IMPROVING THE EMULSION STABILITY OF SPONGE CAKE BY THE ADDITION OF ?-POLYGLUTAMIC ACID

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IMPROVING THE EMULSION STABILITY OF SPONGE CAKE BY THE ADDITION OF γ-POLYGLUTAMIC ACID

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ABSTRACT

The batter properties of sponge cake with the addition of γ-polyglutamic acid (PGA) at different levels (0.05, 0.1, 0.5 gkg^{-1}, w/w) was evaluated. The viscosity, emulsion stability and foam stability increased at the addition of 0.5 gkg^{-1} PGA level. PGA caused significant declines in the differential scanning calorimetry (DSC) enthalpy, onset and peak temperatures of ice-melting transition of sponge cake. Sponge cakes manufactured with 0.5 gkg^{-1} PGA addition were in general lighter on crumb color and white index than the control. Scanning electron microscopy showed that sponge cake with the addition of 0.1 and 0.5 gkg^{-1} PGA exhibited microstructures having thinner walls of pores on cake crumb. The internal structure of sponge cake with the addition of PGA (0.1 and 0.5 gkg^{-1}) was finer and smoother than without. The addition of γ-PGA was shown to delay staling of cake crumb as well as improve texture of sponge cake.

I. INTRODUCTION

Gamma-polyglutamic acid (γ-PGA) is a high molecular anionic polymer formed from glutamic acid. Several strains of Bacilli were cultivated for the microbial production [1, 20, 27].

γ-PGA was first found in the capsule of *Bacillus anthracis* [10, 11]. Other *Bacillus species*, *B. licheniformis* and *B. subtilis*, also make γ-PGA [5, 6, 9, 15, 16, 19, 30]. The γ-PGA is a food additive of generally regarded as safe (GRAS) and can easily be produce by *Bacillus* spp.

It has no toxic or immunogenic effect on mice, rats, and human [7, 21, 22, 24, 27]. And it has been shown that PGA has antifreeze properties [26]. Its antifreezing ability increases as molecular weight decrease. Several salts of γ-PGA have little taste of their own [23]. Therefore, they could be used in greater concentrations than glucose which was often used as antifreeze [26]. Adding γ-PGA to health food holding physiologically active substances, such as calcium salt, vitamins, polyphenols, or carotenoids, could help their absorption in the small intestine [2, 12, 29]. Low concentrations of γ-PGA were showed to make better taste and drinkability [25] in fruit juice and other drinks.

The addition of γ-PGA and its salts were shown to retard aging, improve texture, and maintain shape in bread, cakes, and pasta [14, 28].

The objectives of this research were to evaluate the effects of PGA on the viscosity, emulsifying properties and foam stability of sponge cake batter. The temperature and enthalpy of ice-melting transition, microstructure and the level of staling of PGA-containing sponge cake were also determined in order to gain a better understanding regarding the feasibility of using PGA on baked food products.

II. MATERIALS AND METHODS

1. Materials

Cake flour, milk, and vegetable oil were obtained from Uni-President Enterprises Co. Ltd., Tainan, Taiwan. The sodium salt of high molecular weight γ-Polyglutamic acid (PGA) (>10^6 Da) was obtained from Vedan Enterprises Corporation, Taichung, Taiwan.

2. Preparation of Sponge Cake

The formulation for sponge cake included cake flour, 100 gkg^{-1} fine granulated sugar, 150 gkg^{-1} salt, 3 gkg^{-1} vegetable oil, 20 gkg^{-1} milk, 20 gkg^{-1} eggs, 175 gkg^{-1} PGA, (0, 0.05, 0.1 and 0.5 gkg^{-1}, w/w) of flour w/w. First, the eggs and salt were mixed in a mixing bowl. Vertical mixer attached with whip is used to whip the eggs with high speed until light and fluffy. Sugar was added during whipping. The sifted flour was poured into the blended liquid mixture and blended for only five seconds. Vegetable oil was then blended into the batter slowly. Five hundreds twenty grams of cake batter were placed into

