochemical changes of DEW/HEW dough model systems

During storage, several physicochemical properties of the dough systems stored at three temperatures were analysed at designated time intervals. Firstly, the a_w of the samples was determined using the AquaLab 3TE Water Activity Meter (Decagon Devices). Secondly, the degree of hardness of the samples was analysed using the TA.XTPlus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA) fitted with a flat-ended cylinder stainless steel probe (3 mm diameter). Thirdly, the remaining free amino groups in the samples were determined using the *o*-phthalaldehyde method (Goodno, Swaisgood, & Catignani, 1981) with modifications. Fourthly, the colour (L^* value) of the samples was analysed using the Minolta Chroma Meter CR-200 (Minolta Camera Co., Osaka, Japan). Finally, the presence of fluorescent Maillard compounds in the samples was measured through the determination of fluorescence intensity (FI). All relevant methods are described in detail in our previous studies (Rao & Labuza, 2012; Rao et al., 2012). Additionally, reaction kinetics related to three dynamic parameters (L^* , FI and hardness) were analysed using an apparent zero-order model, which is described in our previous study (Rao et al., 2012). The activation energy (E_A) related to quality changes in different dough model systems was also calculated using the Arrhenius equation (Labuza & Kamman, 1983).

2.4. Statistical analysis

Each sample condition was tested at least in duplicate at each time. To compare the change in the dynamic parameters during storage, one-way ANOVA with Tukey's post test was performed. The Pearson correlation between FI and the other two dynamic parameters (L^* value and hardness) were measured. $P < 0.05$ was considered to be statistically significant. The software, GraphPad Prism for Windows (version 5.04, GraphPad Software, La Jolla, CA, USA), was used to analyse the data.

3. Results and discussion

3.1. Water activity (a_w) assessment

From Table 1, the initial moisture content in three dough systems was not significantly different ($P > 0.05$). However, their initial a_w increased slightly, but significantly ($P < 0.05$), with increasing ratio of DEW:HEW in the formulation. According to Raoult's law, the a_w of the aqueous ideal solution depends only

Table 1

Initial sample information of three DEW/HEW dough systems.

Dough system	Moisture content $\pm$ SEM* (% wb)	$a_w \pm$ SEM	Protein content (g protein/100 g dough)
100%HEW + 0%DEW	24.6 $\pm$ 1.2 ^a	0.810 $\pm$ 0.001 ^a	53.8
75%HEW + 25%DEW	26.4 $\pm$ 3.0 ^a	0.822 $\pm$ 0.002 ^b	54.3
50%HEW + 50%DEW	21.7 $\pm$ 2.4 ^a	0.844 $\pm$ 0.004 ^c	54.9

*SEM: the standard error of the mean.

^{a,b,c}Values in the same column with different letters are significantly different ($P < 0.05$).

on the total moles of solute molecules. The smaller the molecular weight of the solute such as HEW (<10 kDa), the larger the total moles of the solute, the greater its a_w -lowering effect (humectancy) per unit of weight dissolved (Taoukis & Richardson, 2007). In the DEW/HEW dough systems, when the percentage of HEW, i.e., the moles of HEW, increased from 50% to 100%, the a_w of the system decreased significantly from 0.844 to 0.810 ($P < 0.05$, Table 1), indicating the humectancy of HEW.

During storage at three different temperatures (23, 35 and 45 °C), for both the 100%HEW + 0%DEW and the 75%HEW + 25%DEW systems (Fig. 1A and B), their a_w s did not change significantly ($P > 0.05$, data not shown) compared with their initial a_w s, respectively. For the 50%HEW + 50%DEW system (Fig. 1C), its a_w decreased linearly during storage at three different temperatures, especially in the samples stored at 35 °C ($R^2 = 0.862$) and 45 °C

