CHAPTER IV

RESULTS AND DISCUSSION

4.1 Chemical composition and yield of mucilage extracted from okra.

After extracting the mucilage from ground deseeded okra pod using distilled water, the crude mucilage was centrifuged to remove the residue. Then, it was precipitated with ethanol (20, 40, 60, 80, and 95% v/v) at 1:4 (crude extract: ethanol) ratio. The results showed that 20 and 40% ethanol were unable to precipitate polymer from the crude extract. The mixture appeared slimy and no precipitation was observed. On the contrary, when using 60, 80, and 95% ethanol at the same crude extract to ethanol ratio, fibrous material precipitation having brown color was obtained. It could be observed by bare eyes that, when ethanol with higher concentration was used for precipitating, the crude precipitates appeared browner. Since ethanol at the concentrations of 60, 80, and 95% could precipitate out the polymer in the crude extract, only 60, 80, and 95% ethanol precipitated polymers were further determined for proximate composition and extraction yield.

Table 4.1 shows the yield and proximate composition of okra mucilage precipitated by ethanol at different concentrations. The results showed that the percentage of ethanol used during precipitation did not significantly affect proximate composition of the mucilage ($p>0.05$), but did significantly affect the precipitation yield ($p\leq0.05$). Using 60%, 80%, and 95% ethanol in polymer precipitation gave 39.48, 41.73, and 45.02 g mucilage per kg dried okra, respectively. For proximate composition, carbohydrate (72.46-74.33% dry wt.) was the major part of okra mucilage. The fat content (0.31-0.63% dry wt.) of the mucilage was relatively low. This was because okra seeds, which is a good source of fat (Calisir *et al.*, 2003), were removed before extraction. The results showed that the protein contents of okra mucilage were similar to that reported by Ndjouenkeu *et al.* (1997), which was about 20% dry weight. This protein could possibly be bound protein linking to polysaccharide chain. Many researchers were interested in the protein content of okra mucilage. Lengsfeld *et al.* (2004) studied the bacterial inhibition of okra mucilage against *Helicobactor pylori*, a gram-negative bacterium that plays a major role in developing chronic gastritis and adenocarcinoma, in an in-situ adhesion model on sections of human gastric mucosa. The results revealed that okra mucilage had the inhibitory effects and this anti-adhesive quality was due to a combination of glycoprotein and highly acidic sugar compounds. In addition, in an in vitro condition, okra polysaccharides strongly inhibited the adhesion of *Campylobactor jejuni* to colonic tissue (Lengsfeld, Faller, and Hensel, 2007).

Table 4.1 Yield and proximate composition of okra mucilage precipitated by using 60, 80, and 95% ethanol.

Ethanol (% v/v)	Yield (g/kg dried okra)	protein (% dry wt.)[ns]	fat (% dry wt.)[ns]	ash (% dry wt.)[ns]	Carbohydrate (% dry wt.)[ns]
60	39.48[a] ± 0.30	21.04 ± 0.82	0.43 ± 0.01	4.39 ± 0.21	74.14 ± 0.97
80	41.73[b] ± 0.39	21.13 ± 0.74	0.31 ± 0.03	4.24 ± 0.60	74.33 ± 0.18
95	45.02[c] ± 0.10	22.32 ± 0.04	0.63 ± 0.05	4.59 ± 0.09	72.46 ± 0.50

[a-c] Different letters in the same column denote significant difference ($p \leq 0.05$).

[ns] There was no significantly difference ($p > 0.05$).

The precipitating yield was the highest for 95% ethanol (45.02 g/kg dried okra) followed by 80% ethanol (41.73 g/kg dried okra) while 60% ethanol (39.48 g/kg dried okra) gave the lowest yield. Since 95% ethanol gave the highest precipitation yield without giving mucilage with different proximate composition, it was further used as the precipitating solvent for okra mucilage in this study.

4.2 FTIR analysis of okra mucilage.

The FTIR spectrum of okra mucilage is shown in figure 4.1. It can be seen that significant transmission peaks were obtained at 3358, 2926, 1722, 1628, 1543, 1412, 1148, 1070, and 1043 cm^{-1}. The intensity of bands around 3358 cm^{-1} is assigned to ~OH stretching frequency and, as expected, it was broad. At the wavelength of 2926

cm^{-1}, there was a weak intensity of band, which is a characteristic of C-H group stretching. The next band in the region of 1722 cm^{-1} corresponds to carboxylic acid C=O stretching vibration, whereas the band in the region of 1628 cm^{-1} corresponds to amide C=O stretching vibration. The band at 1543 cm^{-1} was due to an amide, which is a combination of C-N stretching and in plane N-H deformation modes of the peptide group (Jongjareonrak *et al.*, 2008). There was also absorption intensity at 1412 cm^{-1}, which is CH_2 bending vibration. The band at 1148 cm^{-1} was caused by C-O group stretching (Kaurfikovfi and Mathlouthi, 1995). The characteristic band at 1070 was resulted from the glycosidic linkage (~C-O-C). The band of the C-O stretch showed up at 1043 cm^{-1} (Nagaoka, Watanabe, and Kouchi, 2005). The FTIR spectra presented in figure 4.1 was similar to the previous study on IR spectrum of okra mucilage of Mishra and Pal (2007).

