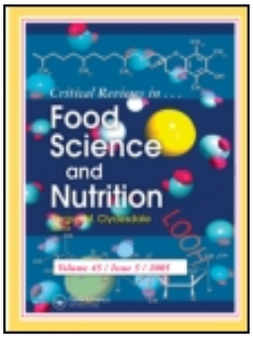

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Storage Stability of Food Protein Hydrolysates–A Review

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In recent years, mainly due to the specific health benefits associated with (1) the discovery of bioactive peptides in protein hydrolysates, (2) the reduction of protein allergenicity by protein hydrolysis, and (3) the improved protein digestibility and absorption of protein hydrolysates, the utilization of protein hydrolysates in functional foods and beverages has significantly increased. Although the specific health benefits from different hydrolysates are somewhat proven, the delivery and/or stability of these benefits is debatable during distribution, storage, and consumption. In this review, we discuss (1) the quality changes in different food protein hydrolysates during storage; (2) the resulting changes in the structure and texture of three food matrices, i.e., low moisture foods (LMF, $a_w < 0.6$), intermediate moisture foods (IMF, $0.6 \leq a_w < 0.85$), and high moisture foods (HMF, $a_w \geq 0.85$); and (3) the potential solutions to improve storage stability of food protein hydrolysates. In addition, we note there is a great need for evaluation of biofunction availability of bioactive peptides in food protein hydrolysates during storage.

Keywords Water activity, moisture, bioactive peptide, disulfide, Maillard reaction, biofunction

INTRODUCTION

The global use of protein ingredients in formulated foods, beverages, and dietary supplements is estimated to be at 5.5 million metric tons by 2018 (Figure 1A) (Frost and Sullivan, 2012a, b) and exceed $24.5 billion by 2015 (Global Industry Analysts, 2010). The United States, which accounts for more than one-fifth of the global protein ingredients market, is projected to expand at an annual average growth rate ranging between 8 and 9% over the period 2010–2015 (Global Industry Analysts, 2010).

Based on their molecular integrity, food protein ingredients can be classified into two types: intact proteins (native or denatured) and their hydrolysates. In this review, protein hydrolysates are defined as mixtures of polypeptides, oligopeptides, and amino acids that are produced from various animal and plant protein sources using physical (heat or shear) or chemical (acid, alkali, or enzyme) hydrolysis. For the reader's convenience, the characteristics of the major intact proteins in three important foods, i.e., cow's milk (Table 1), hen egg white (Table 2), and soy (Table 3), are summarized, respectively. In addition, the manufacturing characteristics of several commercial powdered protein hydrolysates discussed in this review are shown in Table 4.

Protein hydrolysates actually have been used in human food for several thousand years. For example, the earliest known ancestor of today's soy sauce, a condiment produced from hydrolyzed soy proteins, was made in China in 160 AD (Shurtleff and Aoyagi, 2012). In recent years, mainly due to the specific health benefits associated with (1) the released bioactive peptides, (2) the reduction of protein allergenicity, and (3) the improved protein digestibility and absorption, the utilization of protein hydrolysates in functional foods and beverages for both protein supplementation and clinical use has significantly increased. Between 2005 and 2010, the global production of protein hydrolysates increased about 32% (Dairymark.com, 2010). The global production of whey protein hydrolysates (WPH), one of the major food protein hydrolysates, is projected to have an annual average growth rate of about 3.4% between 2008 and 2018 (Figure 1B) (Frost and Sullivan, 2012a). In the United States, protein hydrolysate-based baby formula accounted for about 29% of all 2011 sales (Mintel, 2012a). For the specific health benefits from different food protein hydrolysates, the readers can refer to many excellent review articles related to animal sources (Kristinsson and Rasco, 2000; Moskowitz, 2000; Terracciano et al., 2002; Bello and Oesser, 2006; Manninen, 2009; Ahhmed and Muguruma,

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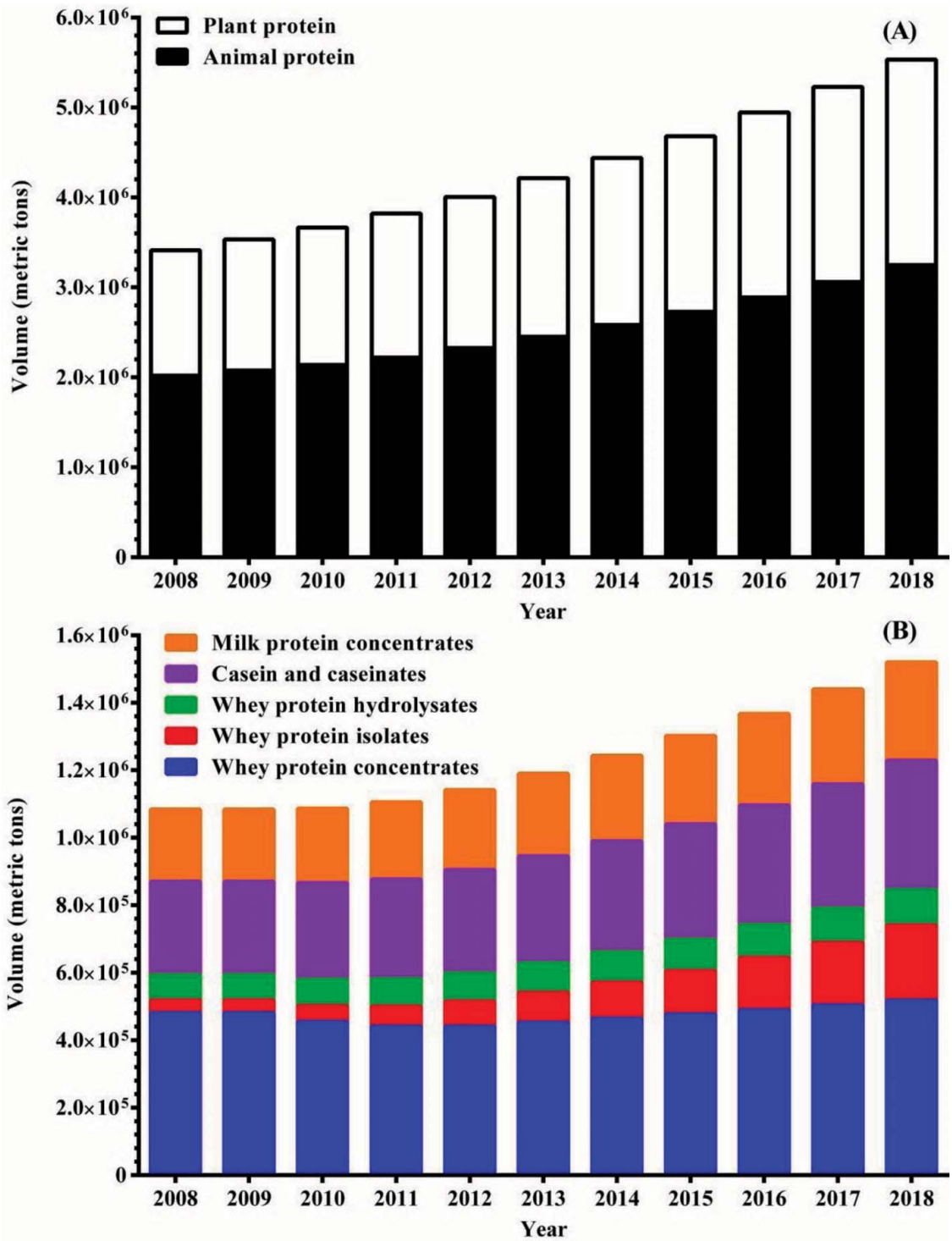

Figure 1 Total market volume of (A) global food protein ingredients and (B) global cow's milk protein ingredients (2008–2018) (Frost & Sullivan, 2012a, b).

2010; Di Bernardini et al., 2011; Herpandi et al., 2011), plant sources (Aluko, 2008; Sun, 2011), or both sources (Kitts and Weiler, 2003; Potier and Tome, 2008; Udenigwe and Aluko, 2012). In addition, protein hydrolysates have been widely used by the food industry to improve the quality of finished products, especially their storage stability. These functionalities are summarized in Table 5.

Although the specific health benefits from different hydrolysates are mostly supportable scientifically, the consistency of these benefits is debatable because of quality changes

Table 1 Major proteins in cow's milk

Protein	% of milk proteins[a]	Major genetic variants[b]	Isoionic point[c]	Isoelectric point[b]	Molecular weight (kDa)[b]	Denaturation temperature (°C)[e]	Sulfhydryl group[f]	Disulfide group[f]
Caseins	78.3					#		
α_{S1}-Casein	32	B	4.92–5.05	4.44–4.76	23.6			
		C	5.00–5.35		23.5			
α_{S2}-Casein	8.4	A			25.2		0	1
β-Casein	26	A^1	5.41		24.0			
		A^2	5.30	4.83–5.07	24.0			
		B	5.53		24.1			
κ-Casein	9.3	A	5.77 (5.35)	5.45–5.77	19.0		0	1
		B	6.07 (5.37)	5.3–5.8	19.0			
γ-Casein	2.4		5.8–6.0[d]					
γ_1-Casein					20.5[d]			
γ_2-Casein					11.8[d]			
γ_3-Casein					11.6[d]			
Whey proteins	19							
β-Lactoglobulin	9.8	A	5.35	5.13	18.4	78	1	2
		B	5.41	5.13	18.3			
α-Lactalbumin	3.7	B	4.2–4.5[d]	4.2–4.5	14.2	62	0	4
Serum albumin	1.2	A	5.13	4.7–4.9	66.4	64	1	17
Immunoglobulin (Ig)	2.4							
IgG	1.8					72	0	32
IgG1			5.5–6.8[d]	5.5–6.8	161			
IgG2			7.5–8.3[d]	7.5–8.3	150			
IgA	0.4				385–417			
IgM	0.2				1000			

[a]Data are from Walstra et al. (2006).
[b]Data are from Farrell et al. (2004).
[c]Data are from Eigel et al. (1984).
[d]Data are from Belitz et al. (2009).
[e]Denaturation temperature in 0.7 M phosphate buffer (pH 6.0). Data are from Dewit and Klarenbeek (1984).
[f]Data are from Owusu-Apenten (2005).
[#]Casein has no characteristic denaturation temperature (Dickinson, 2006).

during storage that complicate digestibility. Storage stability (shelf life stability) of foods is a measure of how long food products retain optimal quality after production (Labuza, 1982).

