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Comparison the stability and physical properties of salad dressing obtained with egg yolk and gelatin

Sansanee Thimthong[1], Supuksorn Masavang[2*]

[1] *Department of Food Service Industry, Faculty of Home Economics Technology, Rajamangala University of Technology Phra Nakhon, Dusit District, Bangkok, Thailand*

[2] *Department of Food Technology, Faculty of Home Economics Technology, Rajamangala University of Technology Phra Nakhon, Dusit District, Bangkok, Thailand.*

[*] *Corresponding author: supuksorn.m@rmutp.ac.th*

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Abstract - Emulsifiers are essential for emulsion formation and stabilization. Egg yolk and gelatin play an important role as effective emulsifiers in salad dressing. This study aimed to examine the impacts of different emulsifiers, including egg yolk (EY), gelatin (GE), and combination of egg yolk and gelatin (YG) on emulsion stability and selected physical properties of salad dressings. The results showed that GE-salad dressing showed the highest emulsion stability, followed with YG-emulsified salad dressing during 14 days of storage at room temperature. The separated serum layer at a bottom phase were observed for EY- and control-salad dressing, indicating instability of emulsion. GE-emulsified sample exhibited the higher lightness and whiteness and lower redness and yellowness than EY-emulsified sample ($p \leq 0.05$). YG-emulsified salad dressing had the highest viscosity ($p \leq 0.05$). From the microscopic results, all formulations presented a visible increase in the drop size throughout the storage period. GE- and YG-emulsified samples remained stable and protected them against creaming, flocculation, and coalescence. Emulsifiers had a strong influence on the structure and physical stability. The egg yolk and gelatin used to stabilize the dressing-type emulsions are additively promising in microstructured food design.

Keywords: Salad dressing, emulsifier, egg yolk, gelatin, stability

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1. Introduction

Emulsion usually refers to a mixed system consisting of small droplets of one liquid dispersed in another immiscible liquid (Hoffmann & Reger, 2014). Salad dressing is an example of oil-in-water emulsions with a semi-solid structure that traditionally produced by a mixture of egg yolk, vinegar, salt, oil and flavored material. Egg yolk is a common protein used as an emulsifier in the emulsion systems due to the combination of lipoprotein and phospholipid fractions in yolk which contributes to the formation and stabilization of salad dressing (Anton, 2013). Egg yolk could be used for reducing the interfacial tension between oil-water and facilitating the disruption of emulsion droplets during homogenization step (McClements & Demetriades, 1998). Nowadays, the health-conscious consumers demand healthier and nutritious foods, therefore, the different attempts have been made to develop low-cholesterol or cholesterol-free salad dressing with similar characteristics to conventional recipe dressings, such as use of soy bean Diftis *et al.*, 2005a; Sirison *et al.*, 2017), wheat gluten (Liu *et al.*, 2018), gelatin and whey protein (Sanmartín *et al.*, 2018) and gums (Bouyer *et al.*, 2011; De Cássia da Fonseca *et al.*, 2009) as emulsifiers to replace egg yolk in salad dressing. In addition to the emulsifiers mentioned above, gelatin is a type of a high-molecular-weight hydrocolloid produced by hydrolysis of collagen from the skin, bones and connective tissue (Karim & Bhat, 2009). This hydrocolloid is also particular valued in food emulsion products and can act as a thickener and emulsifier where its surface-active and film-forming functions can be exploited during the emulsification process and its stabilization (Bouyer *et al.*, 2011). Gelatins are generally considered as weaker emulsifiers than casein and whey protein (Dickinson & López, 2001; Karim & Bhat, 2009) and mainly produce relatively large droplet sizes during homogenization (Zhang *et al.*, 2020). Gelatin is widely used in emulsion foods such as low-fat mayonnaise (Ataie *et al.*, 2019), restructured ham and bacon (Lee & Chin, 2022; Villegas *et al.*, 1999), cheese (Hesarinejad *et al.*, 2021; Hee *et al.*, 2008) and ice cream (Milliatti an & Lannes, 2018). However, gelatin is not found in salad dressing products as emulsifier. Gelatin is natural amphiphilic macromolecule and has unique emulsifying and gel properties as well as good surface-active abilities (Karim & Bhat, 2009). Therefore, it has attracted more attentions in the field of salad dressing development.