From the functional groups observed, it can be said that the major contents were carbohydrate and proteins, respectively. This assumption was in agreement with the results on okra mucilage proximate composition. The C-H and C-O group stretching and a bending CH_2 group come from internal structure of sugar, whereas glycosidic linkage (~C-O-C) comes from the linkage between each monosaccharide. The presence of C=O group, C-N stretching, and N-H could possibly be attributed to sugar acid that is either conjugated with protein or in free forms. The FTIR spectra obtained in this study was similar in the peak positions to the FTIR spectra of *Aloe barbadensis* Miller (Aloe vera) (Liu *et al.*, 2007). Their results showed that the polysaccharide-enriched fraction extracted from gel and skin of *Aloe barbadensis* Miller contained arabinose, rhamnose, xylose, mannose, galactose, and glucose. FTIR and ^{13}C NMR study of the polysaccharide indicated that the polysaccharides contained β-(1->4) mannopyranosyl structure and glycosylated carbon atoms. Hence, as a result of the similarity of FTIR spectrum between okra mucilage and the mentioned polysaccharides, it can be assumed that the polysaccharide in okra mucilage composed of the similar sugars and possibly similar arrangement of polymer structure. This assumption was in an agreement with the results of Lengsfeld *et al.* (2004), which indicated that okra mucilage composed of arabinose, rhamnose, xylose, mannose, galactose, and glucose.

Further, the result of Wu *et al.* (1995) showed that the mucilage from okra composed of galactose, galacturonic acid and rhamnose

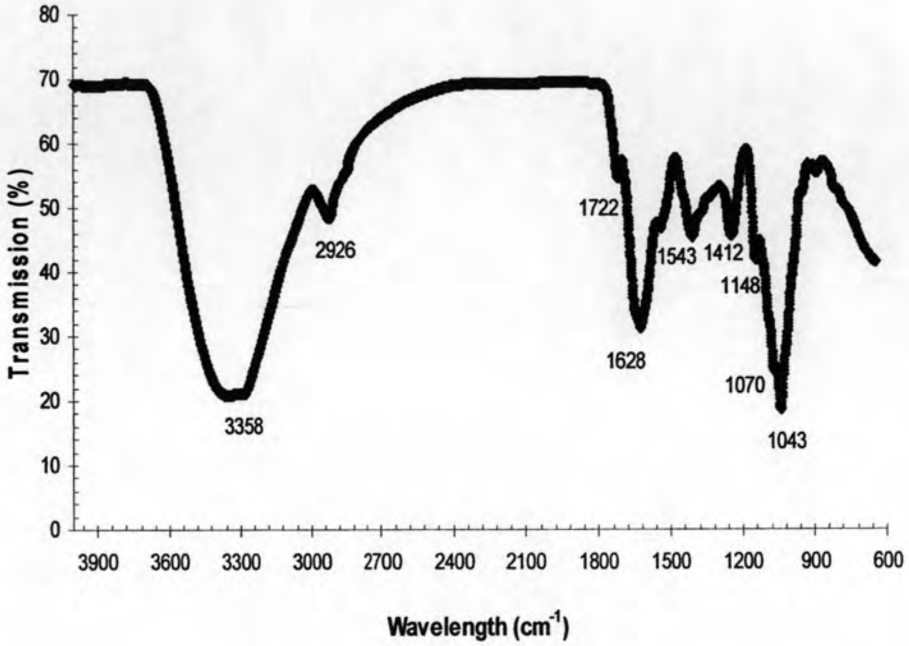


Figure 4.1 FTIR spectrum of okra mucilage that contained 99% moisture.

4.3 Surface tension of gum solutions.

The surface tension of all gum solutions was measured after 12 hours of gum preparation to ensure equilibrium. Figure 4.2 illustrates that an increase in concentration of gum solutions from 0.2%-1.0% w/w yielded a reduction in surface tension of gum in water solution as compared with the reference value of distilled water at air/water interface (72.5 dyn/cm, 25°C). At the gum concentration of 0.2% w/w, all gum solutions had similar effect on surface tension. At 0.4%w/w, xanthan gum solutions had better surface activity, whereas surface activity okra mucilage and guar gum were similar. But, at higher concentration (0.6-1.0% w/w), xanthan gum had the highest effect on decreasing the solution surface tension, followed by guar gum and okra mucilage, respectively.

It could be said that okra mucilage can be considered as a weak surfactant because it could lower the surface tension of water, in which it was dissolved in this study. This property was due to the presence of protein which could possibly bind to polysaccharide in okra mucilage. In the solution, protein, an amphiphilic molecule, migrates to an air-water interface, because the free energy of protein is lower at the interface than it is in the bulk phase. Once the equilibrium is established, the concentration of protein in the interfacial region is greater than that in the bulk aqueous phase, resulting in a lower in total free energy. Hence, the surface tension is reduced. Other reports on the ability of gum arabic, an arabinogalactan-protein complexes, and fenugreek gum are noted (Huang *et al.*, 2001). Huang *et al.* (2001) reported that the surface activity of gum arabic and fenugreek gum to reduce the surface tension was due to protein. Brummer, Cui, and Wang (2003) reported that, when the protein component of fenugreek gum was removed by the enzymatic treatment with proteinase, its surface acitivity was reduced.

Xanthan gum, which is a non-absorbing biopolymer (Makri and Doxastakis, 2007), reduced surface tension of air-water with increasing concentration. The observed increase in surface activity could be linked to the increased viscosity of the gum solutions that promote emulsion stabilization.

According to the results, guar gum, a galactomannan, was found to reduce the surface tension. Garti and Reichman (1994) stated that the surface activity guar gum was due to the polysaccharide itself, not the presence of protein. They found that the purified guar gum containing 0.8% protein still had the surface activity similar to the unpurified guar gum. Therefore, the surface activity of guar gum was the property of polysaccharide that was able to bind to the interface through the slight hydrophobicity of the polymannose backbone.