($R^2 = 0.893$). These a_w changes in different dough systems could be derived from the coaction of three mechanisms. Firstly, the Maillard reaction could produce up to 3.5 mol of water per mole of sugar consumed (Taoukis & Richardson, 2007). Several studies have observed the increase in a_w of different food bar model systems (a_w : ~0.6) with the addition of reducing sugar such as high-fructose corn syrup (HFCS) (Davis, 2005; Li, Szlachetka, Chen, Lin, & Ruan, 2008; McMahon, Adams, & McManus, 2009; Taterka, 2009; Tran, 2009). It must be noted that instead of an increase caused by the Maillard reaction, two studies (Li et al., 2008; McMahon et al., 2009) suggested that the increase in a_w of the bar models during storage was caused by a decrease in the amount of water capable of acting as a plasticizer in the product. In both DEW and HEW powders, the reducing sugar content was really low (<0.1%, g/g) (Rao & Labuza, 2012). The quantity of water produced through

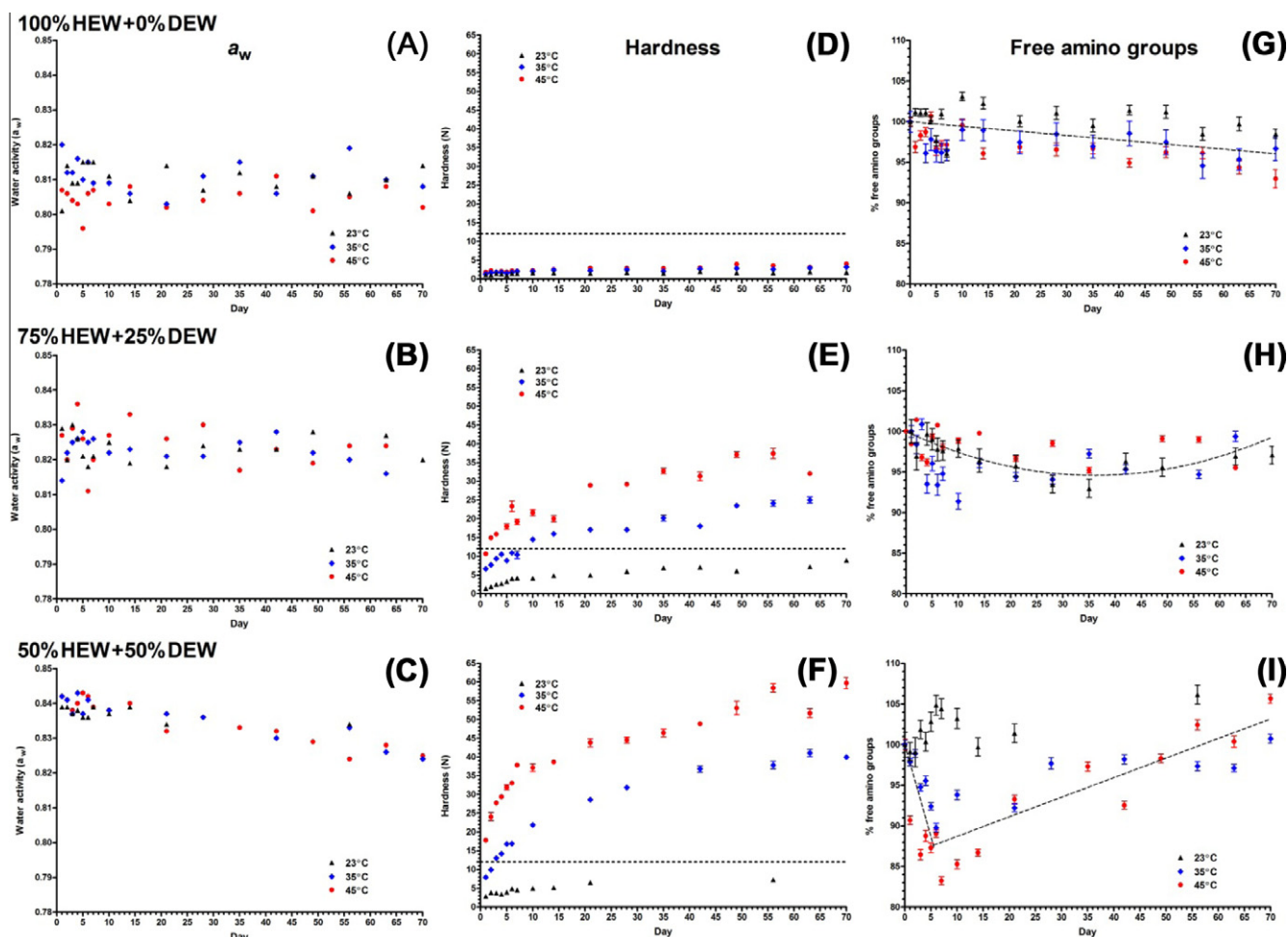


Fig. 1. Effect of storage temperature on the a_w (A–C), hardness (D–F), and free amino groups (G–I) of three DEW/HEW dough systems. The dotted line (D–F) indicates 12 N. The overall trend of the remaining free amino groups is indicated by the dashed line during storage at three temperatures (G and H) or at two temperatures (35 °C and 45 °C, I). A, D and G: 100%HEW + 0%DEW; B, E and H: 75%HEW + 25%DEW; C, F and I: 50%HEW + 50%DEW.

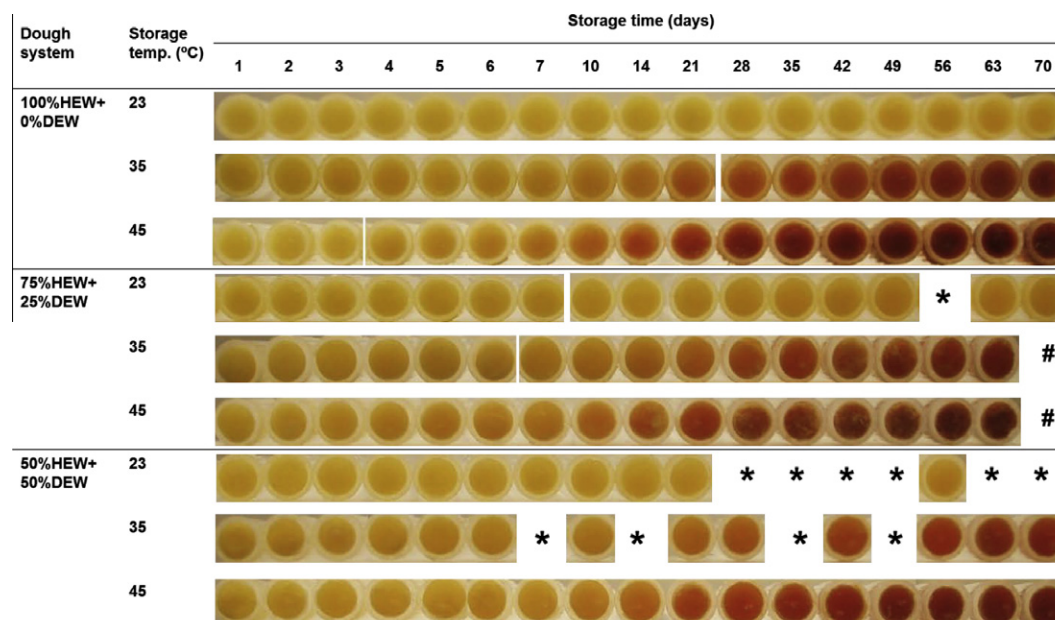


Fig. 2. Colour change of three DEW/HEW dough systems during storage at different temperatures. *Indicates that the sample became mouldy at that time. #Indicates that the sample was not collected.