In general, food products can be classified into three types according to their water activities (a_w) at room temperature, i.e., low moisture foods (LMF, $a_w < 0.6$) such as powdered foods, intermediate moisture foods (IMF, $0.6 \leq a_w < 0.85$) such as high protein nutrition bars (HPNB), and high moisture foods (HMF, $a_w \geq 0.85$) such as protein beverages (Labuza et al., 1972). In this review of more recent studies, we discuss the quality changes occurring in different food protein hydrolysates during storage, and the resulting changes in the structure and texture of three food matrices (LMF, IMF and HMF) as well as the potential solutions to improve storage stability of food protein hydrolysates.

GENERAL MOISTURE SORPTION PROPERTIES

It is well known that the moisture sorption isotherm is an extremely valuable tool for the prediction of potential changes in food stability (Labuza et al., 1970). The moisture sorption isotherm depicts the relationship between equilibrium moisture content and a_w at a constant temperature. In general, different powdered protein hydrolysates show a type II moisture sorption isotherm (Figure 2) that can be modeled well using the Guggenheim–Anderson–deBoer (GAB) equation (*Equation 1*) (Van den Berg and Bruin, 1981; Labuza et al., 1985). The GAB monolayer moisture values (m_0) of different protein hydrolysate systems were similar to their intact protein at room temperature (~23°C, ~6 g H_2O/100 g solid, Table 6), indicating that protein hydrolysis exposes few, if any, new adsorption sites (Zhou and Labuza, 2007). The m_0 is generally around an a_w of 0.2–0.3 (Table 6) (Bell and Labuza, 2000b). It must be noted that the optimal moisture for maximum shelf life is below the GAB m_0 where no aqueous phase reactions take place (Bell and Labuza, 2000a).

$$m = \frac{m_0 k C a_w}{(1 - k a_w)(1 - k a_w + k C a_w)} \quad (1)$$

Where m_0 is the monolayer moisture value, k is a multilayer factor, and C is the surface heat constant.

Table 2 Major proteins in hen egg white*

Protein	% of egg white proteins [a]	Isoelectric point [ab]	Molecular weight (kDa) [abd]	Denaturation temperature (°C) [ac]	Sulfhydryl group	Disulfide group
Ovalbumin	54.0	4.5 (5.1–5.3)	45.0 (42.4)	84.0 (71.5)	4[e]	1[e]
Ovotransferrin	12.0	6.1 (6.2–6.7)	76.0 (85–75)	61.0 (57.3)	0[f]	15[f]
Ovomucoid	11.0	4.1 (5.0–5.3)	28.0 (37.2–43.1)	79.0	0[g]	9[g]
Ovomucin	3.5	4.5–5.0				+i
α_1-Ovomucin			[150]			
α_2-Ovomucin			[220]			
β-Ovomucin			[400]			
Lysozyme	3.4	10.7	14.3 (15.0)	75.0 (81.5)	0[h]	4[h]
Globulin				(72.0)		
Ovoglobulin		(6.1–5.3)				
G2 globulin	4.0	5.5	30.0–45.0	92.5		
G3 globulin	4.0	4.8				
Ovoinhibitor	1.5	5.1 (6.2–6.4)	49.0 (69.5–63.6)			
Ovoglycoprotein	1.0	3.9 (5.0–5.4)	24.4 (37.2–43.1)			
Ovoflavoprotein	0.8	4.0 (5.0–5.2)	32.0 (37.4–43.1)			+
Ovomacroglobulin	0.5	4.5	769			+
Cystatin	0.05	5.1 (6.1)	12.7 (17.0)			+
Avidin	0.05	10.0	68.3	85.0		+

*Table was reprinted with permission from the study of Rao et al. (2012a). Copyright (2012) American Chemical Society.
[a]Data are from Li-Chan et al. (1995).
[b]Data shown in parentheses are from Guerin-Dubiard et al. (2006).
[c]Denaturation temperature in water or buffer. Data shown in parentheses are from Johnson and Zabik (1981).
[d]Data shown in square brackets are from Itoh et al. (1987).
[e]Data are from Fothergill and Fothergill (1970).
[f]Data are from Williams (1982).
[g]Data are from Kato et al. (1987).
[h]Data are from Canfield (1963).
[i] +: protein molecule contains disulfide bonds. Data are from Li-Chan and Kim (2007) and Nagase et al. (1983).

Table 3 Major proteins in soy

Protein	% of soy proteins [ab]	Isoelectric point	Molecular weight (kDa) [ed]	Denaturation temperature (°C) [f]	Sulfhydryl group [gk]	Disulfide group [g]
Glycinin (11S)	36.5–51.0	4.7[c]	300–380	94.1	12–20[#]	5–13
Acidic polypeptides					6/mole glycinin	
A3 chain			42.0		4	
A1,2,4 chains			33.6–37.0		6	
Basic polypeptides			20.7		6/mole glycinin	
β -Conglycinin (7S)	27.8–40.7	4.9–5.0[c]	150–200	76.7	2[#]	0
α' polypeptides			72.0–82.2		1	
α polypeptides			68.0–70.6		1	
β polypeptides			48.4–52.0		0	
γ-Conglycinin	5.0–6.2		163–177[j]			
Basic 7S globulin	3.6	9.1–9.3[i]	168[i]			
Kunitz trypsin inhibitor (2S)	2.9–4.1	3.8[h]	20.1[h]		4[h]	2[h]

[a]Data are from Murphy and Resurreccion (1984).
[b]Data are from Sato et al. (1986).
[c]Data from Koshiyama (1972).
[d]Data from Fontes et al. (1984).
[e]Data from Sathe et al. (1987).
[f]Denaturation temperature in powder equilibrated at 50% relative humidity. Data from Tang et al. (2007).
[g]Glycinin data are from Wolf (1993).
[h]Data are from Koide and Ikenaka (1973).
[i]Data from Sato et al. (1987).
[j]Data from Sato et al. (1984).
[k]Data from Utsumi et al. (1997).
[#]Total sulfhydryl groups.

Table 4 Manufacturing characteristics of several commercial powdered protein hydrolysates

Protein hydrolysates			Degree of hydrolysis (%)	Average molecular weight (kDa)	Free amino acids (%)	Protein (% dry basis)	Sugar (% dry basis)	Fat (% dry basis)	a_w	Moisture content (g H_2O/ 100 g solid)	Reference
Origin	Brand name	Manufacturer*									
Whey	BioZate 1	Davisco	5.2			97.1	0.08	0.3	0.24	6.9	Zhou and Labuza (2007)
	BioZate 3		8.5			95.1		0.3		4.5	Tran (2009)
	BioZate 7		14.9			89.4		0.3		6.0	
	WE 80-M	DMV	16	3.0	2						Netto et al. (1998)
	WE 80-BG		30	0.5	4						
	LE 80-BT		41	2.0	35						
Casein	CAS 90-F	DMV	4	16.7	<1						Netto et al. (1998)
	CAS 90-GBT		23	0.8	13						
	CAS90-STL		44	0.4	17						
Egg	EP-1 #400	Deb-El	7–14	<10		76	0.07		0.29	6.0	Rao and Labuza (2012)

*Davisco: Davisco Foods International, Inc. (Eden Prairie, MN, USA); DMV: DMV International (Lacrosse, WI, USA); Deb-El: Deb-El Food Products, LLC (Elizabeth, NJ, USA).

For formulated foods containing protein hydrolysates, during postproduction (storage and distribution), the external factors impacting shelf life are light intensity, oxygen level, packaging permeability, temperature, and relative humidity, while the intrinsic factors of storage stability are surface hydrophobicity, presence of reducing sugars, moisture content (a_w), pH, glass transition temperature (T_g) and degree of hydrolysis (DH), etc. DH is defined as the proportion of the total number of peptide bonds that are cleaved during hydrolysis and is calculated as follows:

$$\mathrm{DH}(\%) = \mathrm{h}/\mathrm{h}_{\mathrm{tot}} \times 100$$

Where h is the number of hydrolyzed peptide bonds, and h_{tot} is the total number of peptide bonds present which is dependent on the amino acid composition of the raw material

Table 5 Functionality of food protein hydrolysates in the food industry

Type*	Functionality	Origin	Typical reference
General applicable	Flavor	Mollusca	Silva et al. (2011)
	Release bioactive peptides	Meat	Ahhmed and Muguruma (2010)
	Reduction of allergenicity	Casein, whey, soy, rice	Terracciano et al. (2002)
		Pea, bean	Aluko (2008)
	Improved protein digestibility	Whey, casein	Manninen (2009)
		Whey, casein, soy, pea	Potier and Tome (2008)
LMF	Oxidation inhibition	Fish	Thiansilakul et al. (2007)
IMF	Plasticizer	Whey	McMahon et al. (2009)
		Soy	Cho Myong (2010)
	Lipid oxidation inhibition	Egg	Sakanaka et al. (2004)
HMF	Emulsion	Whey	Singh and Dalgleish (1998)
		Whey	Lajoie et al. (2001)
		Whey	Turgeon et al. (1996)
		Milk	Agboola and Dalgleish (1996)
	Lipid oxidation inhibition	Whey, soy	Pena-Ramos and Xiong (2003)
		Potato	Wang and Xiong (2005)
		Fish	Samaranayaka and Li-Chan (2008)
	Microbial inhibitor	Soy	Vallejo-Cordoba et al. (1987)
	Increase of water holding capacity	Fish	Slizyte et al. (2005)
	Decrease rate of protein denaturation	Fish	Khan et al. (2003)
		Crustacean	Zhang et al. (2002)
		Mollusca, crustacean	Yamashita et al. (2003)
	Flavor	Soy	Sun (2011)

*LMF: low moisture foods ($a_w < 0.6$); IMF: intermediate moisture foods ($0.6 \leq a_w < 0.85$); HMF: high moisture foods ($a_w \geq 0.85$).

