Effects of Sodium Chloride and Acetic Acid Concentrations on Dispersion Stability of Mayonnaise

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Abstract: Mayonnaise is an oil-in-water (O/W) emulsion containing oil at the weight fraction of about 70%, which is stable for over a year when stored at room temperature. The stability examination of more stable mayonnaise can be time-consuming. Mayonnaise rapidly separates into oil and aqueous phases when water in the mayonnaise evaporates, which increases the ionic strength of the aqueous phase and reduces electrostatic repulsion among oil droplets. Simulating this phenomenon under reduced pressure, the stability of mayonnaises with different sodium chloride concentrations [0, 1, 3, 5 or 8% (kg/kg-aqueous phase)] and acetic acid concentrations [0, 1, 5, 10, 15 or 20% (kg/kg-acetic acid solution)] was evaluated by comparing the duration of time before each mayonnaise sample separated into oil and aqueous phases. The durations (destabilization times) correlated with the sodium chloride concentrations for mayonnaises with 1% (kg/kg-acetic acid solution) acetic acid solution. Destabilization times were independent of the sodium chloride concentration, however, for mayonnaises with greater than 10% (kg/kg-acetic acid solution) acetic acid solution. The differences in destabilization times were ascribed to denaturation of egg yolk granules. The destabilization time of commercially available mayonnaise can be similarly explained. The results of this study, which showed that the increase in the ionic strength of the aqueous phase by evaporation assessed the stability of mayonnaise in expedition way, were consistent with previously reported findings.

Key words: dried egg yolk, dispersion stability, ionic strength, mayonnaise, pH

1 Introduction

Mayonnaise, a popular consumer seasoning, is an oil-in-water (O/W) emulsion in which small droplets of vegetable oil are dispersed in an aqueous phase consisting mainly of egg yolk, vinegar and salt^{1, 2)}. Since the O/W emulsion is thermodynamically unstable, the coalescence of oil droplets proceeds over time, resulting in oil-water separation (destabilization). Generally, the higher the fraction of oil phase in O/W emulsion is, the more easily the emulsion destabilizes³⁾. Mayonnaise is an O/W emulsion with a very large fraction, approximately 70% (w/w), of oil phase. Despite the large fraction of oil phase, mayonnaise is stable over a long period and its mouthfeel does not change for more than a year when stored at room temperature²⁾. Egg yolk largely contributes to the high dispersion stability of the mayonnaise^{1, 4, 5)}. Stability examinations of more stable mayonnaise can be time-consuming. Therefore, we proposed a simple and expeditious method for assessing dis-

persion stability of mayonnaise or mayonnaise-like dressing in one day⁶⁾. The basic principle of the method is as follows: when mayonnaise is subjected to reduced pressure at room temperature, water in the mayonnaise evaporates to increase the ionic strength of the aqueous phase. Stability of mayonnaise can be therefore be evaluated in a short time, because the increased ionic strength reduces electrostatic repulsion acting among oil droplets and promotes destabilization^{4, 7)}.

A strip of filter paper was dipped into mayonnaise. The filter paper begins absorbing oil when oil-water separation occurs. Before the mayonnaise separates into oil and water, the absorption of water or oil by the filter paper cannot be visually observed. By analyzing the oil penetration into the filter paper, it is possible to evaluate the time the mayonnaise began to separate into oil and aqueous phases (destabilization time). It was shown, using this method, that mayonnaise and mayonnaise-like dressing had different

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destabilization times, *i.e.*, different dispersion stability⁶⁾. Since the major difference between mayonnaise and mayonnaise-like dressing is the composition of the aqueous phase, characteristics of the aqueous phases can be assessed through this method.

This study used the aforementioned method to investigate dispersion stability of mayonnaise consisting of aqueous phases with different concentrations of sodium chloride and acetic acid. The effects of the compositions of the aqueous phase on the dispersion stability of mayonnaise were examined.

2 Experimental

2.1 Materials

Soybean oil (first grade) and acetic acid (special grade) were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). Sodium chloride (reagent grade) and dried egg yolk (No. 1) were purchased from Nacalai Tesque (Kyoto, Japan) and Kewpie Egg Corporation (Tokyo, Japan), respectively.