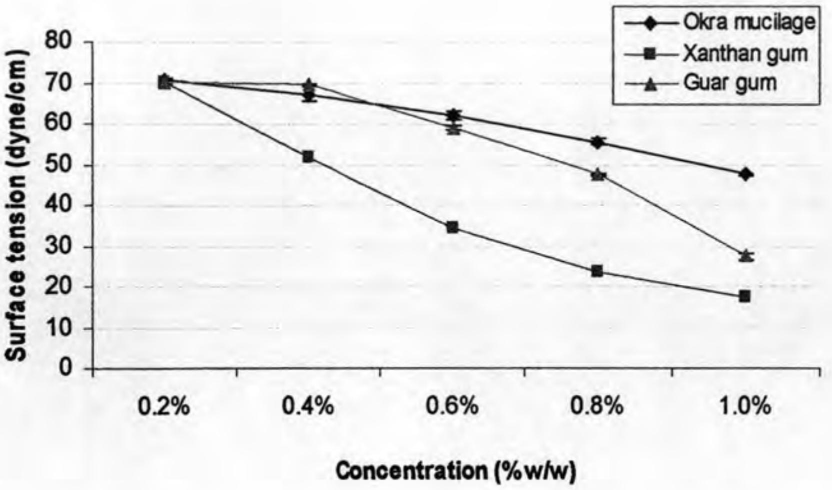


Figure 4.2 Surface tension of okra mucilage, xanthan gum, and guar gum at neutral condition.

4.4 Flow behavior of okra mucilage, xanthan gum, and guar gum.

4.4.1 Linear viscoelastic range (LVR)

LVR covers the range of strain that moduli are constant. A decrease in modulus is the result of the change of sample's structure. In order to determine LVR, aqueous okra mucilage (OM), xanthan gum (XG), and guar gum (GG) solutions were prepared at 0.2, 0.4, and 1.0 w/w at pH 6.6 ± 0.1. Figure 4.3 illustrates that complex modulus (G*) of 0.4, and 1.0% w/w of OM and GG, and 0.2, and 1.0% w/w of XG were constant in the range of 0.01-1 strain or 1-100% strain. An increase in strain (more than 1) led to a decrease in complex modulus, indicating that the structures were broken down with increasing strain. Thus, it could be concluded that, at strain less than 1, the structures of OM, XG, and GG were not destroyed. Since LVR of 0.2% w/w of OM and GG could not be determined, they were not further tested for viscoelastic behavior.

G* of 0.2% XG was quite similar to G* of 0.4% OM and GG, whereas, at 1% w/w, G* of XG was the highest, followed by GG, and OM, respectively. This was due to the structure of gum. Xanthan gum, which has an ionic side chain, is a stiff ordered chain conformation, whereas, the structure of guar gum is more flexible polymer chain. Therefore, the modulus of xanthan was higher than that of guar gum and okra mucilage.

The LVR ranges of the three samples were similar. The strain that G* starts to decrease indicates the ability of the sample as a stabilizer in emulsions. If G* decreases at higher strain, the sample is better in stabilizing emulsion. From the results, although the LVR ranges were similar, G* of XG was the highest among the three samples. Thus, it was expected that XG is the best in stabilizing emulsions as it is able to increase G*, which is related to viscosity and restricts the movement of oil droplets.

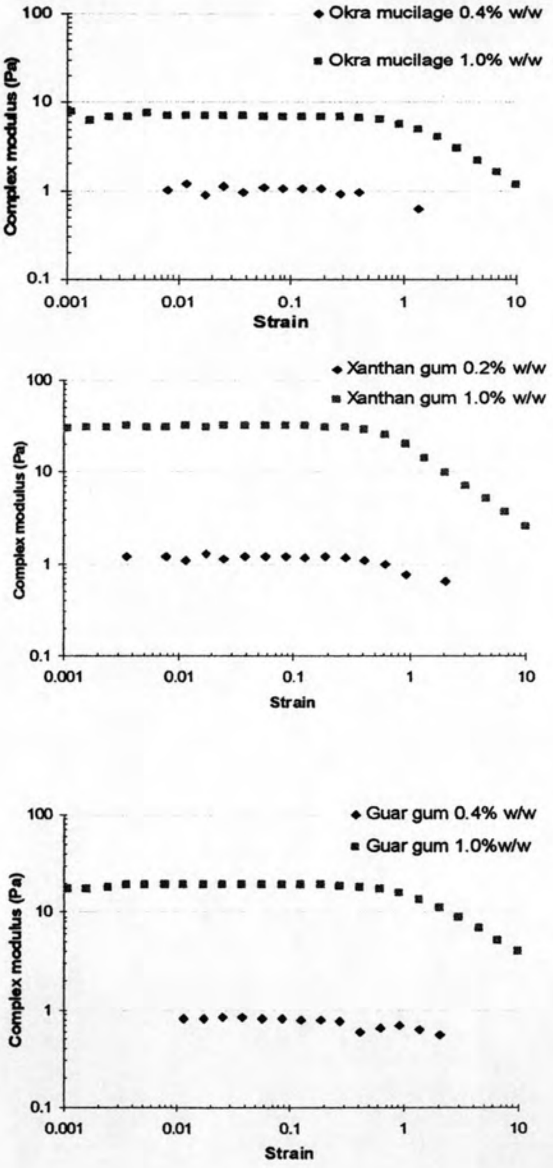


Figure 4.3 Linear viscoelastic range of gum solutions at various concentrations (0.2%-1.0% w/w).

4.4.2 Viscoelastic behavior of okra mucilage, xanthan gum, and guar gum.

Figure 4.4 and 4.5 show moduli and complex viscosity (η^*) of 0.4-1.0% w/w OM at neutral (pH 6.6 ± 0.1) and acidic pH (pH 3.7 ± 0.2), respectively. The results presented that, for 0.4% w/w of OM at pH 6.6, loss modulus (G'') was higher than elastic modulus (G') at low frequency. A crossover between G' and G'' was observed at around 0.1 Hz frequency, then G' became higher than G''. This indicated entanglement of polymer in a concentrated polymer solution. At pH 3.7, the crossover shifted to a higher frequency. This means that low pH might cause the polymer chain to contract slightly, thus lessened the extent of polymer entanglements. For 0.6, 0.8, and 1.0% w/w OM at pH 6.6 and 3.7, $G'$and G'' crossover frequency was close to 0.01 Hz. From 0.01 Hz up, G' was higher than G'' in all samples. This result suggested a weak network formation in the okra mucilage solution. These networks could be the result of more entanglement due to higher polymer concentration. Figure 4.5 shows η^* of 0.4-1.0% w/w OM. 1.0% w/w OM gave higher complex viscosity than 0.8, 0.6, 0.4, and 0.2% w/w, respectively. At every concentration, the complex viscosity of okra mucilage at pH 6.6 was higher than that at pH 3.7. The results suggested that a decrease in pH might cause the chain contraction of polymer molecules; hence, the hydrodynamic volume and viscosity decreased.