In this study, egg yolk, gelatin, and a combination of egg yolk and gelatin were used as emulsifier in salad dressings, and the effects of these different emulsifiers were evaluated by characterizing the properties of the salad dressing. The emulsion stability, microstructure, viscosity, and color of salad dressing were assessed and compared to the control salad dressing without an emulsifier.

2. Materials and methods

Fish gelatin granules (250 g Bloom) were purchased from JRF & B Company Limited. Bangkok, Thailand. Chicken eggs, soybean oil, salt, sugar and vinegar were obtained from the local supermarket

2.1 Preparation of salad dressing

Salad dressings were prepared using the different recipes. The formulations of the salad dressing without emulsifier was used as control sample, and the salad dressing samples containing emulsifiers (egg yolk and gelatin) were shown in Table 1 (in g/100 g-1). Firstly, gelatin was slowly added to total amount of water used in each formulation to form a gelatin-water dispersion, and then the dispersion was heated at 80 °C for 3 min to obtain a complete melting and cooled to 25 °C. Then, egg yolk and vinegar were combined in a glass beaker after with the melted gelation solution had been cooled to 25 °C. To make sure all the ingredients were dissolved, a Daihan homogenizer HG-15D (Gangwondo, Korea) was used to continuously stir at 1,000 rpm for 1 min. Then, the soybean oil was slowly added and the mixture was homogenized at 10,000 rpm for 2 min. For dressing with gelatin, the solid components were added and dissolved in water at room temperature, then stirred at high speed at 10,000 rpm during the homogenization step. Salad dressings were transferred to a plastic-sealed jar and protected against light and moisture, then stored at 4 °C in the refrigerator overnight until further analysis. The above steps were repeated to make three different protein-emulsified formulations, except the control sample was prepared without any emulsifier.

Table 1. Recipes used for the preparation of different formulated salad dressings.

Ingredients ($g\ 100\ g^{-1}$)	Formulation			
	Control	EY	GE	YG
Soybean oil	62.500	56.604	61.855	56.075
Water	31.250	28.302	30.928	28.037
Apple vinegar	2.083	1.887	2.062	1.869
Sugar	3.125	2.830	3.093	2.804
Salt	1.042	0.943	1.031	0.935
Egg yolk	-	9.434	-	9.346
Gelatin	-	-	1.031	3.935

Control recipe prepared without emulsifier; EY is egg yolk-emulsified salad dressing; GE is gelatin-emulsified salad dressing; YG is combination of egg yolk and gelatin-emulsified salad dressing.

Emulsion stability

The salad dressing samples were poured into 250 mL glass cylinders and stored at room temperature. The stability of the samples was evaluated by monitoring the development of separated serum layer at a bottom phase during 14 days of storage. The volume of serum layer of salad dressing at the bottom of cylinders and the initial volume of the samples was measured and the emulsion stability was calculated as percentage using equation (1).

$$\text{Emulsion stability (\%)} = \left(\frac{V_O - V_c}{V_O}\right) \times \mathbf{100} \qquad (1)$$

where v_c is the volume of the visible separated serum layer and v_o is the initial volume of the salad dressing (Anton *et al.*, 2000).

2.2 Optical microscopy

Freshly made samples of salad dressings were examined for their microstructure, as well as the evolution of the emulsion's structure over time. Samples were dropped onto a microscope slide, gently covered with a cover slip, and examined with an optical microscope at 400x magnification (BX41-TF, Olympus, Tokyo, Japan). On each slide, three separated fields were manually photographed using a digital camera (Panasonic Lumix DMC-GF8, Osaka, Japan).