a 200 mm diameter and 75 mm height, metallic, tin-coated a baking paper at the bottom. Sponge cake was produced by baking the cake batter in an oven at upper and lower oven temperature of 180°C for 30 min. Sponge cake was sealed in 1 kg polyethylene (PE) bags after cooling and held at room temperature (25°C) for 4 days storage duration. A color meter (Nippon Denskoku Color Measuring System, Japan) was used to determine lightness (*L*), red content (*a*) and yellow content (*b*) values on sponge cake crumb at first day. White index (WI) was calculated as following formula: $WI = 100 - ((100 - L)^2 + a^2 + b^2)^{1/2}$. The calibration was conducted using a standard white plate (X = 93.49, Y = 95.43, Z = 113.21).

Sponge cakes were evaluated by 4 panelists. Two male and two female bakers between the ages of 20 and 45 who were enrolled in a sensory evaluation course evaluated the samples. Panelists were instructed to evaluate how much they liked appearance, texture, and overall acceptability of sponge cakes on a hedonic scale.

3. Rotational Viscometry

About 300 ml of batter was taken for viscosity measurements. The reading of the viscometer output started 2 min after the experiment onset. The viscosity of the cake batter was monitored at 25°C ± 0.1°C by using Brookfield digital viscometer (Model RTV, Brookfield Engineering Labs., Inc., Middleboro, MA, USA) equipped with a LV No. 2 spindle head at the spindle rotational speed of 60 rpm.

4. Foam Stability of Egg White

Foam stability of 0-2.0 gkg^{-1} PGA adding egg white was evaluated using the modified method of Min *et al.* [18]. One hundred grams of egg white was weighed and mixed in a mixer (Hobart, Troy Ohio, USA) at high speed for 3 min. The foam was transferred into a 1,000 ml graduated volumetric cylinder. The volume was measured by recording the volume every 30 min of holding the foam at room temperature for 3 hours. The foam stability was reported as foam volume × 100/ original foam volume.

5. Emulsion Activity and Emulsion Stability

The emulsifying properties of egg yolk (10 ml), water (45 ml), soybean oil (45 ml) were determined by the method of Yasumatsu *et al.* [35] with slight modification. For the preparation of emulsions, 45 gkg^{-1} (w/w) water, 45 gkg^{-1} soybean oil (w/w), (0, 0.05, 0.1, 0.5, 0.8, 1.0, and 2.0 gkg^{-1}, w/w) PGA and 10 gkg^{-1} (w/w) of egg yolk solution were homogenized with homomixer (model L4R, Silverson Co., England) at 7,000 rpm for 2 min. The emulsion obtained was divided evenly into ten 10ml centrifugal tubes and centrifuged at 1,500 × g for 20 min (SCR 20B, Hitachi Co., Japan). The emulsion activity was determined as (the height of emulsified layer/the height of whole layer in the centrifugal tube) × 100. To measure the emulsion stability, the emulsion prepared by the method for emulsion activity measurement was heated for 30 min at 80°C, cooled with tap water for 15 min, divided into ten 10 ml cen-

trifugal tubes evenly and centrifuged at 1,500 × g for 20 min. Emulsion stability was calculated as (the height of remaining emulsified layer/the height of whole layer in the tube) × 100.

6. Texture Profile Analysis (TPA) of Sponge Cake

The hardness, springiness, cohesiveness and chewiness of 0-0.5 gkg^{-1} PGA sponge cake were measured by a texture analyzer (TA-XT2 Texture Analyzer, Stable Micro Systems Co. Ltd., UK) with a cylinder probe (No. P/0.5 S, 1/2 in diameter) [32]. The sponge crumbs were cut into cubes with the lengths of 3 × 3 × 3 cm and were compressed by the probe for a distance of 15 mm (strain 50%) at a speed of 1 cm/min in a two-cycle test.