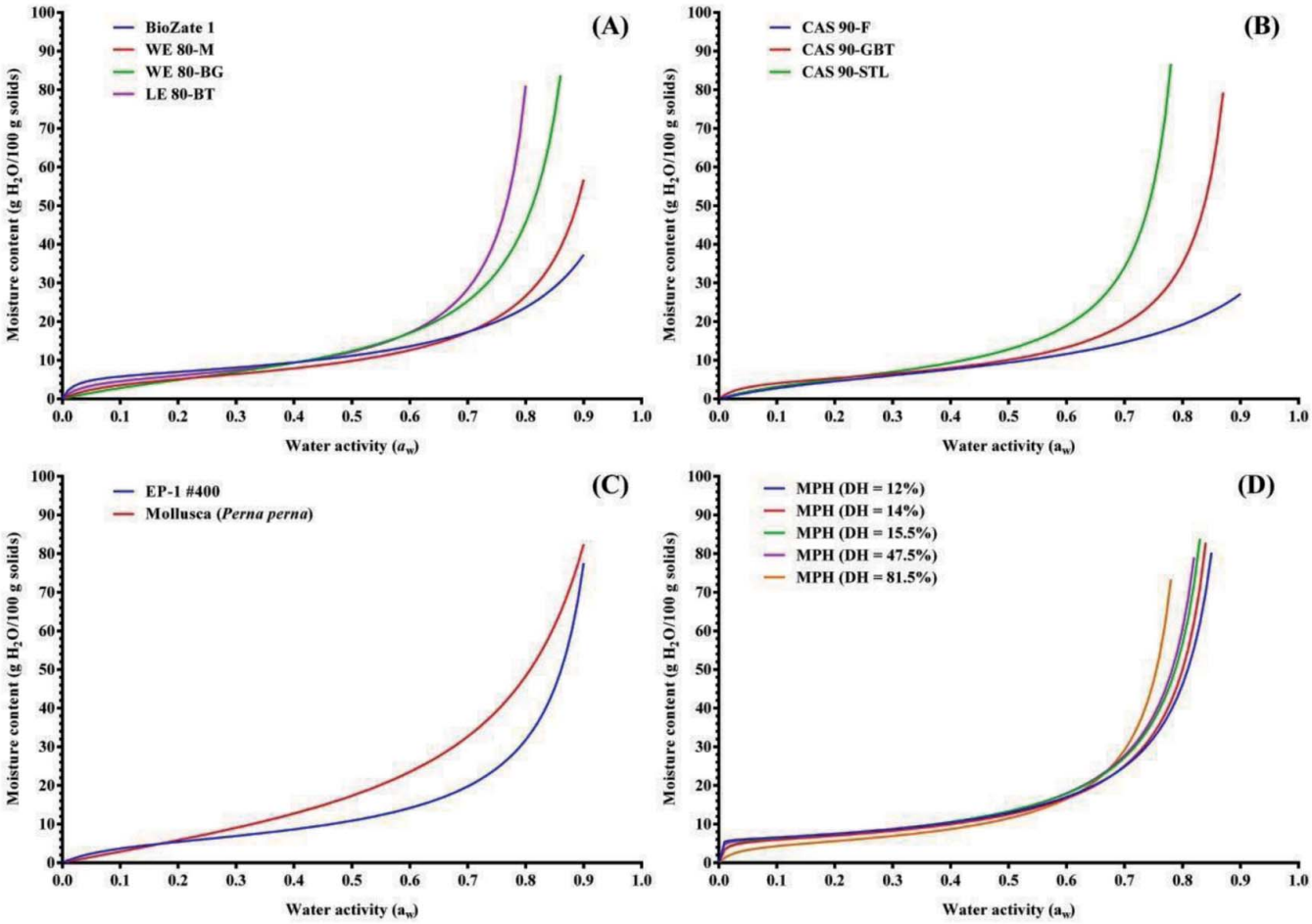

Figure 2 Moisture sorption isotherms of: (A) WPH (BioZate 1, WE 80-M, WE 80-BG and LE 80-BT); (B) casein hydrolysates (CH: CAS 90-GBT and CAS 90-STL); (C) hydrolyzed hen egg white (HEW: EP-1 #400) and hydrolyzed mussel meat from *Perna perna*; (D) myofibrillar protein hydrolysates (MPH) from Nile tilapia (degree of hydrolysis (DH): 12%, 14%, 15.5%, 47.5%, and 81.5%). Note: All the samples were stored at room temperature (23–25°C). (A) Was plotted based on the results of Zhou and Labuza (2007) and Netto et al. (1998). (B) Was plotted based on the results of Netto et al. (1998). (C) Was plotted based on the results of Rao and Labuza (2012) and Silva et al. (2011). (D) Was plotted based on the results of Jardim et al. (1999). The characteristics of these powdered protein hydrolysates are summarized in Tables 4 and 6.

(Nielsen et al., 2001). There is no standard method for determining DH. Instead, many methods have been developed and are commonly used to determine the DH of protein hydrolysates (Rutherfurd, 2010). As seen in Figure 2 (A, B and D), when the a_w is higher than 0.7, the higher the DH, the greater is the moisture holding capacity in the powdered protein hydrolysates. It seems that at the higher DH, more hydrophilic groups in the hydrolyzed protein are exposed. This obviously is part of the increased plasticizing effect at higher DH thereby lowering the T_g.

It is also very clear that the physical changes in food powders are affected by the matrix composition. One of the mechanisms, formation of liquid bridges, is postulated for these changes (Downton et al., 1982; Masuda et al., 2006). A liquid bridge can be formed at the contact point between two particles by moisture condensation due to vapor pressure depression between the particles. This adhesive force is determined by the size of the particle, the surface tension of liquid, the capillary pressure inside the liquid bridge, and the distance between particles (Masuda et al., 2006). Compared with intact proteins, the average molecular weight of protein hydrolysates is usually smaller. Therefore, at the same a_w, it is easier to form a liquid bridge between two hydrolyzed protein particles.

Several studies have reported that when different hydrolyzed protein powders were stored at different temperatures, after short-term storage at different a_ws, similar physical changes in the powder systems, such as agglomeration, stickiness, caking (inter-particle bridging), and structural collapse (flow under the force of gravity), were noted in the range of middle to high a_w (Table 9).

GENERAL GLASS TRANSITION PROPERTIES

It is well known that the physical storage stability parameters of food powders is closely related to the T_g (Levine and

Table 6 Guggenheim–Anderson–de Boer (GAB) equation parameters of several powdered food protein hydrolysates stored at room temperature (23–25°C)

Protein hydrolysates		Degree of hydrolysis (%)	m_0* (g H_2O/ 100 g solid)	a_w at m_0	k*	C*	MAPE*	Reference
Origin	Brand name							
Whey	BioZate 1	5.2	6.1	0.10	0.93	60.4	3.1	Zhou and Labuza (2007)
	WE 80-M	16	5.2	0.25	1.01	14.4	4.6	Netto et al. (1998)
	WE 80-BG	30	6.8	0.30	1.07	4.9	2.1	
	LE 80-BT	41	5.2	0.13	1.17	26.1	4.8	
Casein	CAS 90-F	4	6.4	0.31	0.86	6.9	1.7	Netto et al. (1998)
	CAS 90-GBT	23	4.8	0.13	1.08	24.9	3.5	
	CAS 90-STL	44	5.6	0.33	1.20	7.2	3.4	
Egg	EP-1 #400	7–14	5.7	0.22	1.03	11.9	3.7	Rao and Labuza (2012)
Chicken	LM*	N/A*	14.1		0.33	5.8		Kurozawa et al. (2009)
Mollusca	LM	N/A	13.7		0.94	2.3		Silva et al. (2011)
Fish	LM	12	5.9	0.03	1.09	963.2		Jardim et al. (1999)
		14	5.6	0.07	1.11	136.9		
		15.5	5.9	0.08	1.12	119.1		
		47.5	5.8	0.04	1.13	420.1		
		81.5	4.7	0.13	1.20	30.8		

*m_0 is the monolayer moisture value; k is a multilayer factor; C is the surface heat constant; MAPE: mean absolute percentage error; LM: laboratory-made; N/A: not available.

Slade, 1986). The T_g is the temperature and corresponding moisture point, below which at that moisture content, a product is glassy. Such a powder would be free flowing. Raising the temperature and/or increasing the moisture content to a point above the T_g brings the powder into the rubbery state, converting the system from a free flowing powder into a rubbery system with high hydrophilic surface interactions causing stickiness, caking, and eventually flow induced by gravity (Roos and Karel, 1990; Slade and Levine, 1991; Peleg, 1993; Netto et al., 1998; Labuza and Labuza, 2004). It should be clarified that moisture itself has effect on T_g, which is mentioned below and different from the effect of storage temperature.