Color

The colors of all salad dressing samples were evaluated using a Minolta CM-3500d spectrophotometer (Konica Minolta, Inc., Tokyo, Japan) at room temperature calibrated with a white calibration plate. The data was expressed in terms of L*, a* and b* values, where L* represents lightness (from 0 - black to 100 -white); a* and −a* for redness and greenness respectively; and b* and −b* for yellowness and blueness respectively. The whiteness index was calculated using the equation (2). All analyses were performed in triplicate.

$$\text{Whiteness (\%)} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (2)$$

Viscosity

Apparent viscosities of salad dressing samples were determined using a RVDV-II Brookfield viscometer (Engineering Laboratories Inc, Stoughton, MA, USA) using the modified method of Paraskevopoulou *et al.* (2007). The analysis was carried out at 25 °C by using spindle No. 3 at rotational speed of 20 rpm for all freshly prepared samples. The results were recorded in centipoises (cP) after 30 s of shearing.

Statistical analysis

Salad dressing samples were produced in triplicates and each replication was subjected to three parallel measurements in a randomized complete block design (RCBD). The mean and standard deviation of the data are reported in tables. A one-way analysis of variance (ANOVA) was conducted. The mean values of the parameters were compared using Duncan's multiple comparison tests to identify significant differences between the results ($p \leq 0.05$).

3. Results

3.1 Emulsion stability

As demonstrated in Table 2, the kind of emulsifier and storage period had a highly significant impact ($p \leq 0.05$) on the salad dressing emulsion stability. The emulsion stability for the control salad dressing without emulsifier dropped dramatically during storage from 97.77 to 67.70%. For EY-emulsified sample, emulsion stability decreased progressively from 99.41 to 92.33%. Emulsion stability of YG-emulsified sample slightly decreased from 100.00 to 98.83%, and GE-emulsified sample had the maximum stability throughout the storage of 14 days ($p \leq 0.05$).

Table 2. Emulsion stability of salad dressing samples during storage for 14 days.

Storage time	Emulsion stability			
	Control	EY	GE	YG
1	97.77±0.30^{b}	99.41±1.02^{a}	100.00±0.00^{a}	100.00±0.00^{a}
3	93.47±0.59^{c}	98.08±0.26^{b}	100.00±0.00^{a}	100.00±0.00^{a}
5	84.19±0.60^{c}	96.76±0.51^{b}	100.00±0.00^{a}	100.00±0.00^{a}
7	67.70±0.60^{d}	94.10±0.51^{c}	100.00±0.00^{a}	98.78±0.26^{b}
14	67.70±0.60^{d}	92.33±0.51^{c}	98.83±0.29^{a}	97.71±0.00^{b}

The results are expressed as mean ± standard deviation. Means with different letters in the same row are significantly different (p≤0.05).

3.2 Microscopy

A visual comparison of the emulsion prepared with different emulsifiers, including egg yolk (EY), gelatin (GE), a combination of egg yolk and gelatin (YG), and a control sample without emulsifier was evaluated. All salad dressing samples showed visible increase in droplet size throughout the experimental period of 14 days (Figure 2). In particular, there was on change in emulsion stability values of the control salad dressing (Table 2) which related to the complete phase separation after 7 days of storage (Figure 1), during which the creaming phenomenon took place. Nevertheless, the droplets of GE- and YG-emulsified salad dressings stored for 14 days still appeared to have a good droplet structure with slightly increased sizes, as shown in the optical microscopy images (Figure 2).