Figures 4.6 and 4.7 show moduli and η^* at 0.2-1.0% w/w XG at pH 6.6 and 3.7, respectively. It could be observed that the viscoelastic behavior and η^* of 0.2% XG were dependent on pH. For 0.2% w/w XG at pH 6.6 and 3.7, G'' was higher than G' at low frequency, but, as the frequency increased, G' became higher than G''. The crossover of 0.2% w/w XG at pH 3.7 was higher and the moduli were lower than that at pH 6.6. This indicated that the polymer of 0.2% w/w XG at pH 3.7 was more disentangled. For 0.4, 0.6, 0.8, and 1.0% w/w XG at pH 6.6 and 3.7, in the experimental range of frequency, G' was higher than G''. This suggested that they tended to form macromolecular network with elastic properties. The pH of the solution had an effect on 0.2% XG, in which the η^* at pH 3.7 was lower than that at pH 6.6 (figure 4.7). This result was in agreement with the effect of pH on the moduli. The η^* of 0.4-1.0% w/w XG at pH 6.6 and 3.7 were equal.

Figures 4.8 and 4.9 show moduli and η^* at 0.4-1.0% w/w GG at pH 6.6 and 3.7, respectively. For 0.4, 0.6, and 0.8% w/w GG at pH 6.6 and 3.7, G'' was higher than G', indicating viscous flow behavior of the solutions. At 1.0% w/w, G'' was higher than G' at low frequency. From 0.6 Hz up, which was the crossover frequency, G' became higher than G'', indicating the presence of polymer entanglement. The results showed that, at every concentration, the moduli and η^* of guar gum at pH 3.7 were lower than that at pH 6.6, and the difference decreased systematically with increasing concentration. Similar observations were found in Medina-Torres *et al.* (2000).

Comparative to OM and GG, XG exhibited superior strength at the same concentration as evidenced by higher G'. It could be observed that viscoelastic properties of OM were similar to XG. At 0.4% w/w OM and 0.2% w/w XG, they exhibited entangled polymer solution, in which G'' dominated over lower tested frequency range. As concentration increased, 0.6-1.0% w/w OM and 0.4-1.0% w/w XG, they exhibited gel-like behavior, in which G' dominated indicating that the structure did not dissipate energy. In contrast, GG had different viscoelastic properties. At every concentration, GG exhibited entangled polymer solution that flowed well. However, it could be observed that the structure arrangement of GG was the least sensitive to shear force among the three gums, as evidenced by the extent of η^* reduction over the test frequency range.