7. Differential Scanning Calorimetry (DSC) of Sponge Cake

The thermal properties of sponge cake samples were determined by a DSC (Modulated DSC 2010, TA instrument, New Castle, USA) according to the procedure reported by Biliaderis *et al.* [3]. About 10 mg sponge cake sample with 0-0.5 gkg^{-1} (w/w) PGA was sealed in a sample pan and scanned from -50°C to 20°C with a heating rate of 5°C min^{-1}. An empty pan was used as the reference. The onset temperature (T_0), peak temperature (T_p) and enthalpy ($\triangle H$) of the transition of ice-melting were measured.

8. Scanning Electron Microscopy (SEM) of Sponge Cake

The method was based on the report of Kim *et al.* [13]. The 0-0.5 gkg^{-1} PGA-containing sponge cake was frozen at -50°C for 24 hours. Once the samples were completely frozen, they were cut into small chunks with a size about 0.8 × 0.8 × 0.3 cm and then were freeze-dried for about 2 days. After drying, the surface of the sample was sputter coated in a vacuum with an electrically conductive layer of gold (LADD No. 30800 Sputter Coater, Vermont, USA). The microstructure of the sample was scanned with a Scanning Electron Microscopy (SEM) (Hitachi S-2500 Scanning Electron Microscopy 20 kV, Tokyo, Japan).

9. Statistical Analysis

One-way analysis of variance (one-way ANOVA) was conducted using a package (SAS Institute Inc., Cary, NC). A significance level of 5% was adopted for all comparisons. Duncan's multiple-range test was used to determine the significant difference between different treatments.

III. RESULTS AND DISCUSSION

Figure 1 shows the effect of the addition of PGA on the viscosity of sponge cake batter. The viscosity of control batter was 6,300 cps: however, it increased to 34,050 cps with the addition of 2 gkg^{-1} PGA ($p < 0.05$). γ-PGA is a high molecular weight natural biopolymer for use as thickener or moisturizer. The possible explanation for PGA to raise the viscosity of batter was that PGA can work as cross-links and serve as hy-
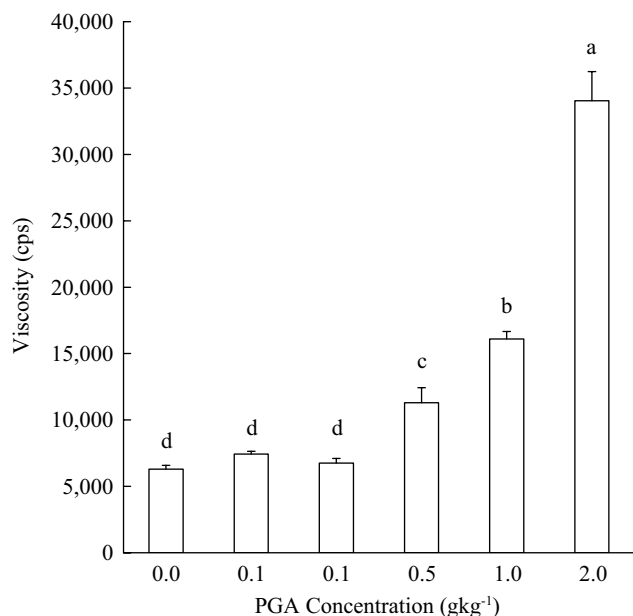
Fig. 1. Effects of different PGA concentrations on the viscosity of sponge cake batter. Bars with the different letter are significantly different ($p < 0.05$).
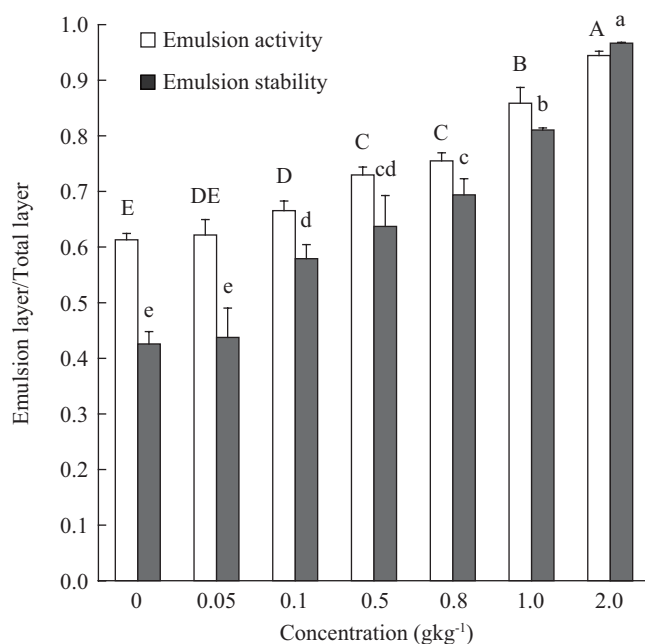