Since the T_g of a food product is an important parameter to determine its storage stability, many different models have been developed to predict this value (Khalloufi et al., 2000; Katkov and Levine, 2004). Among these prediction models, the Gordon–Taylor equation (*Equation 2*) (Gordon and Taylor, 1952) has several advantages: (1) it recognizes a food product as a binary mixture (water and solids); (2) it is easy to calculate; (3) it requires knowledge of only a minimum number of easily measurable parameters; and (4) it has a good estimate of

Table 7 Gordon–Taylor equation parameters of several powdered food protein hydrolysates stored at room temperature (23–25°C)

Protein hydrolysates		Degree of hydrolysis (%)	T_{gs}*	K*	MAPE*	Reference
Origin	Brand name					
Whey	BioZate 1	5.2	138.9	3.04		Zhou and Labuza (2007)
	BioZate 3	8.5	157.6	4.69		
	BioZate 7	14.9	142.3	4.60		
	WE 80-M	16	119.4	6.83		Netto et al. (1998)
	WE 80-BG	30	73.03	3.91		
	LE 80-BT	41	87.02	4.46		
Casein	CAS 90-GBT	23	108.0	5.27		Netto et al. (1998)
	CAS 90-STL	44	68.6	3.75		
Egg	EP-1 #400	7–14	118.9	4.38	5.9	Rao and Labuza (2012)
Chicken	LM*	N/A*	44.4	2.59		Kurozawa et al. (2009)
Mollusca	LM	N/A	64.4	3.60		Silva et al. (2011)
Fish	LM	12	59.2	2.11		Jardim et al. (1999)
		14	73.9	2.60		
		15.5	67.4	2.05		
		47.5	132.7	5.32		
		81.5	N/A	N/A		

*T_{gs} is the Glass transition temperature of the solid component; K is a constant; MAPE: mean absolute percentage error; LM: laboratory-made; N/A: not available.

the experimental data in most cases (Hancock and Zografi, 1994). Therefore, it has been widely used in many food studies (Table 7).

$$T_{g,blend} = \frac{w_1 T_{g1} + K w_2 T_{g2}}{w_1 + K w_2} \quad (2)$$

Where $T_{g,blend}$ is the T_g of the binary mixture; w_1 and w_2 are the weight fractions of the components; T_{g1} and T_{g2} are the T_gs of the components; K is a constant. It can be modified as shown in *Equation 3* to predict the effect of moisture content on the T_g of a food product. T_{gs} is the T_g of the solid component in its dry form; w_w is the weight fraction of water. The commonly accepted T_g of pure water, i.e., T_{g2} in *Equation 2*, is −135°C (Johari et al., 1987). It must be noted that the T_g of pure water is still uncertain, it is sometimes taken as a fitting parameter in the Gordon–Taylor equation (Velikov et al., 2001; Le Meste et al., 2002; Katkov and Levine, 2004). The T_g curve for several food protein hydrolysates using this model is shown in Figure 3.

$$T_{g,blend} = \frac{(1 - w_w) T_{gs} - 135 K w_w}{(1 - w_w) + K w_w} \quad (3)$$

In addition, both the physical and chemical reaction rates are increased with an increase in moisture content because the water molecule plasticizes the amorphous structure increasing particle mobility and causes the T_g of the food matrix to decrease below the storage temperature (Pittia and Sacchetti, 2008). With greater moisture content or higher temperature in the rubbery zone, more reactants dissolve and their mobility increases, resulting in faster reaction rates (Bell, 2007).

As seen in Figure 3A, it is commonly accepted that the higher the DH, the smaller the average molecular weight, and the lower the T_g. However, it must be mentioned that several studies did not follow this relationship (Figure 3B, C and D). Actually, this relationship to some extent depends on (1) the instrument used and (2) the experimental analyst whether he/she can correctly determine the T_g of the food sample, especially when the food sample has high moisture content.

It has been reported that protein hydrolysis can dramatically decrease the T_g (Rao and Labuza, 2012). For example, at a_w 0.844, the difference of T_g between intact hen egg white powder (64°C) and hydrolyzed hen egg white powder (HEW, −48°C) is 112°C (Rao and Labuza, 2012). This makes the powder system very unstable and subject to the results of increased molecular mobility (Netto et al., 1998; Zhou and Labuza, 2007). In amorphous powdered protein hydrolysates, the resistance to flow (storage modulus or local viscosity) has an inverse function for the difference between the storage temperature ($T_{storage}$) and T_g ($\Delta T = T_{storage} - T_g$) (Aguilera et al., 1995; Netto et al., 1998; Labuza et al., 2004). At very low moisture content, powdered protein hydrolysates generally exist in the amorphous glassy state, i.e., the T_g is well above room temperature (Figure 3) (Chuy and Labuza, 1994). Hydrophilic agglomeration (hydrogen bonding) generally dominates at 10 to 20°C above the T_g. Agglomeration is associated under conditions at which the force required to stir food powders increases dramatically because of stickiness (Chuy and Labuza, 1994; Aguilera et al., 1995). As more moisture is gained or the product is stored longer, the second stage–caking occurs. Caking involves recrystallization of the sugars (e.g., lactose or sucrose) and forms physical bridges between the particles which upon drying makes the system very rigid. It generally occurs about 20 to 40°C above the T_g depending on the types of sugars present. Collapse is the stage where the powder loses its structure and begins to flow. It usually occurs about 60°C above the T_g (Labuza et al., 2004; Rao and Labuza, 2012). For example, combined with the glass transition diagram of a commercial HEW (Figure 3E), these visible physical changes during storage can be explained clearly (Figure 4B).

CHEMICAL REACTIONS DURING STORAGE

Nonenzymatic browning (NEB) has been observed in different powdered protein hydrolysates during storage at medium to high a_w, indicating that the Maillard reaction occurred, even when only small amounts of residual reducing sugars were present (Netto et al., 1998; Rao and Labuza, 2012; Rao et al., 2012b). These changes usually occur over time as a function of increased storage temperature and relative humidity. For example, the effect of moisture content on the color change in HEW after four months of storage at 23°C is shown in Figure 4. It was noted that although the amount of residual glucose (reducing sugar) in HEW involved in the Maillard reaction is very small ($\leq$ 0.07%) (Rao and Labuza, 2012), it has significant impact on product quality.

During product processing, including hydrolysis and subsequent heat treatments (pasteurization and spray-drying), the extent of peptide aggregation is influenced by the type of enzyme used (Otte et al., 1997; Otte et al., 2000; Groleau et al., 2003a; Spellman et al., 2005; Creusot and Gruppen, 2007a), the hydrolysis time (Su et al., 2008), acidic pH (Groleau et al., 2003b), the DH, temperature and ionic strength (Creusot et al., 2006), high pressure (Penas et al., 2004; Quiros et al., 2007; Bruins et al., 2009), and the presence of other proteins (Creusot and Gruppen, 2007b, 2008).

During postproduction, peptide aggregates also have been observed in different powdered protein hydrolysates obtained from hen egg white and soy proteins (Table 9). The term "aggregates" refers to any self-associated state of proteins/peptides, involved in covalent bonding, that is effectively irreversible under the conditions it forms (Weiss et al., 2009). It must be noted that during *in vitro* studies, protein/peptide aggregates can be classified into two categories based on their solubility in the selected buffer: either soluble or insoluble. For

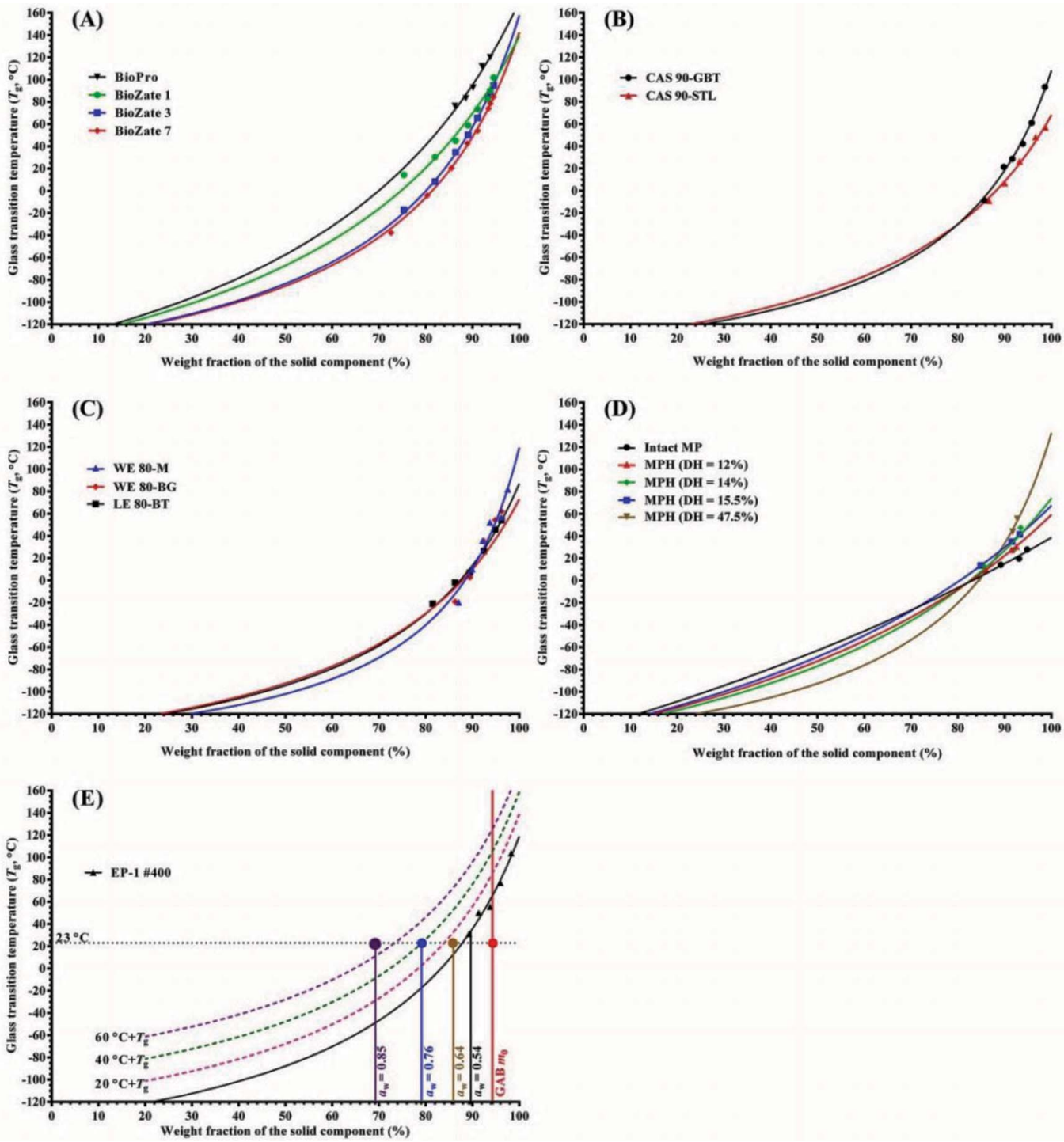

Figure 3 The glass transition diagrams of: (A) WPI (BiPro) and WPH (BioZate 1, BioZate 3 and BioZate 7); (B) casein hydrolysates (CH: CAS 90-GBT and CAS 90-STL); (C) WPH(WE 80-M, WE 80-BG and LE 80-BT); (D) intact myofibrillar protein (MP) from Nile tilapia and its hydrolysates (MPH, the degree of hydrolysis (DH) were 12%, 14%, 15.5%, and 47.5%, respectively); (E) hydrolyzed hen egg white (HEW: EP-1 #400). Note: (A) was plotted based on the results of Zhou and Labuza (2007) and unpublished results from Dr. Labuza. (B) and (C) were plotted based on the results of Netto et al. (1998). (D) Was plotted based on the results of Jardim et al. (1999). (E) Was reprinted and modified from the study of Rao and Labuza (2012) with permission from Elsevier Ltd. The characteristics of these powdered protein hydrolysates are summarized in Tables 4 and 7. BiPro, whey protein isolate (WPI), was obtained from Davisco Foods International, Inc. (Eden Prairie, MN, USA).