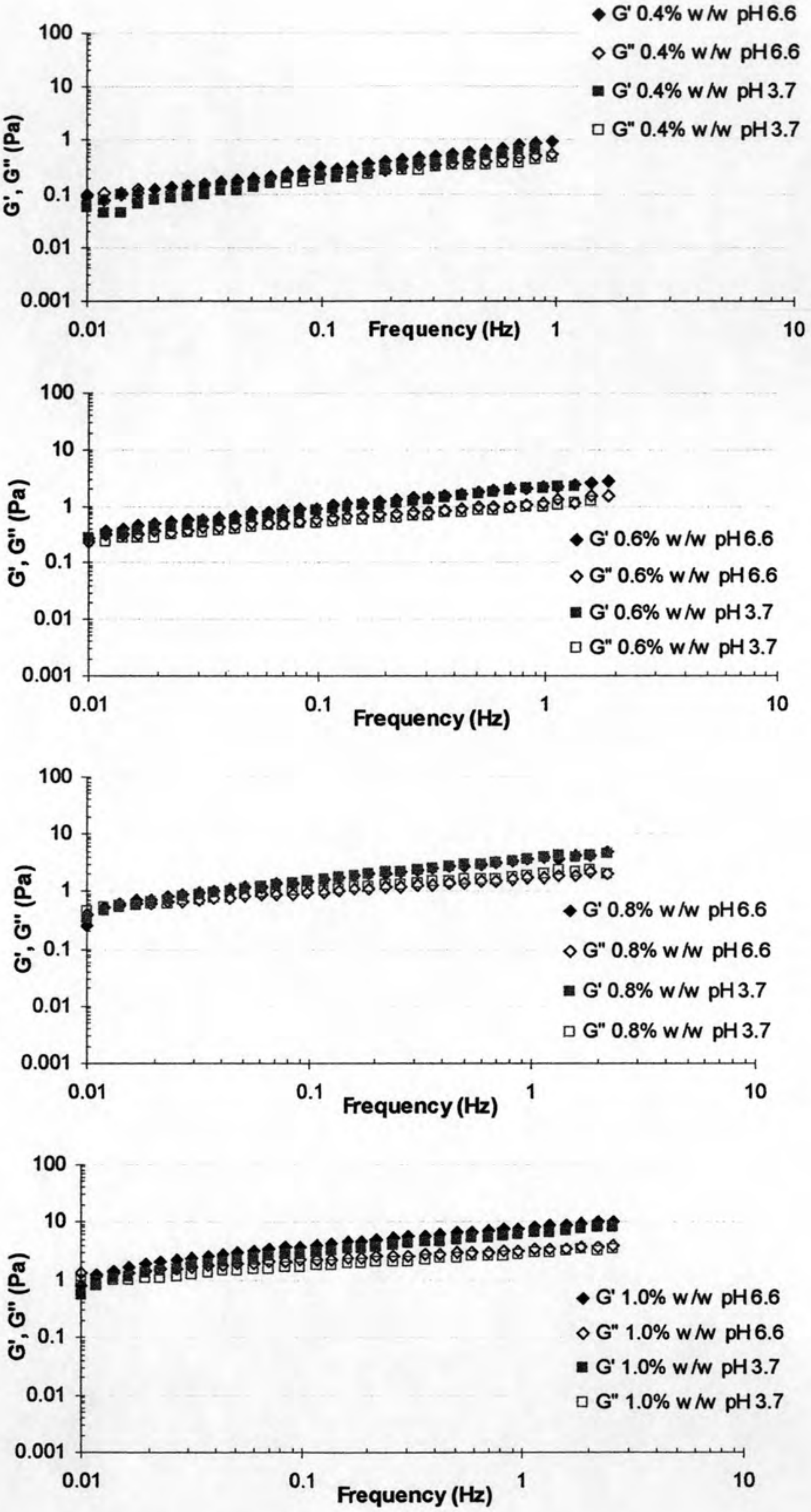


Figure 4.4 Storage (G') and loss (G") modulus of 0.4-1.0% w/w okra mucilage at pH 6.6 ± 0.1 and pH 3.7 ± 0.2.

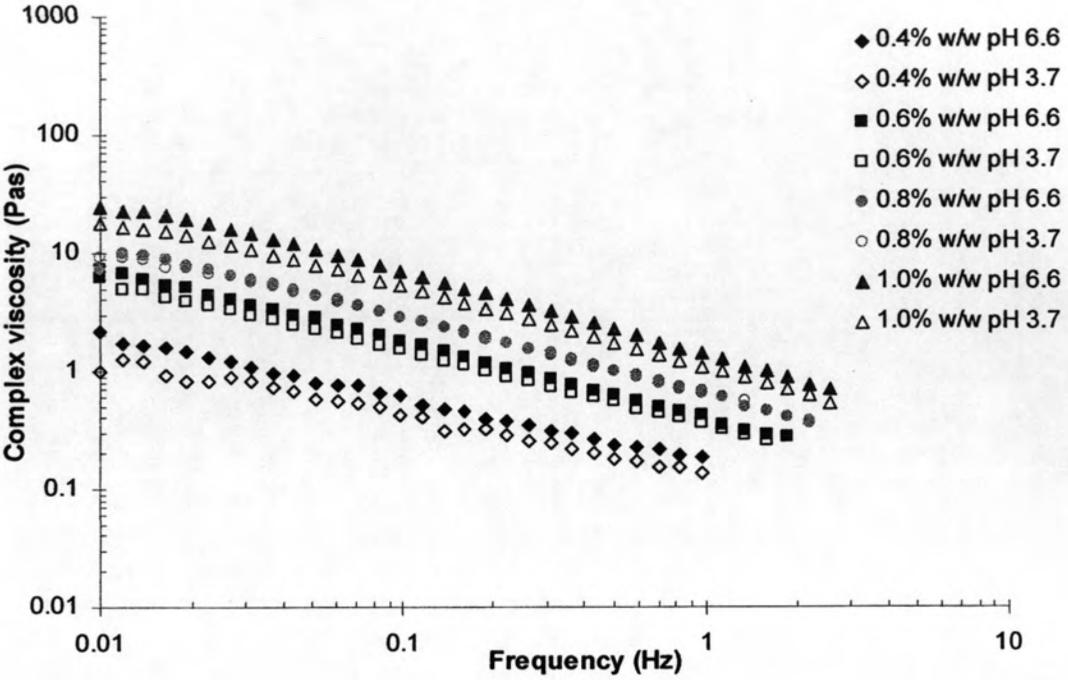


Figure 4.5 Complex viscosity (η^*) of 0.4-1.0% w/w okra mucilage at pH 6.6 ± 0.1 and pH 3.7 ± 0.2.