Fig. 2. Effects of different PGA concentrations on the emulsion activity and emulsion stability of egg yolk solution. Bars with the different letter are significantly different ($p < 0.05$).

Fig. 3. Effects of different PGA concentrations on the foam stability of egg white.

drogels. We expected that PGA addition made the sponge cake batter thicker than the control batter. Incorporation of air bubbles into the batters will be more uniform and stable. In fruit juice and other drinks, low concentrations of γ-PGA were found to enhance taste and drinkability [25, 33].
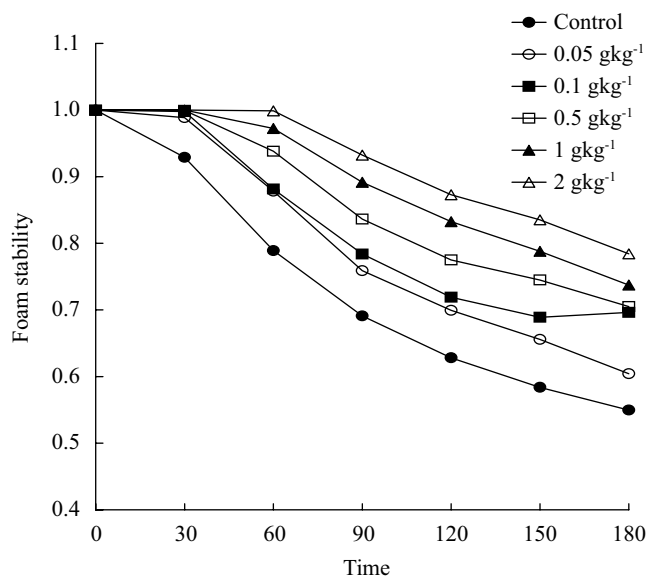
The effects of PGA on emulsion activity and emulsion sta-

bility are shown in Fig. 2. The emulsion activity and emulsion stability of egg yolk solution increased significantly at the addition level of 0.1 gkg^{-1} PGA. It was found that the emulsion activity and emulsion stability in 0.05 gkg^{-1} PGA group did not differ with the control, but higher emulsion activity and emulsion stability were observed above 0.5 gkg^{-1} PGA level. The increasing emulsion activity and emulsion stability effect might be due to the cross-links between the triglyceride of liquid and the polyhydroxyl group of PGA. The high emulsion activity and emulsion stability, 0.94 and 0.97, respectively, in 2 gkg^{-1} PGA group further agreed that PGA might slow down the separation of soybean oil and water in the emulsion system. Since the addition of 2 gkg^{-1} PGA hindered the aggregation of liquid, a higher viscosity was observed in the sponge cake batter.