Figure 2 showed shows that YG produced fine droplets with no change in the size of the emulsion, but the EY-emulsified emulsion displayed significantly larger droplet sizes. The salad dressing made with gelatin did not exhibit any obvious coalescence or flocculation, and after 14 days, the emulsion stability was less than 5% serum separation

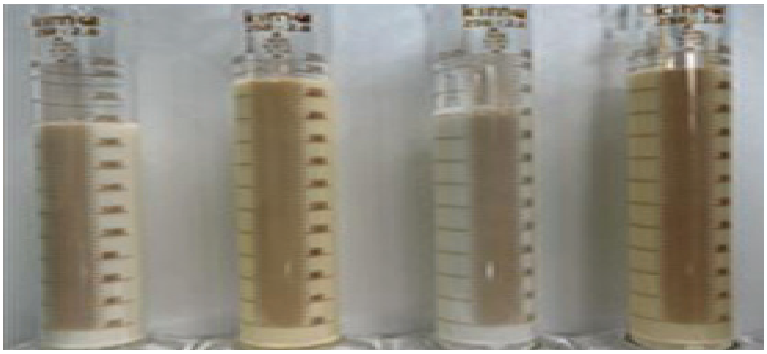
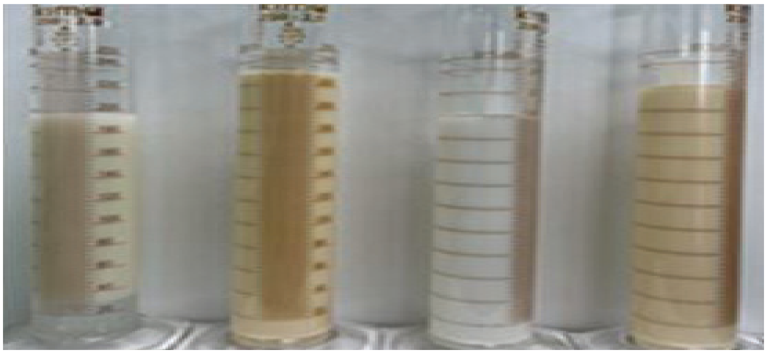
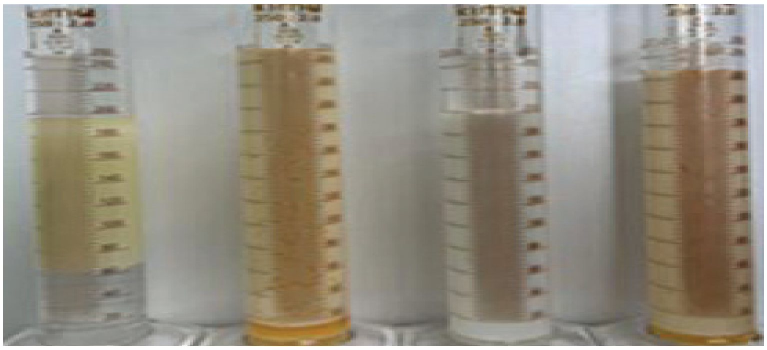
Storage time	Control EY GE YG
1 day	
3 days	
5 days	
14 days	

Figure 1. Stability of salad dressing emulsified with egg yolk (EY), gelatin (GE) and the combination of egg yolk and gelatin (YG) comparing with salad dressing without emulsifier addition (control) at 1, 3, 5 and 14 days.