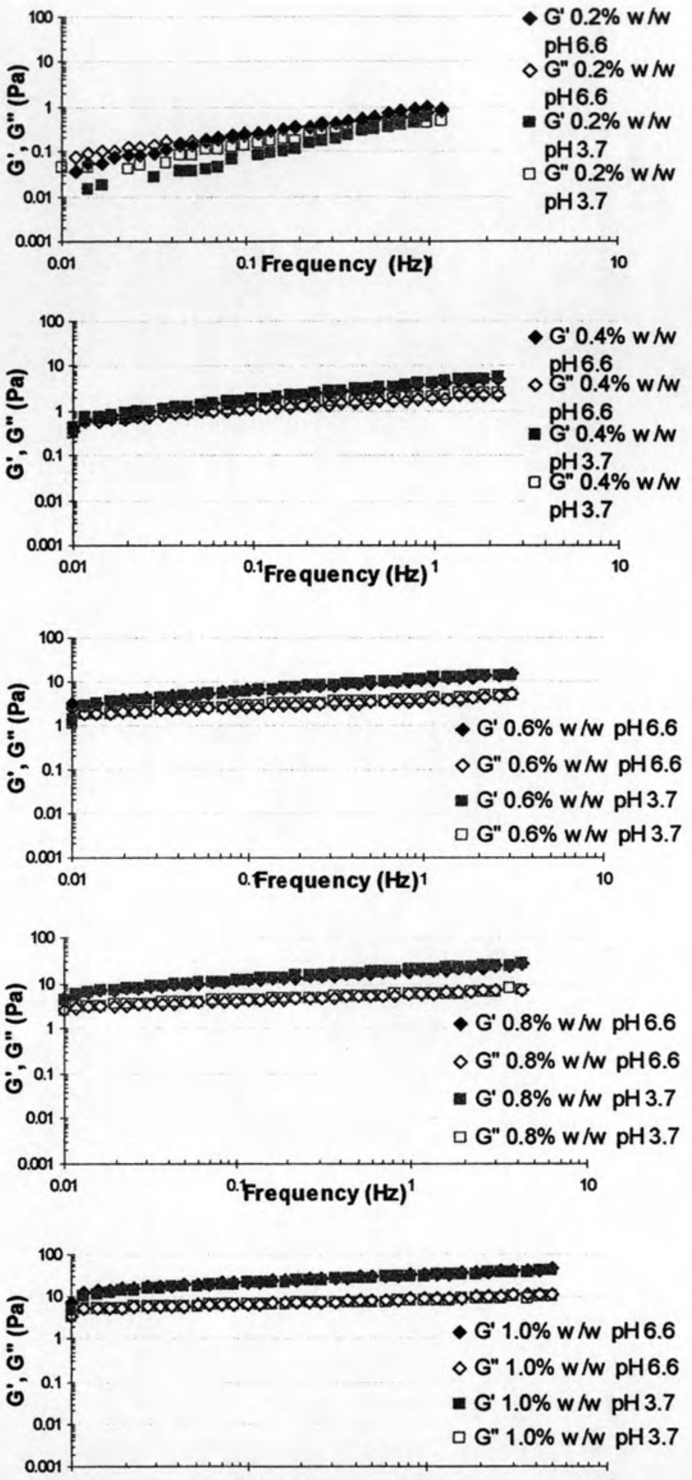


Figure 4.6 Storage (G') and loss (G") modulus of 0.2-1.0% w/w xanthan gum at pH 6.6 ± 0.1 and pH 3.7 ± 0.2.

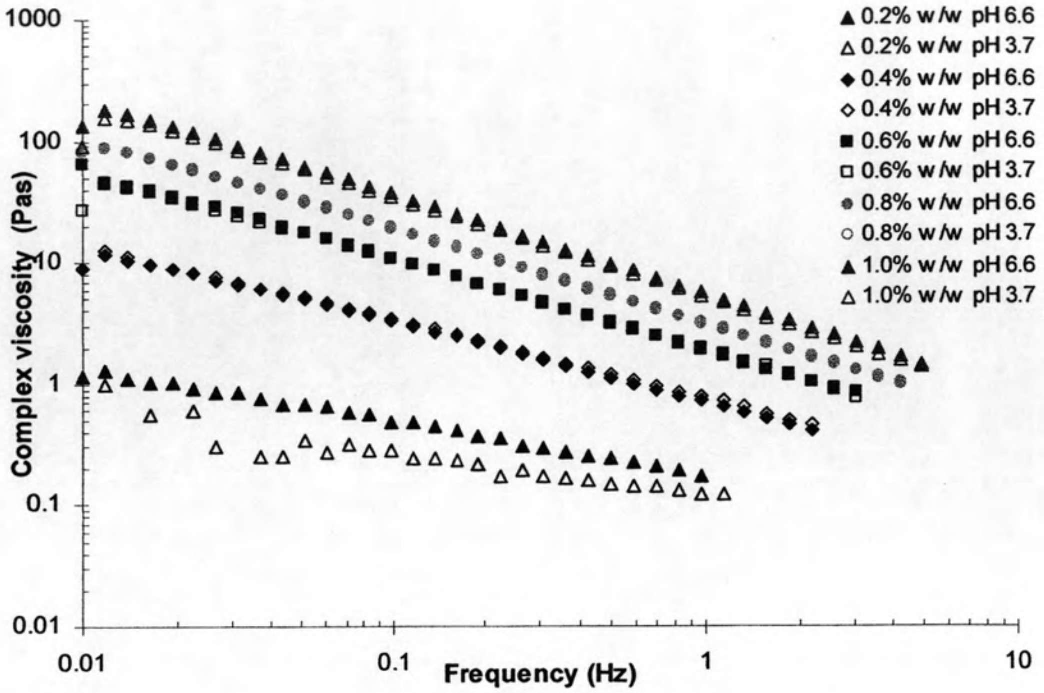


Figure 4.7 Complex viscosity (η^*) of 0.2-1.0% w/w xanthan gum at pH 6.6 ± 0.1 and pH 3.7 ± 0.2.