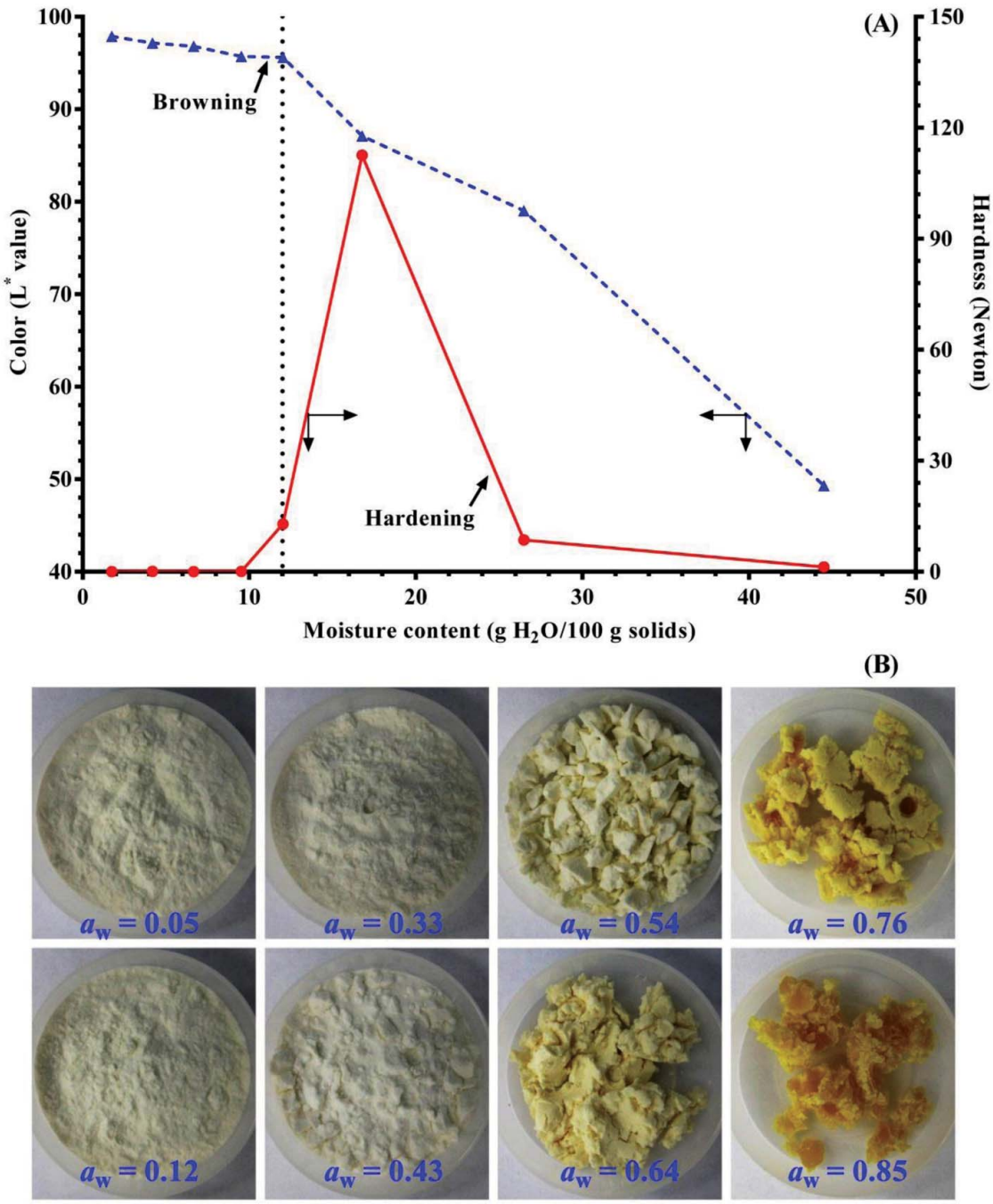

Figure 4 (A) Effect of moisture content on the color (L^* value) and hardening of hydrolyzed hen egg white (HEW: EP-1 #400) after four months of storage at 23°C. The vertical dotted line indicates the minimum moisture content (12.0%, $a_w = 0.54$) that showed the peptide aggregation. (B) Images of color changes in HEW after four months of storage at 23°C. Note: Figure was reprinted and modified from the study of Rao and Labuza (2012) with permission from Elsevier Ltd.

example (Zhou et al., 2008b), in Table 8, after the addition of sodium dodecyl sulfate (SDS), guanidine HCl, or urea, the solubility of phosphate buffer (PB)-insoluble aggregates increased slightly compared with the control, i.e., PB. This suggested that neither hydrophobic interactions nor hydrogen bond formation was the major factor causing protein aggregation in this protein/buffer dough model. However, after the addition of dithiothreitol (DTT), more than 90% PB-insoluble aggregates were dissolved, indicating that the formation of intermolecular disulfide bonds played an important role in protein aggregation during storage at 35°C for three weeks (Table 8). It must be noted that the digestibility of these buffer-soluble and buffer-insoluble aggregates still needs to be confirmed through *in vivo* studies as this is critical to ensure

Table 8 Solubility of buffer-insoluble whey protein aggregates* in different buffers containing denaturing and/or reducing chemicals†

Buffer#	Solubility of whey protein aggregates (% ± SD)
10 mM phosphate buffer (PB, pH 7.0)	4.4 ± 0.6
PB with 0.1% SDS (g/mL)	8.2 ± 0.8
PB with 6 M guanidine HCl	10.9 ± 0.7
PB with 8 M urea	11.6 ± 1.7
PB with 10 mM DTT	92.2 ± 0.9
PB with 0.1% SDS (g/mL) and 10 mM DTT	97.1 ± 1.7

†Table was reprinted and modified with permission from the study of Zhou et al. (2008b). Copyright (2008) American Chemical Society.
*The buffer-insoluble aggregates refer to those formed in a protein/buffer dough system ($a_w = 0.98$) after storage at 35°C for three weeks. The buffer used was 10 mM phosphate buffer (PB, pH 7).
#HCl: hydrochloric acid; SDS: sodium dodecyl sulfate; DTT: dithiothreitol.

that the protein/peptide bioactivity is preserved. Unfortunately, the studies related to the influence of peptide aggregation during storage on the biofunction availability of bioactive peptides in the food products are very limited.

During storage, moisture-induced aggregation of powdered protein hydrolysates also can result in dramatic changes in their structure and matrix texture (Netto et al., 1998; Zhou and Labuza, 2007; Lv et al., 2009; Rao and Labuza, 2012). When the relative humidity is high, moisture-induced aggregates in protein hydrolysates can form physically (noncovalent interactions) and/or chemically (covalent interactions). For noncovalent interactions, there is a positive correlation between the hardness (protein/peptide aggregation) and the surface hydrophobicity of protein hydrolysates. The hydrophobicity mainly depends on the amount of hydrophobic peptides after hydrolysis. This can also significantly affect their water solubility. For covalent interactions, mainly depending on the amount of (1) sulfhydryl and disulfide groups and (2) carbonyl groups in the powdered protein hydrolysates, the presence of moisture can induce two above-mentioned chemical reactions. One is the disulfide interaction; another is the Maillard reaction. For example, after four-month storage at different a_ws at 23°C, in the range of a_w 0.54 to 0.64, aggregation increased the hardness of HEW significantly mainly due to the hydrophilic and disulfide interactions (Figure 4). In the range of a_w 0.74 to 0.84, Maillard reaction-induced aggregates could form through peptide polymerization (Rao and Labuza, 2012). In addition, it was assumed that the Maillard reaction and/or its resulting products might have a negative influence on intermolecular disulphide bonds (Rao and Labuza, 2012; Rao et al., 2012b). It is noted that many proteins in these foods contain sulfhydryl groups and/or disulfide bonds, although the relevant number differs and little is known about their distribution in peptides (Tables 1–3). Compared to whey, hen egg white and soy proteins, casein has the fewest number of sulfhydryl and disulfide groups (Tables 1–3), which could be the reason that casein hydrolysates are relatively stable in relation to physicochemical changes as compared to WPH under abusive storage conditions (Netto et al., 1998).

Another chemical reaction, lipid oxidation, can occur in formulated food matrices during storage, especially LMF and IMF systems. In general, lipid oxidation shows a minimum in the 0.2 to 0.35 a_w range (around the GAB m_0) and increases in rate on both sides (Labuza, 1971). However, when the formulated food products contain protein hydrolysates, the negative effect of lipid oxidation maybe reduced significantly during storage because many protein hydrolysates have been reported to exhibit antioxidative activity (Table 5). Even so, the antioxidative ability of different protein hydrolysates still needs to be confirmed experimentally. However, it must be noted that fish protein hydrolysates are prone to oxidation due to the high content of unsaturated fatty acids (Sohn et al., 2005; Yarnpakdee et al., 2012a; Yarnpakdee et al., 2012b).

LOW-MOISTURE FOODS (LMF)

As mentioned above, the a_w of LMF is usually much less than 0.6. Obviously, food protein powders belong in this category. In 2012, the market size of global protein powder for sports nutrition will exceed $4.5 billion (Figure 5A) (Euromonitor International, 2012). In 2011, more than 76% of the sales of baby formula in the United States ($3.7 billion) is powder, of which more than 32% of the turnover contains protein hydrolysates (Figure 6), mainly WPH (79%) (Mintel, 2012a). For the physicochemical changes during storage in LMF containing protein hydrolysates, such as powdered protein hydrolysates, the reader can refer to the previous sections in this review and Table 9. It must be noted that for LMF the optimal moisture for maximum shelf life is below the GAB m_0 where no aqueous phase reactions take place (Bell and Labuza, 2000b). If kept below the moisture content for the T_g at room temperature, several physicochemical changes, i.e., stickiness, caking, and collapse, can be prevented in LMF (Labuza and Labuza, 2004).