the Maillard reaction during storage should not be enough to change the initial a_w (~ 0.8) of the dough system, which was confirmed in both the 100%HEW + 0%DEW and the 75%HEW + 25%DEW systems (Fig. 1A and B). Secondly, some chemical reactions which could use water as a reactant at high a_w level, such as proteolytic hydrolysis, were involved during storage. These reactions could decrease the a_w of the system because its moisture content decreased. It is noted that no antimicrobial agent was added into the dough systems in order to avoid any side effects such as the cross-reaction between proteins/peptides and antimicrobial. According to the product specification, both powders still contained a very small amount of microbes such as yeast, mould and coliform after pasteurisation. The mouldy dough samples were observed during storage as expected (Fig. 2), which is mainly because the a_w of the dough systems was much higher than the minimum a_w (0.61) for microbial growth (Tapia, Alzamora, & Chirife, 2007). Therefore, during storage, the proteases produced by these microbial could further hydrolyze proteins/peptides using water in the formulation. Further research is needed to understand the microbiological implications in these dough systems. Thirdly, it is known that HEW contains different kinds of antimicrobial peptides after enzymatic hydrolysis (Mine & D'Silva, 2007; Mine, Ma, & Lauriau, 2004). Therefore, mould did not occur in the 100%HEW + 0%DEW

system (Fig. 2). With increasing ratio of DEW:HEW in the formulation, the antimicrobial effectiveness of HEW decreased, and the incidence of mouldy samples occurred sooner.

3.2. Hardness assessment

During storage at three different temperatures (23, 35 and 45 °C), the hardness changes for the three DEW/HEW dough systems are shown in Fig. 1(D–F). As expected, the effect of storage temperature increased as a function of time in three systems. The changes in hardness could be expressed using an apparent zero-order model over the 70-day storage at different temperatures. The reaction rate constant (k) of dough hardening increased with both increasing storage temperature and increasing ratio of DEW:HEW (Table 2). According to our previous studies (Taterka, 2009; Tran, 2009), at a hardness value of 12 N, consumers would indicate the hardened bar was unacceptable. In our dough systems, after seven days at 35 °C and one day at 45 °C, the hardness of the 75%HEW + 25%DEW samples was beyond 12 N (Fig. 1E). For the 50%HEW + 50%DEW system, it took only three days at 35 °C and less than one day at 45 °C to reach 12 N (Fig. 1F). From our previous study (Rao et al., 2012), in a 100% DEW dough system, two chemical reactions, disulfide interaction and the Maillard reaction,

Table 2
Reaction rate constant (k) values and activation energy (E_a) related to quality changes in three DEW/HEW dough systems.

Quality change	Dough system	$k \times 10^2$ (day ⁻¹)			E_a (kJ/mole)
		23 °C	35 °C	45 °C	
Hardness	100%HEW + 0%DEW	0.9 ^{ax}	2.0 ^{bx}	3.0 ^{cx}	41.9
	75%HEW + 25%DEW	8.4 ^{ay}	27.8 ^{by}	35.5 ^{cy}	52.6
	50%HEW + 50%DEW	7.1 ^{ay}	44.5 ^{bz}	45.5 ^{bz}	68.5
Colour (L^* value)	100%HEW + 0%DEW	10.5 ^{ax}	37.7 ^{bx}	44.0 ^{bx}	52.6
	75%HEW + 25%DEW	10.9 ^{ax}	33.5 ^{bx}	47.1 ^{bx}	53.1
	50%HEW + 50%DEW	10.4 ^{ax}	28.0 ^{bx}	38.4 ^{cx}	47.4
Fluorescence intensity (FI)	100%HEW + 0%DEW	1458 ^{ax}	12823 ^{bx}	17449 ^{cx}	90.7
	75%HEW + 25%DEW	1297 ^{ax}	8551 ^{by}	12761 ^{cy}	83.2
	50%HEW + 50%DEW	1131 ^{ax}	5236 ^{bz}	7619 ^{cz}	69.3

^{a,b,c}Values in the same row with different letters are significantly different ($P < 0.05$).

^{x,y,z}For the same dynamic parameter, values in the same column with different letters are significantly different ($P < 0.05$).