The foam stability of liquid egg white with the addition of PGA in the range of 0-2 gkg^{-1} is shown in Fig. 3. Egg white is a good foaming agent and it plays an essential role in foam formation in cakes since it improves the dispersion of large volumes of air into cake batter during whipping. Volume of the foam formed from egg white reduced and became watery with the increasing of time. The foam stability was determined by measuring the volume of egg white from the foam in a given time. The data in Fig. 3 indicate that the foam stability of egg white could be improved with the increasing of PGA addition. The development of foam depends on the surface activity and film formation properties of protein compositions present in a food system. The mixture of different kinds of proteins such as egg white can be an excellent foaming agent because each protein achieves a different function during foaming. Globulins help the development of foam and ovomucin and lysozyme responsible for foam stability [34]. Yang and Baldwin [34] also showed that the viscosity of liquid egg white

Table 1. Effects of different PGA concentrations on crumb color of sponge cake.

	L	a	b	W.I.
Control	81.08 ± 0.04^c	-5.07 ± 0.04^d	23.85 ± 0.59^a	69.13 ± 0.44^b
0.05 gkg^{-1}	82.52 ± 0.30^b	-4.91 ± 0.05^c	24.00 ± 0.74^a	69.90 ± 0.49^{ab}
0.1 gkg^{-1}	82.96 ± 1.04^b	-4.69 ± 0.08^b	24.07 ± 0.44^a	70.13 ± 0.94^{ab}
0.5 gkg^{-1}	84.43 ± 0.56^a	-4.53 ± 0.07^a	23.92 ± 0.68^a	71.10 ± 0.73^a

The values are mean ± standard deviation.
The values in the same column followed by different superscripts were significantly different. ($p < 0.05$).

Table 2. Effects of different PGA concentrations on the onset temperature, peak temperature and enthalphy of the ice-melting transition of sponge cake.

	Onset T (°C)	Peak T (°C)	Enthalphy (Jg^{-1})
Control	-21.55 ± 0.64^a	-11.74 ± 0.78^a	35.52 ± 1.09^a
0.05 gkg^{-1}	-21.31 ± 0.66^a	-11.34 ± 0.40^a	34.96 ± 0.69^a
0.1 gkg^{-1}	-22.21 ± 1.08^a	-12.02 ± 0.83^a	31.41 ± 1.72^b
0.5 gkg^{-1}	-23.93 ± 0.78^b	-14.06 ± 1.21^b	25.09 ± 0.57^c

The values are mean ± standard deviation.
The values in the same column followed by different superscripts were significantly different. ($p < 0.05$).
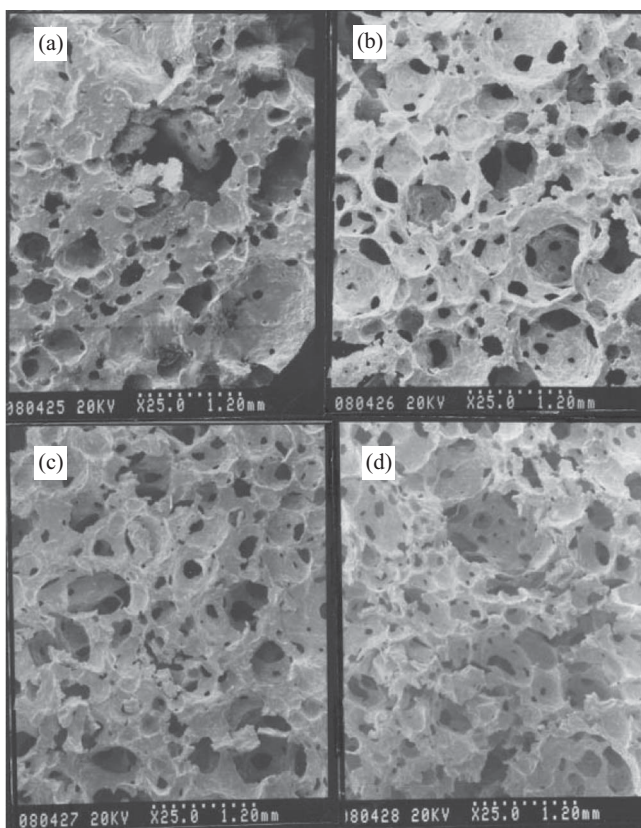