INTERMEDIATE-MOISTURE FOODS (IMF)

IMF are products with a moderate moisture content and a moderate a_w created to be shelf-stable without refrigeration (Pavey and Schack, 1969; Karel and Heidelbaugh, 1973; Taoukis et al., 1988). IMF's moisture is generally in the range of 10 to 40%, and its a_w is generally from 0.6 to 0.85 at room temperature (Labuza et al., 1972; Erickson, 1982; Taoukis and Richardson, 2007).

In recent years, HPNB is a rapidly growing sector of the sports nutrition, muscle building, health supplement, and weight reduction markets (Wright, 2011; Mintel, 2012b). The global market size of protein bars for sports nutrition is projected to grow at an annual average growth rate about 9.8%

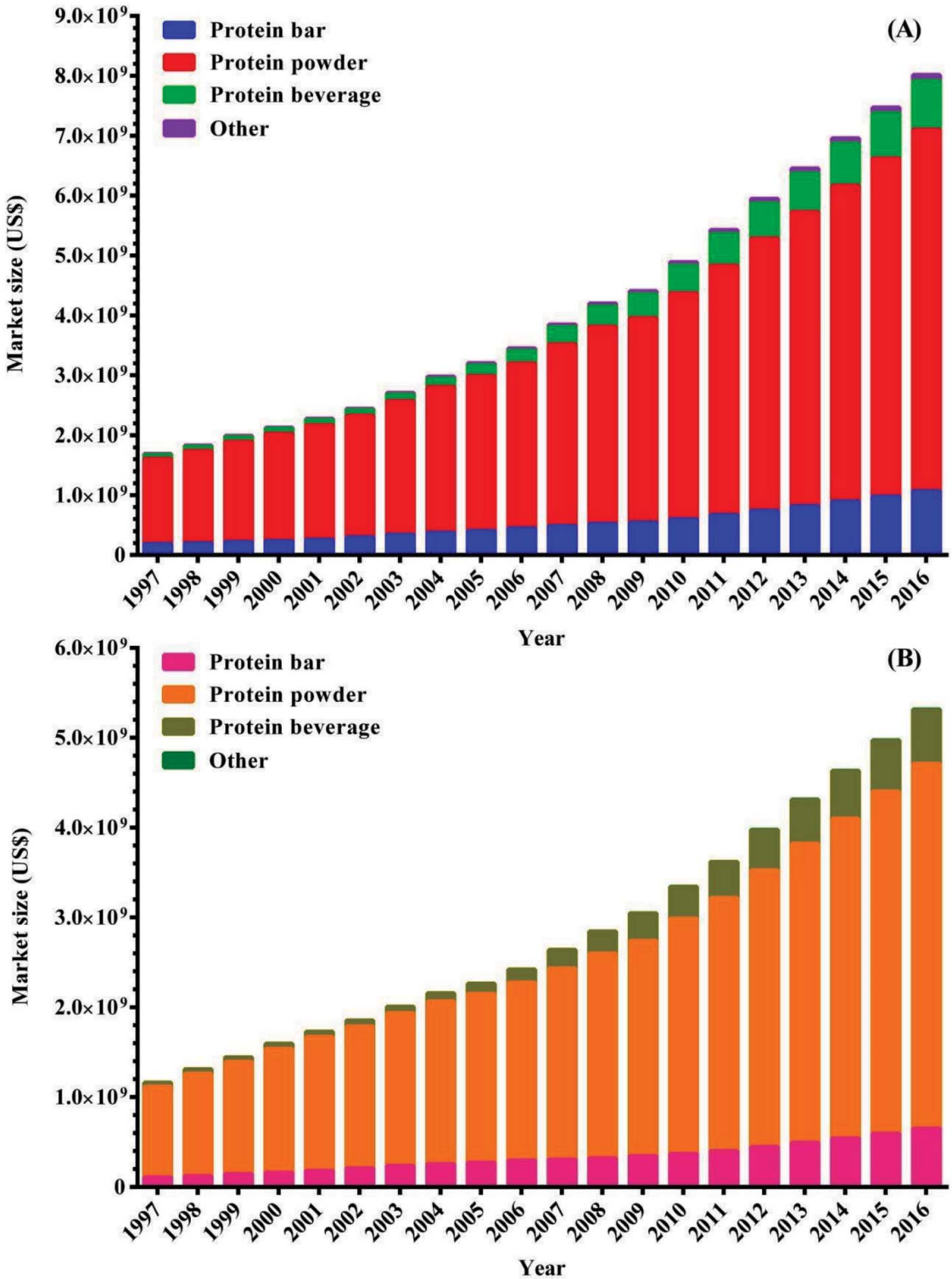

Figure 5 Total market size of (A) global and (B) the US protein production for sports nutrition (1997–2016) (Euromonitor International, 2012).

between 1997 and 2016 (Figure 5A) (Euromonitor International, 2012). Most of the commercial bars fit into the IMF category, and are generally comprised of proteins, various carbohydrates, and other plasticizers (glycerol, maltitol, sorbitol and xylitol) (Liu et al., 2009). HPNB are typically formulated to have an a_w of about 0.6 at room temperature to ensure microbial stability (Davis, 2005; Hazen, 2010). One major problem for commercial HPNB is that they generally

Table 9 Storage stability of food protein hydrolysates in low moisture foods (LMF, $a_w < 0.6$)

	Sample information			Storage conditions				
Origin	Method of hydrolysis	Degree of hydrolysis (%)	Peptide sequence	Time	Temp (°C)	a_w	Major results	Reference
Milk	Fermentation	N/A*	VPP IPP	six months	15–22.5	N/A	• The concentrations of two tripeptides in the powdered fermented milk remained constant after six months of storage at room temperature	Kurosaki et al. (2005) Maeno et al. (2005a) Maeno et al. (2005b) Matsuura et al. (2005) Mizuno et al. (2005)
Whey	Enzyme	16–41	N/A	two–three weeks	22	0.05–0.85	• After one week, varied from hard to a gummy mass and liquefied as a_w increased • The color varied from cream to dark tan • At a_w 0.55, showed some stickiness	Netto et al. (1998)
	Enzyme	5.2	N/A	two weeks	23, 45	0.11–0.85	• Protein solubility remained constant when $a_w < 0.6$	Zhou and Labuza (2007)
Casein	Enzyme	4–44	N/A	two–three weeks	22	0.05–0.85	• Small changes in structure as the a_w increased, varying from powdery to hard • At a_w 0.55, presented some shrinkage in volume and slight hardness • The appearance of the samples did not change after one week under the same storage conditions	Netto et al. (1998)
Egg	Enzyme	7–14	N/A	seven months	23	0.05–0.85	• When moisture content ≥ 12% (dry basis), both color and hardness changed dramatically • Noncovalent bonding and covalent interactions (disulfide interaction and the Maillard reaction) resulted in moisture induced aggregates in the hydrolyzed protein	Rao and Labuza (2012)
	Enzyme	7–14	N/A	two months	45	0.05–0.79	• Structural changes occurred including agglomeration, stickiness and collapse when the storage temperature was greater than the T_g. • A first-order hyperbolic model fit for the change in three storage quality parameters. • The reduction in the remaining free amino groups was about 5% at a_w 0.50 after one month of storage. • Significant quality loss was found at $a_w > 0.31$	Rao et al. (2012b)

(Continued on next page)

Table 9 Storage stability of food protein hydrolysates in low moisture foods (LMF, $a_w < 0.6$) *(Continued)*

	Sample information			Storage conditions				
Origin	Method of hydrolysis	Degree of hydrolysis (%)	Peptide sequence	Time	Temp (°C)	a_w	Major results	Reference
Fish	Enzyme	60	N/A	six weeks	4, 25	N/A	• The antioxidative activities and solubility of round scad protein hydrolysates slightly decreased • Yellowness of the protein hydrolysates became more intense as the storage time increased but the rate of increase was more pronounced at 25°C than at 4°C	Thiansilakul et al. (2007)
	Enzyme	23.8–44.7	N/A	three months	20	N/A	• Color and nonenzymatic browning measurements indicated significant darkening during storage • The formation of brown pigments may result from aldol condensation of carbonyls produced from lipid oxidation upon reaction with basic groups in protein	Hoyle and Merritt (1994)
Soy	Enzyme	N/A	N/A	44 days	−20	N/A	• During storage, some high molecular weight peptides formed from the original soy protein hydrolysates (SPH). The content of the newly formed high molecular weight peptides produced from the highly hydrophobic SPH was considerably large • The results suggested that hydrophobic interaction may promote the aggregation of SPH during storage	Lv et al. (2009)

*N/A: not available.