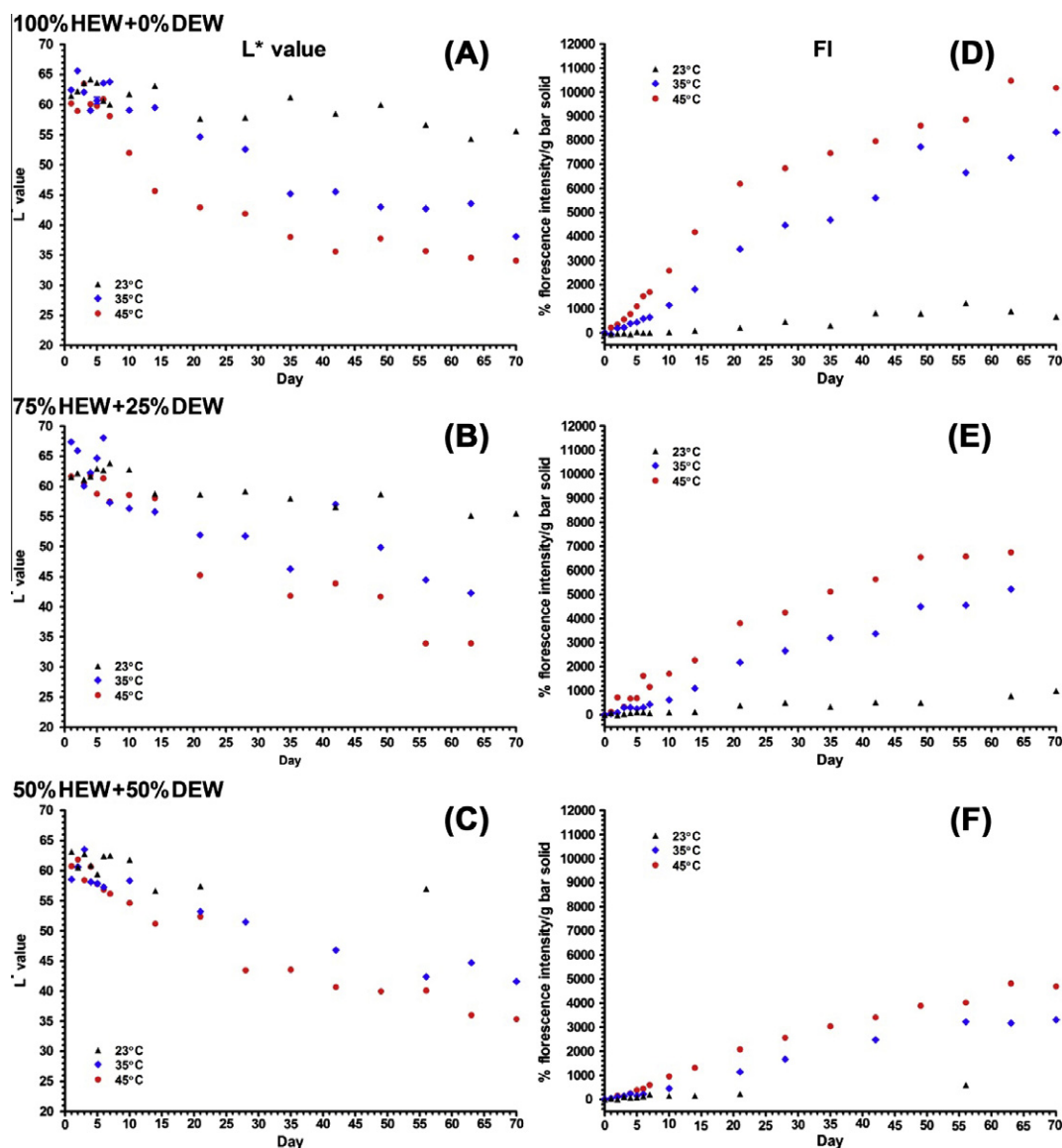


Fig. 3. Effect of storage temperature on the L^* value (A–C) and fluorescence intensity (FI, D–F) of three DEW/HEW dough systems. A and D: 100%HEW + 0%DEW; B and E: 75%HEW + 25%DEW; C and F: 50%HEW + 50%DEW.

caused protein aggregation during storage. Additionally, the decrease in a_w in the 50%HEW + 50%DEW system during storage could be another reason for dough hardening (Fig. 1C), i.e., the interstitial water was decreased so there was less plasticizer.