Fig. 4. Scanning electron microscopy (× 25) of sponge cake with the addition of different PGA concentrations ((a) Control; (b) 0.05 gkg^{-1} PGA; (c) 0.1 gkg^{-1} PGA; (d) 0.5 gkg^{-1} PGA).

large pores after the gelatinization of starch and coagulation of egg proteins.

Sponge cakes manufactured with addition of PGA were in general higher in lightness (L) and redness (a) values than control cakes (Table 1). As can be seen in Table 1 crumb lightness (L) and redness (a) values of with the addition of PGA (0.05, 0.1, and 0.5 gkg^{-1}, w/w) were always notably higher than control.

The addition of PGA had no effect on the crumb yellowness (b), but significant increase in the white index was found at 0.5 gkg^{-1} addition level. Figure 4 presents the pore structure of PGA-containing sponge cake under an electron microscope with 25 magnifications. Thicker walls of pores were found in sponge cake without PGA (the control group. Fig. 4(a)) and with the addition of 0.05 gkg^{-1} PGA (Fig. 4(b)), while those were thinner (Figs. 4(c) and 4(d)). With the addition of PGA (Figs. 4(b)-4(d)), the pore structures were smoother than the control. Rougher surface on the crumb were observed if the walls of pore structure were thicker. When the PGA was added, the resulting cake was brighter than the control as confirmed by the SEM picture. The internal structure of sponge cake with the addition of PGA (0.1 and 0.5 gkg^{-1}) was finer and smoother than without. The PGA additions improved the emulsion stability and foam stability showing a greater effect on the sponge cake microstructure with thinner walls of pore structure. The crumb was smoother indicating more thorough emulsion activity.

Table 2 presents the effects of PGA on the ice-melting temperatures and enthalpy of sponge cake. The onset ice-melting temperature of sponge cake without the addition of PGA was -21.55°C. A lower onset ice-melting temperature (-23.93°C) was found at 0.5 gkg^{-1} PGA level. For the enthalpy and peak temperature of the ice-melting transition, it was also found that peak temperature decrease gradually with the increase of PGA concentration. Vittadini and Vodovotz [31] suggested that addition of soy moved the ice-melting transition to slightly lower temperatures in differential scanning calorimetry and decreased its temperature range. Therefore, the enthalpy of ice-melting transition increased as the amount of freezable water increase in bread crumb. Our data suggesting, the addition of 0.5 gkg^{-1} PGA significantly decreased the amount of freezable water in sponge cake. It can be speculated

is positive related to foam stability. Ma *et al.* [17] found that the foam stability of egg white was improved with the increasing of interfacial viscosity and elasticity of egg white due to structural changes of proteins in egg white, which reduced surface hydrophobicity and increased the viscosity. The development of foam from egg white could be influenced by various factors such as the procedures of beating, pretreatments, and addition of materials [34]. We observed that with the addition of PGA, air could be effectively whipped into cake batter and held their structure before baking. Heating egg foam expands the air bubbles trapped in cake batter, and the volume of sponge cake raises gradually without merging into

Table 3. Effects of different PGA concentrations on texture profile analysis of sponge cake.