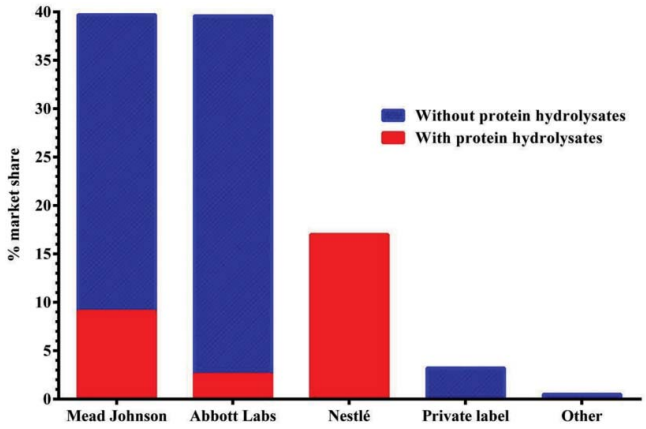

Figure 6 Selected brand sales of baby formula in the United States in 2011 (Mintel, 2012a).

become harder over time without moisture loss, making the product unacceptable to consumers (Ahmed, 2004; Hazen, 2010; Berry, 2011; Hutchinson, 2009; Wade, 2005). Recently, several possible mechanisms related to moisture-induced bar hardening during storage have been elucidated. One chemical mechanism is the above-mentioned protein–protein interactions through disulfide bond formation/exchange and/or non-covalent interactions, resulting in formation of protein aggregates (Zhou et al., 2008a, 2008b; Liu et al., 2009; Zhu and Labuza, 2010; Rao et al., 2012a, 2013). Several other studies stated that during storage, changes in molecular mobility and changes in microstructure of protein bars driven by moisture migration might play an important role for hardening (Taillie, 2006; Li et al., 2008; Loveday et al., 2009). One study suggested that phase separation into large protein-rich and protein-depleted aqueous regions could be the mechanism

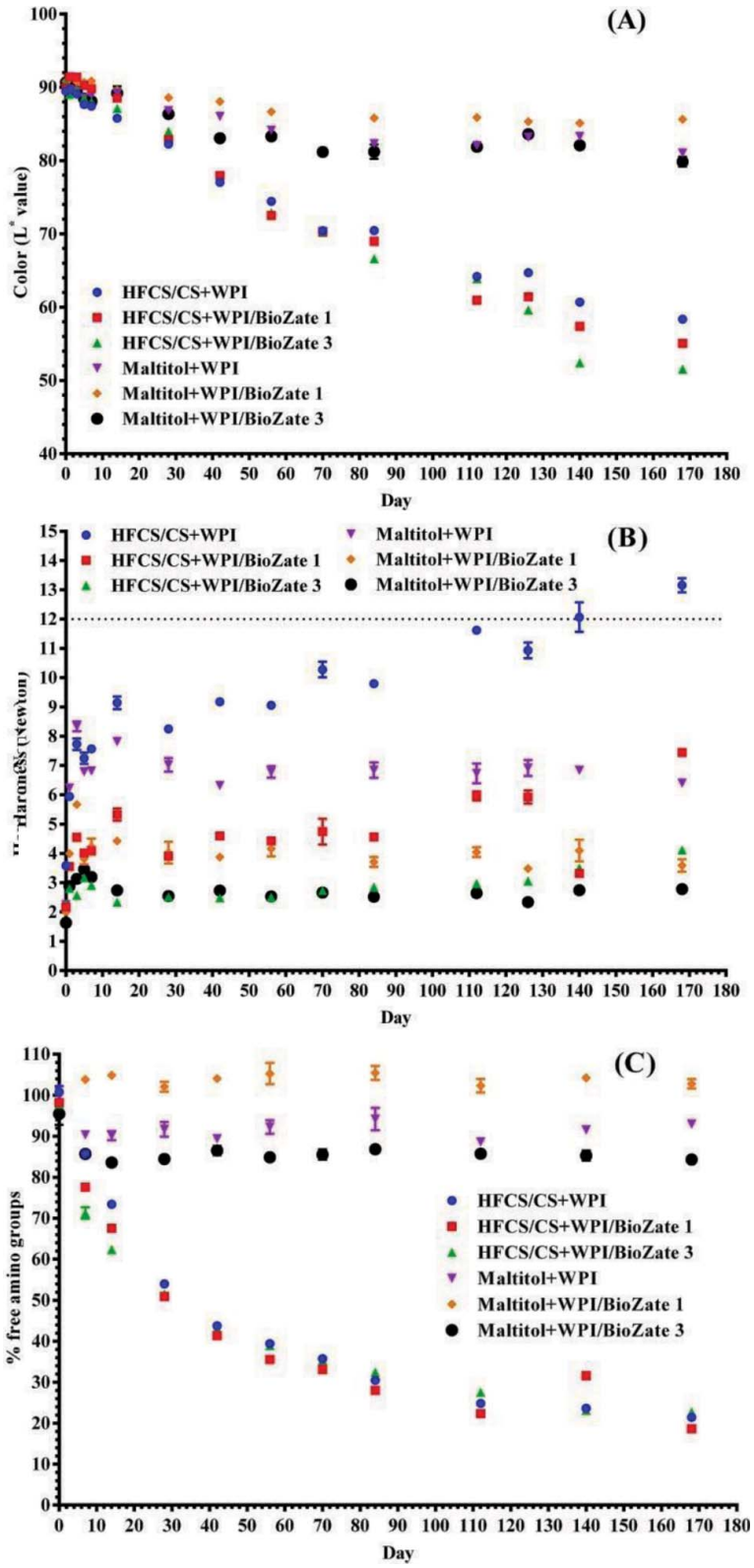

Figure 7 Effect of storage time at 23°C on (A) the color, (B) the hardness, and (C) the free amino groups of six protein bar model systems (Tran, 2009). The dotted line (B) indicates 12 N. The bar models ($a_w = 0.61$) contained 35% protein (WPI:WPH = 26.25:8.75), 50% sugar (either HFCS/CS [25% HFCS + 25% CS] or maltitol), 5% glycerol, and 10% shortening (g/g). WPI: whey protein isolate (BiPro obtained from Davisco); WPH: whey protein hydrolysates (BioZate 1 and BioZate 3); HFCS: high fructose corn syrup; CS: corn syrup.

Table 10 Storage stability of food protein hydrolysates in intermediate moisture foods (IMF, $0.6 \leq a_w < 0.85$)

	Sample information						Storage conditions			
Origin	Method of hydrolysis	Degree of hydrolysis (%)	Concentration (%, w/w)	Reducing sugar (%)	a_w	Matrix	Time	Temp (°C)	Major results	Reference
Whey	N/A*	N/A	9.5–38	0, 43	0.59–0.69	Bar	36 days	32	• Extent of browning was HWPI/HFCS bars > WPI/HFCS bars > HWPI/SS bars > WPI/SS bars.# • Bars made with partially hydrolyzed protein powders remained soft, especially when the carbohydrate was sorbitol rather than the glucose and fructose in HFCS	McMahon et al. (2009)
	Enzyme	5–8.5	8.75	0, 25	0.61–0.68	Bar	6 months	23, 35, 45	• By replacing 25% (g/g) of WPI with WPH, the hardening rate was significantly lowered in the HFCS/CS model systems stored at 45°C • The use of WPH resulted in increased browning in the HFCS/CS model systems • The HFCS/CS+WPI/WPH experienced the fastest loss rate of free amino groups due to an increase in molecular mobility from the use of hydrolysates	Taterka (2009) Tran (2009)
Egg	Enzyme	7–14	N/A	< 0.07	0.81–0.85	Dough	70 days	23, 35, 45	• The addition of HEW could effectively reduce the dough hardening due to the decrease in the T_g of the IMF matrix • The addition of hydrolyzed protein could decrease the storage stability mainly due to the Maillard reaction.	Rao et al. (2013)

*N/A: not available.
WPI: whey protein isolate; HWPI: hydrolyzed WPI; HFCS: high fructose corn syrup; SS: sorbitol syrup.

that initiates bar hardening and increases protein–protein interactions (McMahon et al., 2009). In addition, the Maillard reaction could also cause protein aggregation in IMF during storage through reducing sugar-induced formation of covalent bonds (polymerization) if reducing sugars are present in any ingredients or directly added (Labuza, 1980; Kato et al., 1990; Chevalier et al., 2001).

In order to solve bar-hardening problems, one can substitute some of the protein with protein hydrolysates, which serve as a plasticizer to increase the bar softness (Table 5 and Figure 7B). In general, unacceptable hardness occurs where force exceeds 12 Newton (N). As seen in Figure 7B, the hardness of the HFCS/CS+WPI bar model reached this point after 140-day storage at 23°C. Substituting in WPH, either BioZate 1 or BioZate 3, showed the successful reduction of bar hardness for six months (Tran, 2009). This effect was more obvious when substituting in WPH with higher DH, i.e., BioZate 3 (Figure 7B). This is an effective way to lower the overall T_g of the final product, as discussed previously, 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 caused by higher local viscosity (Figure 7B). 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).

One major problem related to IMF containing protein hydrolysates is that moisture-induced Maillard browning can occur during storage if reducing sugars are present (Figures 7A and 8). As seen in Figure 7A, substituting HFCS/CS (high fructose corn syrup/corn syrup, reducing sugars) with maltitol (sugar alcohol) eliminated a significant increase in darkening (lower L^* value) (Tran, 2009). Sugar alcohols also help maintain bar softness (Figure 7B). The maltitol+WPI bar model was harder than the two maltitol+WPI/BioZate systems (7 N vs. 3 N), but remained below 12 N during the 6-month storage at 23°C. Compared with bar hardening, darkening related to the Maillard reaction is seldom noticed by consumers. The major reason is that these undesirable changes are usually masked intentionally or accidentally by other added ingredients in IMF such as chocolate or caramel. Several studies have reported that protein bars containing WPH remained soft throughout storage yet had excessive browning and became black when HFCS was used (Table 10). In addition, as the Maillard reaction is largely responsible for the loss of free amino groups in IMF, its loss rate can be increased significantly during storage due to an increase in molecular mobility from the use of protein hydrolysates (Figure 7C). This quality loss may eventually lead to reduction of protein quality, such as lysine, an essential amino acid which becomes nutritionally unavailable. This may also cause loss of the claimed biofunction of protein hydrolysates in the products. Unfortunately, to the best of our knowledge, currently, the *in vivo* study related to biofunction quality of protein hydrolysates during storage in both LMF and IMF is very limited.

HIGH-MOISTURE FOODS (HMF)

HMF's moisture is generally greater than 40%, and its a_w is from 0.85 to 1.0 at room temperature (Labuza et al., 1972). In this range, bacteria including pathogens can grow so some pasteurization or sterilization may be needed during processing. Obviously, protein beverages belong in this category. Similar to HPNB, recently, the global market size of protein beverage for sports nutrition also increases rapidly, which is projected to grow at an annual average growth rate of about 14.6% between 1997 and 2016 (Figure 5A) (Euromonitor International, 2012). The typical HMF containing protein hydrolysates can be cheese, salad dressing, yogurt, and beverages (Table 11). To maximize their shelf life, these HMF products are usually required by the manufacturers to store under refrigerated condition (4–8°C) during postthermal processing. Besides storage temperature, the storage stability of HMF containing protein hydrolysates also depends on the peptide sequence, pH, and food matrix (Table 11). As mentioned above, the surface hydrophobicity of protein hydrolysates can significantly affect their water solubility. To prevent coagulation or reduce aggregates (soluble and/or insoluble) in HMF, it is worth using the hydrophilic fraction of protein hydrolysates, especially for high-value HMF products such as liquid baby formula (Table 11). There is a need to optimize the product processing procedure including the selection of enzyme for hydrolysis and the separation of insoluble protein particles.