On the other hand, the addition of HEW effectively lowered the initial hardness of the dough system. For example, replacing 25% of DEW with HEW, the initial hardness of the 75%HEW + 25%DEW system at 23 °C decreased about 50% compared with that of the 50%HEW + 50%DEW system. Furthermore, the addition of HEW efficiently lowered the reaction rate of increased hardness over time at the same temperature (Table 2). It has been reported that in the mixture of two powders, the T_g of the mixture occurred at an intermediate temperature between the T_g s of two pure powders (Biliaderis, Lazaridou, Mavropoulos, & Barbayannis, 2002; Roe & Labuza, 2005). In our dough system, it was assumed that the T_g of the mixture increased with increasing ratio of DEW:HEW since the T_g of HEW is much lower than that of DEW at the same a_w (Rao & Labuza, 2012). For example, at a_w 0.844, the difference of T_g between DEW (64 °C) and HEW (−48 °C) is 112 °C. Therefore, it is a very useful strategy for the food bar industry to add a certain

amount of protein hydrolysates into HPNB as a plasticizer. This way can effectively lower the T_g of the final product, resulting in not only controlling the initial hardness but also decreasing the rate of the reaction which can lead to protein aggregation and bar hardening during storage. However, it must be noted that the real relationship between the percentage of protein hydrolysates and the T_g of the finished product still needs to be studied (Biliaderis et al., 2002). It is noted that in the 100%HEW + 0%DEW system, the rate of dough hardening increased slightly, but significantly ($P < 0.05$), as a function of storage temperature (Table 2). According to our previous study (Rao & Labuza, 2012), this should be the coaction of both hydrophobic interactions and the Maillard reaction during storage.

3.3. Analysis of remaining free amino groups

From our previous studies (Rao & Labuza, 2012; Rao et al., 2012), compared with DEW, the major components in HEW were small peptides and/or amino acids (<10 kDa) after enzymatic hydrolysis. Therefore, when the percentage of HEW increased,