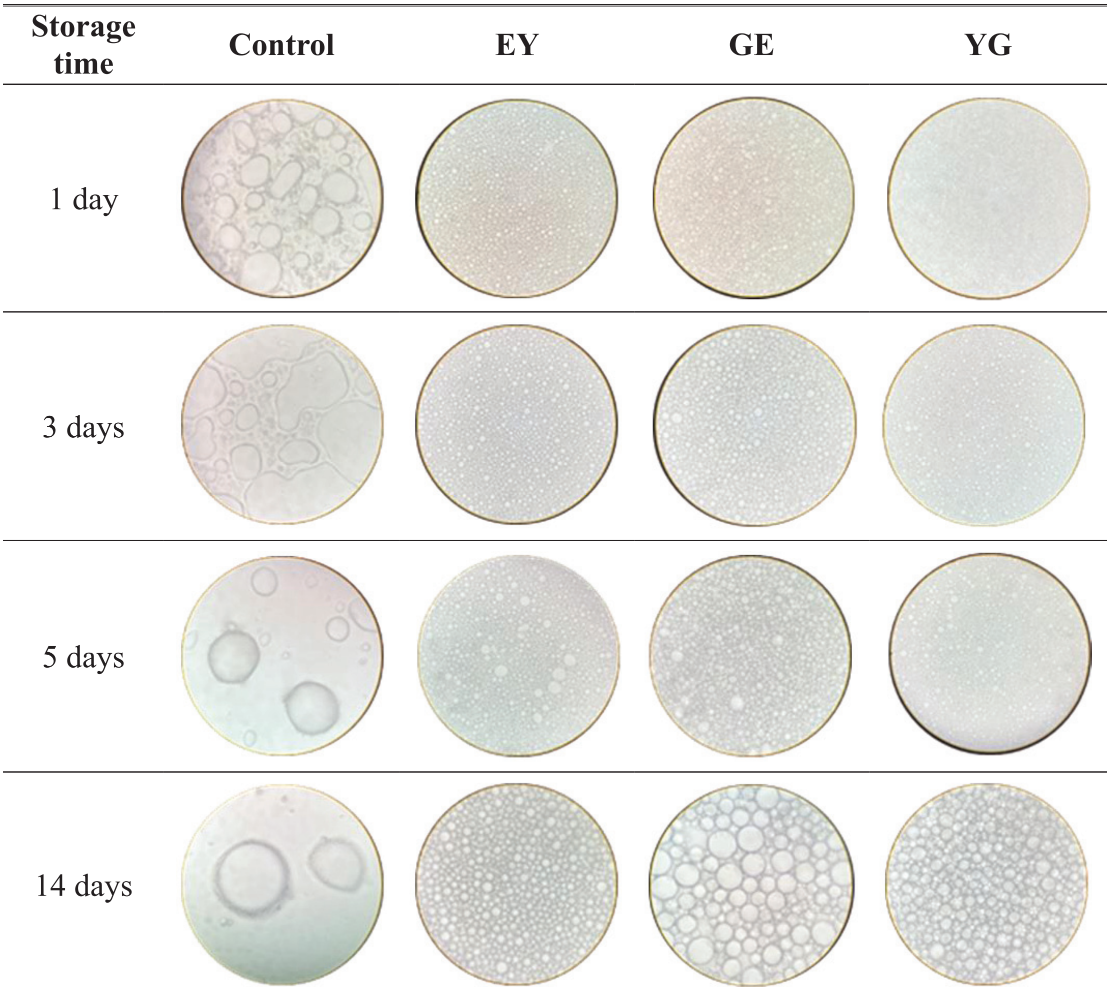


Figure 2. Optical microscopy images of salad dressing emulsified with egg yolk (EY), gelatin (GE) and the combination of egg yolk and gelatin (YG) comparing with salad dressing without emulsifier addition (control) at 1, 3, 5 and 14 days of storage.

3.3 Color and apparent viscosity

Table 3 displays the color coordinate values of the control sample with no emulsifier and the salad dressing with various emulsifiers after one day of storage at room temperature. As seen in Figure 1, following homogenization step, the all salad dressing samples became homogeneous and opaque. After only one day of storage, the salad dressing without an emulsifier developed opaque cream, which was subsequently subjected to flocculation and serum separation.

Color analysis suggested that the highest L* value and whiteness were obtained in GE-emulsified sample compare to EY-, YG- emulsified samples and control ($p \leq 0.05$). Moreover, the redness (a*) value of EY- and YG-emulsified salad dressing showed higher values than that of GE- and control salad dressing. A similar trend was found for the yellowness (b*) value, as expected with salad dressing containing egg yolk.

The YG-emulsified salad dressing showed the highest viscosity after 1-day preparation, followed by GE-, EY-emulsified samples, and control, respectively.

Table 3. Changes of color values and apparent viscosity of salad dressing containing different emulsifiers after 1-day preparation.