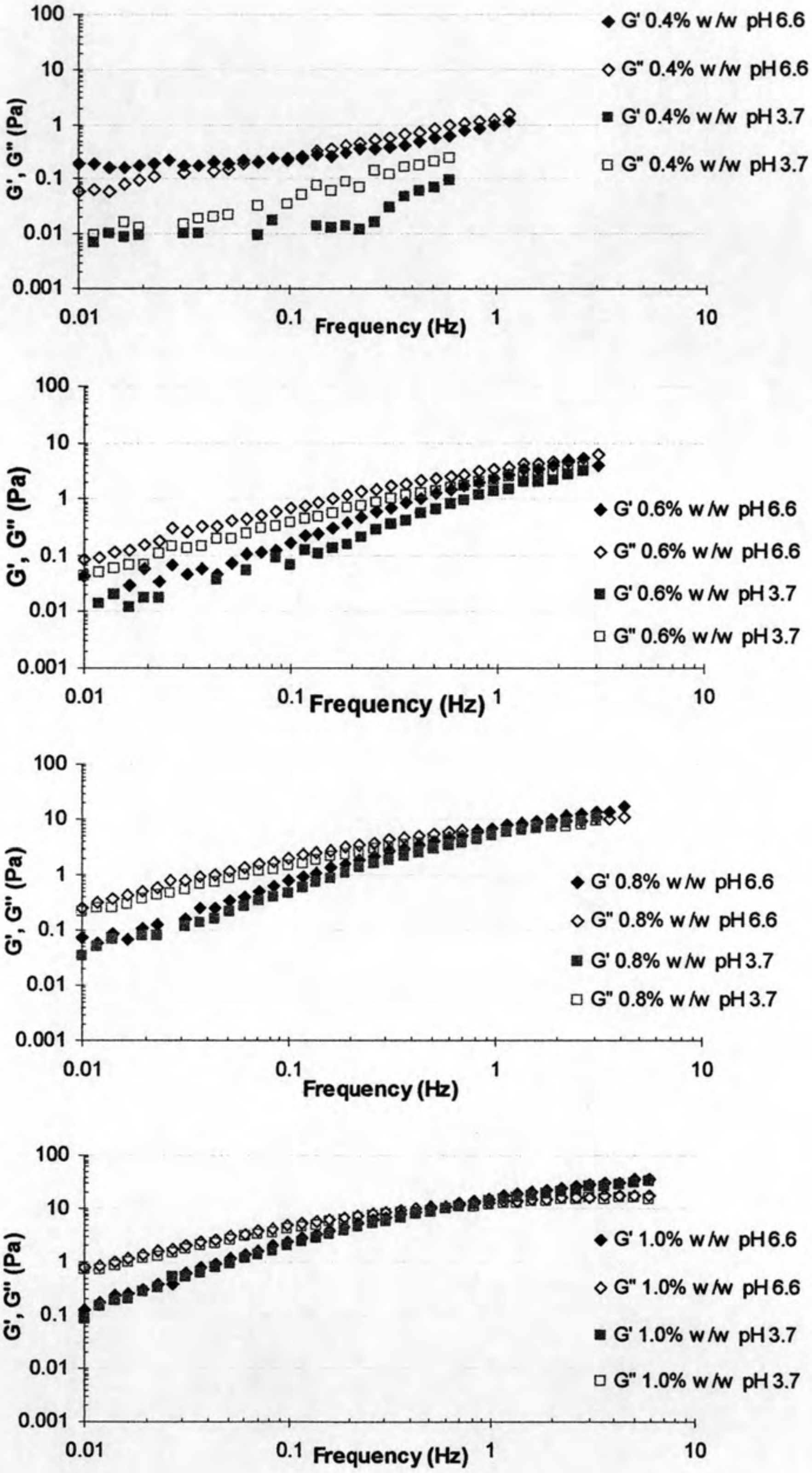


Figure 4.8 Storage (G') and loss (G") modulus of 0.4-1.0% w/w guar gum at pH 6.6 ± 0.1 and pH 3.7 ± 0.2.

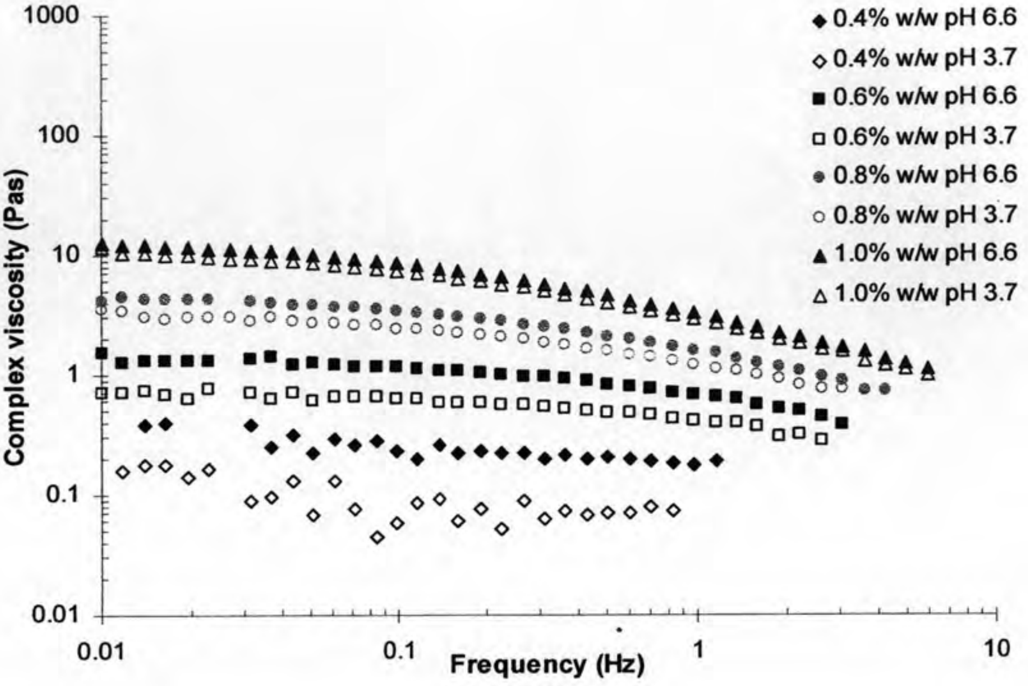


Figure 4.9 Complex viscosity (η^*) of 0.4-1.0% w/w guar gum at pH 6.6 ± 0.1 and pH 3.7 ± 0.2.

4.4.3 Steady shear test.

In this study, the power law model was used to describe flow behavior of each gum solution in the 0.1-50 s^{-1} range. Consistency coefficients (K) and flow behavior indices (n) are shown in tables 4.2 to 4.6. All gum solutions exhibited pseudoplastic, shear thinning flow behavior, in which n is less than 1. There was no hysteresis loop in the shear rate versus shear stress ascending and descending curves of all samples in the tested range. The results indicated that the gum solutions were non thixotropic in the 0.1-50 s^{-1} shear rate range. In this study, concentration, temperature, and pH were concerned as factors that might have an effect on flow behavior. The results showed that concentration, temperature, and pH significantly affected flow behavior of okra mucilage and xanthan gum at every concentration, but only concentration and temperature significantly affected flow behavior of guar gum ($p \leq 0.05$). At 0.8% and 1.0% w/w xanthan gum solutions, yield stresses could also be identified by using a Herschel Bulkley model (table 4.4). This indicated that, at rest, xanthan gum at these concentrations formed static networks and required a stress; yield stress, to make it flow. This might be due to a molecular association network between xanthan gum molecules. For all gum solutions, at the same temperature, K significantly increased and n significantly decreased as concentration increased ($p \leq 0.05$). These results were in agreement with those of dynamic test, which G', G'', and η^* increased as concentration increased. An increase in concentration led to an increase in entanglement and polymer-polymer interaction, resulting in an increase in viscosity. In addition, a disentanglement and alignment of the molecules during flow caused a decrease in n; an increase in pseudoplasticity.