It must be noted that some bioactive peptides in HMF may be degraded partial or totally by bacteria such as lactic acid bacteria in yogurt during fermentation, depending on the peptide sequence, the bacterial strain, and pH (Paul and Somkuti, 2009, 2010). To limit the overall extent of proteolysis, the bioactive peptides may be added at the end of the process (Paul and Somkuti, 2009). Even so, the susceptibility of bioactive peptides in the finished HMF may be still degradable by the living bacteria during post production (Vaslin, 2008). However, a thermal treatment and peptide encapsulation may avoid this adverse activity (Vaslin, 2008).

RECOMMENDATION FOR IMPROVING STORAGE STABILITY

Formulated food products containing protein hydrolysates constitute a large consumer sector due to consumer demand for high-quality nutritional and functional foods. Compared with their intact proteins, the storage stability of protein hydrolysates is compromised. Depending on the ingredients in the food matrix, several physicochemical reactions can occur during postproduction (storage and transportation), such as hydrophobic interactions, disulfide interactions, and the Maillard reaction (browning and polymerization). These undesirable reactions can lead to significant change in the color and the texture of the product. In addition, it must be stated that the

Table 11 Storage stability of food protein hydrolysates in high moisture foods (HMF, $a_w \geq 0.85$)

	Sample information						Storage conditions			
Origin	Method of hydrolysis	Degree of hydrolysis (%)	Peptide sequence	Concentration (%, w/w)	pH	Matrix	Time	Temp (°C)	Major results	Reference
Milk	Enzyme	N/A*	N/A	0.5, 1 (w/v)	N/A	20% soybean oil in water emulsion	seven days	4	• After about two days of storage, a very small population of very large particles (between 40 and 80 μm) appeared in the emulsion formed with 0.5% caseinate that had been hydrolyzed for 30 minutes	Agboola and Dalgleish (1996)
	Fermentation	N/A	α_{s1}-casein *N*-terminal peptides, f(1–9), f(1–7) and f(1–6).	N/A	5.2	Cheese	16 weeks	10	• ACE# inhibitory activity decreased when proteolysis exceeded a certain level during storage	Ryhanen et al. (2001)
	Synthesis	N/A	FFVAPFPEVFGK, RRWQWRMKKLG	500 μg/ml	4.5	Yogurt starter culture	10 days	4	• The stabilizing effect of refrigeration that apparently prevents or minimizes additional peptide loss caused by proteolysis	Paul and Somkuti (2009)
Whey	Fermentation	N/A	N/A	20 (v/v)	3.99	Beverage	81 days	4	• ACE inhibiting capacity was not affected after 81 days	Rivas et al. (2007)
	Enzyme	8–45	N/A	0.02–5	N/A	3% soybean oil in water emulsion	five days	5	• Emulsions prepared from hydrolysates of DH $\leq$ 20% were stable during storage for up to five days However, the emulsions made with hydrolysates of DH >20% were not stable • Neither high nor low concentrations of hydrolysates were capable of producing long-term stability in these emulsions.	Singh and Dalgleish (1998)
	Enzyme	9.9–13.2	N/A	0.5–1.5	N/A	Salad dressing	six months	4, 25	• Peptidic fractions obtained from tryptic hydrolysates produced the most stable salad dressings (over six months at the 1.0% and 1.5% protein level) with rheological properties similar to a commercial mayonnaise	Turgeon et al. (1996)

(Continued on next page)

Table 11 Storage stability of food protein hydrolysates in high moisture foods (HMF, $a_w \geq 0.85$) *(Continued)*

	Sample information						Storage conditions			
Origin	Method of hydrolysis	Degree of hydrolysis (%)	Peptide sequence	Concentration (%, w/w)	pH	Matrix	Time	Temp (°C)	Major results	Reference
	Enzyme	3.9–9.9	N/A	0.005 (w/v)	6.5	Liquid baby formula	six months	20	• With protein hydrolysate-based formulations, the creaming rate of the fat in the product was slightly higher than in the standard formulation (with carrageenan), which is indicative of lower storage stability • Ultrafiltered tryptic hydrolysates in infant formulas may have contributed to the retardation of the separation of fat in the product and improve their storage stability.	Lajoie et al. (2001)
Casein	Enzyme	N/A	RYLGY, AYFYPEL	4	4.2	Commercial yoghurt	28 days	4	• No significant reduction of either peptide was detected during the shelf-life of the product	Contreras et al. (2011)
	Enzyme	N/A	VPP, IPP	0.03	N/A	Water	nine days	2.5–8	• Concentrations of the tripeptides in dosing suspensions were consistently from 101% to 102.8% of concentrations determined immediately after preparation	Mizuno et al. (2005)
Beef	Synthesis	N/A	GFHI, DFHINQ, FHG, GLSDGEW	0.01	6–8	Water	two months	4	• During storage, no significant difference was detected in both the pH adjusted and the temperature abused (70–100°C, for 20 minutes) samples	Jang et al. (2007)

*N/A: not available.
#ACE: angiotensin-converting enzyme

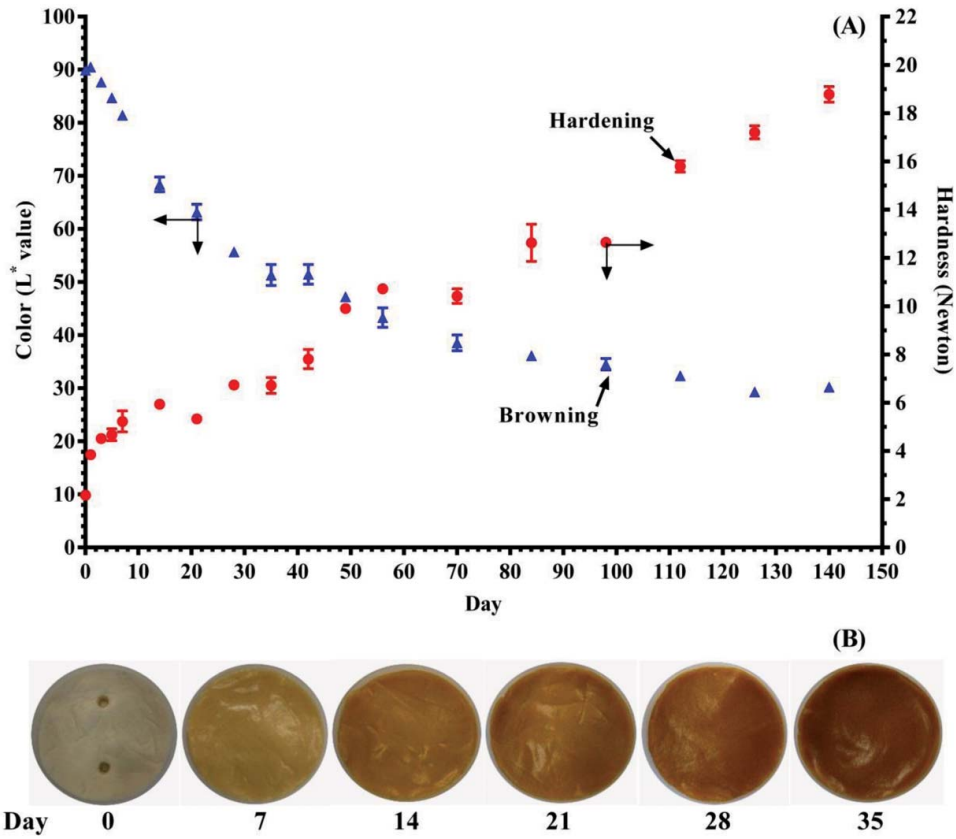

Figure 8 Effect of storage time at 35°C on the color and hardness of a WPI/WPH bar model (26.25% WPI, 8.75% WPH, 25% corn syrup, 25% HFCS, 5% glycerol, and 10% shortening, g/g, $a_w = 0.61$). (B) Images of color changes in the WPI/WPH bar model during storage at 35°C (Tran, 2009). WPI: whey protein isolate (BiPro obtained from Davisco); WPH: whey protein hydrolysates (BioZate 1 and BioZate 3); HFCS: high fructose corn syrup.

higher the DH, the more bitter-tasting the resulting protein hydrolysates may be.

In order to improve the storage stability of food products containing protein hydrolysates, for LMF such as powdered protein hydrolysates, the optimal moisture for maximum shelf life is below the GAB m_0. For IMF such as HPNB containing protein hydrolysates, the problem, bar hardening during storage, should be effectively controlled by substituting with some protein hydrolysates and/or sugar alcohols. However, the Maillard reaction needs to be prevented. That means that the reducing sugar content such as glucose and lactose in the food matrix should be minimized. Therefore, the manufacturers should use the sugar substitutes such as sugar alcohols which do not have residual reducing sugars. In addition, the manufacturers need to control the bitterness of HPNB through optimizing both the DH during protein hydrolysis and the amount of protein hydrolysates in the bar formulation. The food bar industry can also add sugar substitutes into HPNB to mask the bitterness. For LMF and IMF systems, both the moisture sorption isotherm and the glass transition diagram are extremely useful tools for the prediction of potential physicochemical changes in food stability. For HMF such as beverages containing protein hydrolysates, the bioactive peptides added should have high hydrophilicity.

It must be noted that apart from these studies listed in Tables 9–11, very little is known about the effects of storage on protein hydrolysates incorporated into foods. As more and more food products contain bioactive peptides, there is really a need to verify the biofunction availability during postproduction with *in vivo* studies. This new knowledge is especially important with the growth of functional food products derived from plant and animal protein hydrolysates.

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