2.2 Mayonnaise preparation

An acetic acid solution of 0 (water), 1, 5, 10, 15, or 20% (kg/kg-solution) was prepared by diluting the acetic acid with distilled water. An egg yolk solution was prepared by adding distilled water (2.08 g) to dried egg yolk (1.666 g). Sodium chloride was added to the egg yolk solution to give the final concentration of 0, 1, 3, 5, or 8% (kg/kg-aqueous phase of emulsion) and the mixture was mixed well using a vortex mixer. The acetic acid solution (3.00 g) was added to the aqueous solution containing egg yolk and sodium chloride, and the mixture was stirred at 2,000 rpm for 30 s using a mechanical homogenizer (T20SK, Kinematica, Lucerne, Switzerland) to prepare the aqueous phase of the emulsion. Soybean oil (17.50 g) was added in four portions and stirred at 12,000 rpm for 30 s after each addition, producing 25 g of mayonnaise. All materials and equipment were cooled at 20°C during preparation. The preparation was repeated three times yielding a total of 75 g mayonnaise, which was subjected to one stability measurement. Weight fractions of the oil phase, aqueous acetic acid solution and egg yolk solution were 70:12:15. The aqueous phase acetic acid concentration of $x\%$ (kg/kg-solution) and sodium chloride concentration of $y\%$ (kg/kg-aqueous phase) are hereinafter referred to as Ax and Sy , respectively. Mayonnaise consisting of the aqueous phase with composition $AxSy$ was designated as mayonnaise- $AxSy$.

2.3 Measurement of syneresis rate and pH of aqueous phase

The aqueous phase with sodium chloride at 0, 1, 3, 5 or 8% (kg/kg-aqueous phase) was poured into a 15-mL conical

tube and centrifuged at approximately $20,000 \times g$ for 30 min at 5°C. Two layers were obtained: the upper layer was solid layer and contained egg yolk, while the lower layer was liquid. The lower liquid layer was carefully recovered with a Pasteur pipette and weighed. The ratio of the weight of recovered liquid layer to total weight of the aqueous phase was defined as the syneresis rate. The pH of the recovered layer was also measured at, and was the same as that of the original aqueous phase when measured at 20°C. The pH was measured at 0, 10, 20 and 30°C for solutions containing 0 or 5% sodium chloride and an equal weight of distilled water was added instead of egg yolk solution. The pH was measured once under each condition.

2.4 Expeditious assessment of the dispersion stability

The dispersion stability of mayonnaises having different aqueous-phase compositions was assessed using the method briefly described below, in which the time when mayonnaise began to separate into oil and water was evaluated by penetration length of oil absorbed by a strip filter paper⁶⁾.

Approximately 75 g mayonnaise was placed in a plastic Petri dish (ϕ 90 mm $\times$ 10 mm), covered with a wrap, and kept at 30°C for approximately 1 day in a temperature-controlled oven (DO-300FA, As One, Osaka, Japan). A strip of filter paper (5 cm width, 6 cm long; No. 1, Advantec, Tokyo, Japan) was affixed to a plastic support with the lower 1 cm of filter paper dipped in the mayonnaise. The mayonnaise dipped with filter paper was placed in a desiccator inside a chamber (HiTEC, Yamato Scientific, Tokyo, Japan) controlled at 30°C. The pressure in the desiccator was reduced using an oil pump (GLD-051, Ulvac, Kanagawa, Japan), and oil absorption was recorded over time using a digital camera (EOS 70D, Canon, Tokyo, Japan) as shown in the Fig. 1 inset. The height of the liquid penetrated into a capillary; the penetration length L , can be generally expressed by the Lucas-Washburn equation⁸⁻¹⁰⁾.

$$L = \sqrt{\frac{R\gamma \cos \theta \cdot t}{2\eta}} \quad (1)$$

where R is the apparent capillary radius, γ is the surface tension of the liquid, θ is the contact angle, η is the viscosity, and t is the time. The penetration length, L , is proportional to $t^{1/2}$. Plots of L against $t^{1/2}$ could be approximated by a straight line intersecting at the x -axis. Since the liquid penetrating the capillary of filter paper was the oil phase separated from the mayonnaise, the intersection point represents the time when mayonnaise began to separate into oil and water. The time is hereinafter referred to as destabilization time. There is generally a slight delay after the liquid comes in contact with the capillary and the penetration starts, but the delay should be negligibly short compared to the time when the oil-water separation of mayonnaise occurs. For one sample, five penetration lengths were measured at points about 8-mm distance from each on the

Fig. 1 Method for estimating the destabilization time of mayonnaise based on the Lucas-Washburn equation (Eq. (1)).

filter paper. The destabilization times at five places were obtained, and the average value was taken as the destabilization time of the sample. The measurement was performed in triplicate or more, and the results are shown as mean $\pm$ standard error.