Sample	Color values				Apparent viscosity (cP)
	*L**	*a**	*b**	Whiteness	
Control	50.46±2.99^{c}	-2.76±0.32^{d}	9.83±1.53^{c}	49.36±2.62^{c}	248±5.70^{d}
EY	80.92±0.77^{b}	-0.29±0.05^{b}	33.68±1.68^{a}	61.28±1.75^{b}	1,357±5.70^{c}
GE	87.34±0.52^{a}	-2.54±0.99^{c}	11.74±0.98^{b}	82.54±0.84^{a}	2,065±8.37^{b}
YG	84.24±0.65^{a}	0.68±0.13^{a}	32.78±0.68^{a}	63.62±0.71^{b}	2,390±8.6^{1}a

The results are expressed as mean ± standard deviation. Means with different letters in the same column are significantly different (p≤0.05). Salad dressing emulsified with egg yolk (EY), gelatin (GE) and the combination of egg yolk and gelatin (YG) comparing with salad dressing without emulsifier addition (control) at the 1st day of preparation.

4. Discussion and conclusion

4.1 Emulsion stability

The emulsion stability of a salad dressing decreased over the storage time depending on the type of emulsifier. The combination of egg yolk and gelatin caused the higher emulsion stability. The interactions between the protein component and the fat or oil in the salad dressing may be the cause of the higher emulsion stability of the dressing.

Gelatin can be utilized as a thickening, water-holding, and emulsion-forming agent, but egg yolk and gelatin may be more effective at adhering to the surface of oil droplets (Dickinson, 1992). The stability of the emulsion was impacted by these features because they increased the ability to bind and immobilize with water and decreased the repulsive force between oil droplets (Triawati *et al.*, 2016). The most popular food emulsifying additives come from egg proteins, however they are not hydrocolloids (Dickinson, 1992). Gelatin is really the only protein that can be correctly classed as a hydrocolloid due to its distinct hydrophilic nature. Gelatin does have some emulsifying ability, but its more characteristic roles are as a colloid stabilizer and gelling agent (Dickinson, 2009). As a result, EG- and YG-emulsified salad dressing shown greater stability than those of salad dressing without emulsifier.

4.2 Microscopy

The microstructures of the salad dressing samples under an optical microscope revealed the presence of spherical liquid droplets that dispersed with different droplet size depending on the type of emulsifier (Figure 2). The largest droplet size was observed for control sample without emulsifier. Nevertheless, the droplets of GE- and YG-emulsified salad dressing remained more stable throughout 14 days of storage, compared to other samples.

This was probably the evidence for flocculation in the oil phase and coalescence of triglycerides in droplets (Dickinson & López, 2001). A combination of two or more proteins can improve the emulsifying properties by influencing the thickness of the interfacial aqueous film that covers the oil droplets. Gelatin was previously applied in a combination with sodium caseinate and whey protein to improve its emulsifying capacity. The stability of emulsions can be increased by using gelatin, which keeps the droplets apart after formation and thus protects them against creaming, flocculation, and coalescence (Dickinson & López, 2001).

Over the storage period, all sample displayed significantly larger droplet sizes, except the salad dressing emulsified with the mixture of egg yolk and gelatin, which was stable over 14 days as no coalescence or flocculation. Gelatin protein and egg yolk molecules can be absorbed at the oil/water interface due to their amphiphilic nature, acting as an emulsifier and giving salad dressings a creamy and smooth texture (De Cássia da Fonseca *et al.*, 2009). In order to confirm the shelf life of the emulsion system until the separation of layers was observed, resulting in flocculation or coalescence, the temperature assessments in storage circumstances and a longer length of storage time would be performed.

4.3 Color and apparent viscosity

Salad dressings became opaque after homogenization. The control salad dressing without an emulsifier developed opaque cream, which was subsequently subjected to flocculation and serum separation. This demonstrated an unstable emulsion that made the salad dressing less opaque than salad dressing with emulsifiers but still displayed the yellow color of soybean oil. For the unstable emulsion salad dressing, there was a clear solution at the bottom of the cylinder over storage time. Due to the influence of the carotenoids found in the yolk, EY- and YG-emulsified salad dressings formed opaque yellow creams (Nimalaratne *et al.*, 2013), but GE-emulsified sample became opaque white because gelatin is essentially a colorless gel.