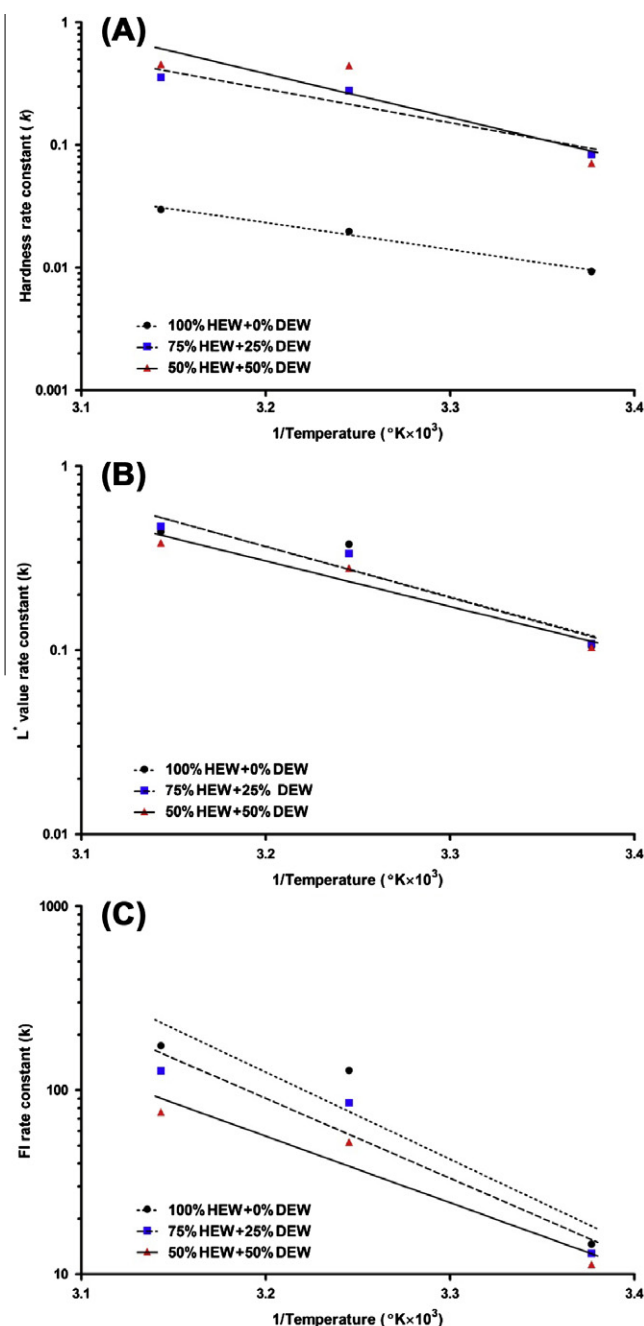


Fig. 4. Arrhenius plot for the rate constants (k) of (A) hardness, (B) L^* value and (C) fluorescence intensity (FI) causing quality changes in three DEW/HEW dough systems.

the initial remaining free amino groups in the dough system increased. As expected, at day 0, the initial remaining free amino groups in the 75%HEW + 25%DEW and the 50%HEW + 50%DEW systems were about 21% and 33% smaller than that in the 100%HEW + 0%DEW system, respectively. During storage at three different temperatures (23, 35 and 45 °C), the change of the remaining free amino groups in the three DEW/HEW dough systems are shown in Fig. 1(G–I). It is noted that three different trends were observed in three dough systems. For the 100%HEW + 0%DEW system (Fig. 1G), the overall trend was a loss of free amines as a function of time. After 70 days of storage, compared with that at day 0, the remaining free amino groups at 35 °C and 45 °C were reduced by about 4% and 7%, respectively, while there was no sig-

nificant change in the sample at 23 °C ($P > 0.05$, Fig. 1G). A similar trend was also observed in HEW powder after one month of storage at different a_{ws} at 45 °C (Rao et al., 2012). For the other two systems containing DEW (Fig. 1H and I), the overall trend was the regeneration of free amino groups after loss over time. Compared with the 75%HEW + 25%DEW system (Fig. 1H), this trend was greater in the 50%HEW + 50%DEW system (Fig. 1I). After one week of storage at 35 and 45 °C, compared with that at day 0, the remaining free amino groups in the 50%HEW + 50%DEW system decreased about 10% and 17%, respectively; however, after 70 days of storage, it was regenerated about 1% and 6%, respectively (Fig. 1I).

The regeneration of free amino groups in the two systems containing DEW during storage is mainly due to two mechanisms. Firstly, the Maillard reaction could not only reduce but also regenerate the free amino groups. 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 (Hodge, 1953; Labuza & Baisier, 1992; Labuza & Massaro, 1990; Mauron, 1981; Wolf, Thompson, & Reineccius, 1977). Secondly, the proteolytic hydrolysis catalysed by microbial proteases could hydrolyze proteins/peptides in the dough systems, resulting in producing more free amino groups during storage. In our previous study (Rao et al., 2012), the change of the remaining free amino groups in a 100% DEW/water dough system during storage at different temperatures was also due to the same two mechanisms.

According to the U.S. regulation for nutrition labelling of food (U.S. Food and Drug Administration, 2012), the nutrient content of proteins is at least equal to 80% of the value for that nutrient declared on the label. Usually, the total protein content in the final product will not change significantly during its shelf life. However, the total protein quality in that product may change significantly over time, resulting in the decrease in its nutritional value and the reduction of the bioavailability of bioactive peptides in HPNB which will be investigated in our laboratory.