	Hardness (g)	Springiness	Cohesiveness	Chewiness (g)
0 day storage				
Control	103.3 ± 2.4[a]	0.79 ± 0.02[a]	0.70 ± 0.02[b]	59.2 ± 4.2[a]
0.05 gkg⁻¹	104.3 ± 2.8[a]	0.80 ± 0.04[a]	0.76 ± 0.01[a]	57.1 ± 3.4[a]
0.1 gkg⁻¹	78.5 ± 4.3[b]	0.81 ± 0.02[a]	0.77 ± 0.01[a]	48.5 ± 2.2[b]
0.5 gkg⁻¹	70.4 ± 1.4[c]	0.80 ± 0.03[a]	0.77 ± 0.01[a]	43.1 ± 2.4[c]
4 day storage				
Control	119.2 ± 4.5[a]	0.76 ± 0.08[a]	0.72 ± 0.01[b]	60.7 ± 8.1[a]
0.05 gkg⁻¹	113.8 ± 3.7[b]	0.74 ± 0.10[a]	0.74 ± 0.08[a]	58.3 ± 7.1[ab]
0.1 gkg⁻¹	95.1 ± 2.6[c]	0.79 ± 0.02[a]	0.74 ± 0.02[a]	55.6 ± 3.1[ab]
0.5 gkg⁻¹	86.3 ± 8.0[d]	0.78 ± 0.01[a]	0.75 ± 0.01[a]	50.6 ± 4.4[b]

The values are mean ± standard deviation.
The values in the same column followed by different superscripts were significantly different. ($p < 0.05$)

that a certain amount of water held in sponge cake matrix in the presence of PGA. Similarly, in our previous studies, PGA caused significant declines in the enthalpy, onset and peak temperatures of ice-melting transition of wheat dough at 5.0 gkg⁻¹ level [28].

The hardness of sponge cake without PGA addition was 103.3 g (Table 3). With the addition of PGA, the hardness was only 78.5 g and 70.4 g at the levels of 0.1 gkg⁻¹ and 0.5 gkg⁻¹, respectively. Our results showed that PGA could decrease the hardness of sponge cake. During storage, the hardness of sponge cake increase significantly, after storage for 4 days, the hardness of sponge cake was only 86.3 g and 95.1 g at the levels of 0.5 and 0.1 gkg⁻¹ PGA addition, respectively. Therefore, our data also indicated that PGA could retard the staling process of sponge cake. During storage, the increase of hardness is often an index of staling in baking products [8]. PGA-containing sponge cake was also found to retard the hardness increase during storage but to a lesser degree than the control group (Table 3). The addition of γ-PGA or its salts was also reported to delay aging and improve texture, and hold the shape of bakery products [14]. There are a number of applications for γ-PGA and derivates in foods containing wheat flour as a main ingredient, such as bread, cakes or pasta [4].

Our data indicated that PGA could decrease the hardness of sponge cake. Cohesiveness is the ratio of the cake bite energy (positive area) during the second to that of the first compression cycle. The cohesiveness of sponge cake significantly increased with the addition of PGA (Table 3), after 4-day storage, the cohesiveness changed slightly. The cohesiveness of all PGA-containing sponge cakes remained higher than 0.74 after 4-day storage. Addition of 0.5 gkg⁻¹ PGA to sponge cake formulation caused a significant decrease in chewiness of cake. Chewiness is the energy required to chew a cake crumb for swallowing. Among PGA-containing sponge cakes, the one with 0.5 gkg⁻¹ PGA adding decreased the chewiness of sponge cake. Therefore, the addition of γ-PGA was shown to delay staling as well as improve texture of sponge cake. However,

there was no statistical differences between those sponge cake samples in appearance, texture and overall acceptability.

IV. CONCLUSION

PGA has an excellent water holding ability and is a non-toxic, biodegradable, and inexpensive polymer for which a great range of applications has been suggested. The use of PGA significantly improves the emulsion activity, emulsion stability and foam stability of sponge cake paste. During storage, it has been confirmed that PGA contributes to the prevention of staling of cake, that is, the decrease of sponge cake hardness and chewiness, and remains the cohesiveness of sponge cake. Another application in emulsifying foods containing soft wheat flour for baking industry was proved. It was shown PGA could be applied to modify the emulsifying food system.

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