The results of color values in Table 3 indicated that the color and appearance of salad dressings depends on the charactering properties of the emulsifiers. The basic shade of this salad dressing recipe is yellow as the color of soy bean oil, yet the color of salad dressing can happen contingent upon the type of emulsifier. A salad dressing generally become opaque and foggy in accordance to the scattering of light at the oil/water interface, which are mainly related to the changing of light transmission due to dispersion of oil droplets and also the air bubbles forming at the air-water interface of emulsion (Kilpatrick, 2012). GE-emulsified salad dressing had the highest L* values, followed with YG-salad dressing. This is probably due to the presence of a branched structure of hydrophilic macromolecules of hydrocolloid that allows the formation of homogenous emulsions with smaller oil droplets, which generates greater light scattering (McClements *et al.*, 2017). Moreover, egg yolk addition as emulsifier in salad dressing showed the effects on redness and yellowness of salad dressing. It was probably due to the increase in pyrrole pigment concentration in yolk caused by oxidation of fat and protein during emulsion preparing (Yang & Chen, 2001) and due to the presence of xanthophylls derived from carotenoids (Balnave & Bird, 1996). Regarding a*, negative values represent green and positive values indicate red (Ly *et al.*, 2020). It was observed that control-, EY-, and GE- emulsified salad dressing presented negatives resulted resulting a trend towards a green color, For the parameter b*, indicating a yellow with positive values, while negative values for blue (Ly *et al.*, 2020), it was found that al salad dressing presented positives resulted, indicating the yellow color. The whiteness was higher in GE- emulsified salad dressing ($p \leq 0.05$), indicating that the sample was the lightest among all sample.

Table 2 showed that salad dressing emulsified with the combination of egg yolk and gelatin had the highest viscosity ($p \leq 0.05$). In this experiment, egg yolk was used as the emulsifier of salad dressing and gelatin was further added the YG-emulsified sample to act as the combination layer of emulsifier. For instance, gelatin is commonly used as an emulsifier and thickening agent, due to its high-water-binding capacity that allow to form viscous emulsion at low concentrations (Diftis *et al.*, 2005b). Gelatin is biopolymer which can form thick interfacial layers, that can prevent droplet flocculation and coalescence (Dickinson & López, 2001). Never the less, the egg yolk had low viscosity, causing by the bridging flocculation effect. This effect is observed when the concentration of biopolymer molecules is not enough to cover the entire oil droplet (McClements & Gumus, 2016). Compared to gelatin, egg yolk displayed a lower affinity for oil-in-water interface, thus the combination of yolk and gelatin is needed to form emulsions that more resistant to flocculation (Bouyer *et al.*, 2011) and resulting in higher viscosity. Furthermore, Gómez-Guillén *et al.* (2009) discovered that the texture of oil-in-water emulsions was significantly affected by aqueous phase viscosity due to functional groups of hydrophilic and hydrophobic molecules of gelatin, likely increasing in gelatin reduced emulsion oil index and kept it more endure. Because of the considerable volume of water included, gelatin may alter the spreadability and diminish the free water to bound water ratio (Williams *et al.*, 2006). As a result, the sticky and viscosity of the low-fat samples were increased.

In this study, demonstrated that the egg yolk, gelatin and combination of yolk and gelatin were used in salad dressing formulation as an emulsifier. The emulsifier stability, color parameters and microstructure of salad dressing depended on the type of emulsifier containing in salad dressing. The combination of yolk and gelatin contributed to the highest viscosity. Based on the result, gelatin was determined as an egg yolk replacer based on good emulsion stability obtained. Further research should be carried out regarding the comparison of stability and rhetorical properties of dressing emulsifying with polysaccharide and/or protein hydrocolloids.

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