3.4. Changes in colour and fluorescent Maillard compounds

The colour change of three dough systems stored at three different temperatures is shown in Fig. 2 from which the trend can be easily visible by the naked eye. The initial L^* values of three dough systems at day 0 were similar (~ 63). During storage, mainly due to the Maillard reaction, two physicochemical changes (colour and fluorescent Maillard compounds) occurred as a function of time: the L^* value decreased (Fig. 3A–C), while the FI value increased (Fig. 3D–F). Similar results were also obtained in a 100% DEW/water dough system (Rao et al., 2012). The changes in the other two dynamic parameters (L^* and FI values) could also be expressed using an apparent zero-order model over the 70-day storage at different temperatures. The reaction rate constant (k) of L^* value increased with increasing storage temperature and decreased with increasing ratio of DEW:HEW (Table 2). Although the rate of browning (L^* value) at 23 °C was significantly lower than those at 35 °C and 45 °C ($P < 0.05$, Table 2), the browning in the three dough systems still could be visible after 70 days of storage at 23 °C (Fig. 2). On the other hand, the change in the k of FI was similar to that of hardening, which increased with both increasing storage temperature and increasing ratio of DEW:HEW (Table 2). According to the Arrhenius plot (Fig. 4), the activation energy (E_A) related to the changes in the three parameters of three DEW/HEW dough systems was calculated (Table 2). The E_A of the L^* value was similar to that of hardness indicating that the Maillard reaction logically caused part of dough hardening (Table 2).

Table 3

Pearson correlation coefficients (r , $P < 0.05$) between the fluorescence intensity (FI) and two other dynamic parameters (colour change and hardness) in the same dough system.

Dough system	Temperature (°C)	Colour change (L^*)		Hardness	
		r	R^2 ^a	r	R^2
100%HEW + 0%DEW	23	−0.787	0.620	0.716	0.512
	35	−0.973	0.946	0.921	0.848
	45	−0.980	0.960	0.931	0.868
75%HEW + 25%DEW	23	−0.879	0.772	0.898	0.806
	35	−0.884	0.781	0.962	0.925
	45	−0.965	0.931	0.961	0.924
50%HEW + 50%DEW	23	−0.582	0.338	0.850	0.723
	35	−0.977	0.954	0.957	0.916
	45	−0.984	0.969	0.940	0.883

^a The value R^2 quantifies goodness of fit.

Additionally, for the same dough system stored at the same temperature, there was a significant Pearson correlation ($P < 0.05$) between the FI and two other dynamic parameters (L^* value and hardness, Table 3).

4. Conclusions

In summary, through studying the physicochemical changes in three simple DEW/HEW dough model systems during storage at three different temperatures, our results clearly showed that the addition of HEW could effectively reduce the dough hardening due to the decrease in the T_g of the IMF matrix. However, the addition of HEW could also decrease the storage stability mainly due to the Maillard reaction. As mentioned above, compared with bar hardening, the browning in HPNB may not be easily noted by consumers. This quality loss may eventually lead to the reduction in the bioavailability of the nutrients in the products. Recently, due to the health concerns, 69% of the U.S. adult consumers claimed that reduced sugar/sugar free was an important factor for them in choosing the nutrition/energy bars (Mintel, 2012b). Therefore, in order to increase the storage stability of HPNB and improve customer satisfaction, the manufacturers can add a portion of protein hydrolysates as a plasticizer into HPNB to control bar hardening during shelf life. It must be noted that the higher degree of hydrolysis, the more bitter-tasting the resulting protein hydrolysates may be. The manufacturers thus will also need to control the bitterness of HPNB through optimising the percentage of protein hydrolysates in the bar formulation. Additionally, the food bar industry can add sugar substitutes such as sugar alcohols into HPNB to mask the bitterness. In order to minimize the reducing sugar content in HPNB, the manufacturers should use the sugar substitutes do not have residual reducing sugars. It is worth mentioning that, from 2007 to 2011, 13.1% of 2152 new products related to snack, cereal and energy bars in the U.S. contained low/no reducing sugar (Mintel, 2012a).

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