3 Results and Discussion

3.1 Conditions under which mayonnaise can be prepared and pH of the aqueous phase

In this study, mayonnaise, with different concentrations of acetic acid and sodium chloride in the aqueous phase, was prepared using dried egg yolk instead of raw egg yolk to maintain constant water content in the aqueous phase. Table 1 shows the effects of acetic acid and sodium chloride concentrations on the mayonnaise preparation. There were four conditions: a mayonnaise-like emulsion with high viscosity (conditions marked by $\bigcirc$), emulsion with low viscosity (conditions marked by $\triangle$), emulsion that could or could not be obtained (conditions marked by $\blacktriangle$), and no emulsion was obtained (conditions marked by $\times$). Under the conditions marked by $\triangle$, the aqueous phase contained no acetic acid or did at 1%. Under the conditions marked by $\blacktriangle$, preparation of mayonnaise depended on the production lot of dried egg yolk. Acetic acid and sodium chloride concentrations were high under the conditions marked by $\times$. It was reported that the higher the concentrations of acetic acid and sodium chloride, the smaller the emulsifying capacity of egg yolk was synergistically¹¹⁾. The same tendency was recognized in this study using dried egg yolk.

Protein structure is related to the emulsifying ability of

Fig. 2 pH of aqueous phase with different sodium chloride and acetic acid concentrations at 20°C with (open symbol) and without (closed symbol) egg yolk. Concentrations of sodium chloride in aqueous phase are 0% (kg/kg-aqueous phase) ($\bigcirc$), 1% ($\triangle$), 3% ($\square$), 5% ($\diamond$) and 8% (∇). Inset shows pH of aqueous phase at 0, 10, 20 and 30°C, in which acetic acid and sodium chloride concentrations are 1% (kg/kg-acetic acid solution) and 0% (kg/kg-aqueous phase) without egg yolk ($\bullet$), 0% and 1% with egg yolk ($\triangle$), and 5% and 5% with egg yolk ($\diamond$).

egg yolk⁵⁾. To consider the charge state of egg yolk protein, therefore, the pH of the aqueous phase was measured at 20°C (Fig. 2). When the aqueous phase did not contain egg yolk (control), the pH of the aqueous phase was about 2 to 3, depending on the acetic acid concentration. The higher the temperature, the lower the pH, and the difference in pH at 0°C and 30°C was 0.14 to 0.28. Typical results of pH at 0, 10, 30°C are shown as a function of temperature in the Fig. 2 inset. Under the conditions marked $\bigcirc$ in Table 1, where mayonnaise was successfully prepared, the difference in pH between 0°C and 30°C was within ± 0.06 , and the temperature dependence of pH was low. On the other hand, the aqueous phase (A0 and A1S0), which produced emulsion with low viscosity, exhibited high temperature dependence. That is, the difference in pH between 0°C and 30°C was -1.2 to 0.43 . These results show that the buffering capacity of egg yolk was smaller under the conditions where mayonnaise could not be prepared than under those where it could be. The isoelectric point of egg yolk is 5.5 to 5.8¹²⁾. Since the aqueous phase of A0 had a pH value close to the isoelectric point where proteins would aggregate, the charge was reduced¹⁾ and the buffer capacity seemed to

Table 1 Relationship between composition of aqueous phase and possibility of emulsification.

Sodium chloride concentration [% (kg/kg-aqueous phase)]	Acetic acid concentration [% (kg/kg-acetic acid solution)]					
	0	1	5	10	15	20
0	△	△	○	○	○	○
1	△	○	○	○	○	▲
3	△	○	○	▲	×	×
5	△	○	○	×	×	×
8	△	○	▲	×	×	×

○: Mayonnaise-like emulsion with high viscosity.

△: Emulsion with low viscosity.

▲: Emulsion could not be constantly obtained.

×: No emulsion was obtained.

Fig. 3 Destabilization time of mayonnaises with different acetic acid and sodium chloride concentrations. The symbols are the same as those used in Fig. 2.

lower. At relatively high pH, the sodium chloride concentration increased and the egg-yolk granules were more easily dissolved¹³. Under the A1 condition, the aqueous phase was presumed to have buffer capacity except for S0, indicating that proteins were solubilized and the ionic concentration of the phase increased.

3.2 Destabilization time of mayonnaise

The destabilization times of the mayonnaises prepared under the conditions marked ○ and ▲ in Table 1 were measured (Fig. 3). Mayonnaise with aqueous phase A1 had a longer destabilization time as the sodium chloride concentration was higher. As mentioned above, more granules were dissolved because sodium chloride concentration in-

creases at relatively high pH¹³. The dissolution of granules enhances the emulsifying ability of egg yolk and stabilizes mayonnaise^{12, 13}. The above-mentioned result regarding longer destabilization time at higher sodium chloride concentrations is consistent with previous findings^{12, 13}. In the cases of A5, the destabilization time of mayonnaise-S5 was extremely long, whereas mayonnaise-S8 had the shortest destabilization time. Destabilization time did not correlate with salt concentration (ionic strength). Egg yolk increases its emulsifying ability due to moderate denaturation through the addition of sodium chloride and lowering of pH, but over-denaturation leads to a decrease in emulsifying ability¹⁴. As shown in Table 1, the conditions for mayonnaise-A5S5 and -A5S8 were located on the boundary where mayonnaise could be prepared or not. Therefore, it is inferred that the egg yolk contained in mayonnaise-A5S5 had moderately denatured to maximize the emulsifying ability, whereas the egg yolk of mayonnaise-A5S8 had over-denatured. For the mayonnaise prepared using aqueous phases A10, A15 and A20, the effect of sodium chloride concentration on destabilization time was not recognized, and the time was slightly reduced with increased acetic acid concentration. At low pH, increasing sodium chloride concentration had no effect on granule dissolution^{13, 14}. It is predicted that mayonnaise may be easily destabilized because the electric charge on the surface of oil droplets is shielded at higher concentrations of sodium chloride. For the mayonnaise prepared using the aqueous phases of A10, A15 and A20, the fact that destabilization time was slightly reduced with increased acetic acid concentrations increases may be due to the ion shielding effect. These results suggest that destabilization times of different mayonnaises can be explained in terms of granule dissolution. Commonly used vinegar has an acetic acid concentration of approximately 5%. The sodium chloride concentration of the mayonnaise, which was commercially available in Japan and used in our previous study⁶, was estimated to be approxi-

Fig. 4 Syneresis rate for aqueous phase with different acetic acid and sodium chloride concentrations.

Fig. 5 Relationship between syneresis rate of aqueous phase and destabilization time for mayonnaise. The symbols are the same as those used in Fig. 2.

mately 6.7% based on its ingredient table. The destabilization time for commercially available mayonnaise was about 35 min, shorter than that of mayonnaise-A5S5 and longer than that of mayonnaise-A5S8. Therefore, the results of this study are consistent when applied to the commercially available mayonnaise.

3.3 Relationship between the syneresis rate of aqueous phase and destabilization time of mayonnaise

Figure 4 shows the syneresis rate after centrifugation of each aqueous phase. The syneresis rates were highest for the aqueous phase of A5. The syneresis rate tended to increase as the sodium chloride concentration increased.

Figure 5 shows the relationship between the syneresis rate of the aqueous phase and destabilization time of mayonnaise. The destabilization time was short for the mayonnaise with a syneresis rate of 0.2 or less, but the destabilization time was long for the mayonnaise with a syneresis rate of 0.3 or more. Gelation of egg yolk destabilizes mayonnaise¹⁵⁾. The low syneresis rate indicates that the interaction between water and egg yolk was strong to enhance the gelation.

4 Conclusions

Mayonnaises with different sodium chloride and acetic acid concentrations were prepared and their dispersion stability was examined using our previous method. The destabilization times of various mayonnaises were explained in terms of the denaturation of egg yolk granules. The destabilization time of commercially available mayonnaise can be explained in the same way.

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