A significant decrease in consistency index was observed with an increase in temperature at all concentrations of gum solutions, indicating a decrease in the apparent viscosity of gum solutions at higher temperature as can be observed in other viscous liquids. An increase in flow behavior index with an increase in temperature indicated that hydrocolloid solutions tended to be closer to Newtonian flow at higher temperatures. These results were in good agreement with Dak, Verma, and Sharma (2006). As temperature increases, the molecules have more energy and the molecular mobility is better. This caused the flow resistance to decrease, hence the viscosity is lowered. The effect of temperature on the apparent viscosity at a specified shear rate is generally expressed by an Arrhenius-type model (Speers and Tung, 1986). If the activation energy (E_a) for flow of the sample is a positive value, it indicates that K increases with an increase in temperature. If the activation energy for flow of the sample is a negative value, it indicates that K decreases with an increase in temperature. Table 4.6 shows that, at 0.2% w/w, the absolute Ea value for flow of all gum solutions were comparable. At the concentrations of 0.4-0.6% w/w, the absolute E_a value for flow of guar gum solutions was the highest and was a negative value. Hence, flow parameters of guar gum were more likely to be more affected by temperature than other gum solutions at the same concentration. At higher concentrations (0.8 and 1.0% w/w), table 4.3 and 4.4, consistency index and yield stress of xanthan gum solution at pH 6.6 tend to increase with increasing temperature indicating the thermal denaturation of xanthan gum (Capron *et al.*, 1998). This also affected the E_a for flow of xanthan gum, in which, at these concentrations, the E_a were positive values (table 4.6). At these concentrations, xanthan gum, a semi-rigid conformation, were characterized as weak gel, which could be observed by the higher G' over G'' throughout the tested frequency range (figure 4.6). As temperature increased, the covalent bonds in the intermolecular cross-links were provoked, resulting in more strength of the network. Therefore, the stress to make the gum solution flow increased (Hill *et al.*, 1998).

Flow behavior of guar gum was not affected by pH as evidenced by an insignificant difference in K and n at neutral and acidic conditions. In contrast, the effect of pH on flow behavior of okra mucilage could be observed. At the same concentration and temperature, a decrease in pH led to a decrease in K and an increase in n, indicating that okra mucilage was less shear thinning. Similarly, for 1.0% w/w xanthan gum, a decrease in pH significantly reduced K and significantly increased n ($p \leq 0.05$). The structure of xanthan gum can change from an ordered (helical) to a disordered conformation, depending on ionic strength, nature of electrolyte, and acetyl and pyruvate contents (Khouryieh *et al.*, 2007). In distilled water, xanthan exists as a disordered structure but highly extended due to the electrostatic or Coulomb repulsions from the charged groups on the side chains. In an acidic condition, positively charged ions may interact with the negatively charged groups of xanthan. The xanthan molecules become more flexible and more compact. This leads to a reduction in viscosity and an increase in the flow behavior index. Since flow behavior of okra mucilage was similar to that of xanthan gum, it is hypothesized that polymers in okra mucilage tended to be ionic. This hypothesis was in agreement with the results of Lengsfeld *et al.* (2004). They found that raw polysaccharides extracted from fresh okra contained acidic polysaccharide fractions, which exhibited a dominant rhamnose-galactose moiety. A similar argument was given by Medina-Torres *et al.* (2000). Their study showed that the pH and ionic strength affected hydrodynamic and thus flow properties of aqueous mucilage solutions from *Opuntia ficus indica*, a negatively charged polyelectrolyte molecule. A decrease in pH and an increase in ionic strength led to a decrease in viscosity. At zero ionic strength condition such as in deionized water, a negative charge produces strong intermolecular repulsion and thus a more expanded molecule. But, an acidic pH, the addition of positive ions reduces repulsion and molecular expansion, resulting in a reduction in viscosity.

Comparatively, the magnitude of flow behavior index was the lowest for xanthan and the highest for guar gum within the range of concentration levels studied. It has been reported that the value of flow behavior index and its change with concentration are highly dependent on the molecular size (Speers and Tung, 1986). According to Achayuthakan (2007), the structure and intrinsic viscosity were also concerned when studying rheological properties. Guar gum is water soluble non-ionic polysaccharide, which has a general structure of galactomannan. It consists of a main chain (1 $\rightarrow$4) β-D-mannopyranosyl unit substituted at O-6 by single-unit side chain of a D-galactopyranose. The ratio of mannose to galactose is approximately 2:1, depending on the source and method of extraction. In contrast, xanthan gum is an anionic extracellular polysaccharide. It consists of a β-(1$\rightarrow$4)-D-glucopyranose glucan backbone with side chains of (3$\rightarrow$1)-α-linked D-mannopyranose-(2$\rightarrow$1)- β-D-glucuronic acid-(4$\rightarrow$1)- β-D-mannopyranose on alternating residues. The mentioned structure makes xanthan molecule a rather stiff rod, ordered chain conformation when dissolved in solutions. The results of Achayuthakan (2007) presented that xanthan gum had lower average molecular weight (3.47×10^6 Da) and higher intrinsic viscosity (112.3 dl/g) than that of guar gum (average MW 6.12×10^6 Da, intrinsic viscosity 9.4 dl/g). Intrinsic viscosity is a measure of the hydrodynamic volume per gram of macromolecular substance at infinite dilution. In dilute solutions, the polymer chains are separate, and the intrinsic viscosity of the polymer in solution depends only on the dimensions of the polymer chain. A more flexible polymer chain of guar gum exhibits a smaller chain dimension and, hence, gives lower intrinsic viscosity. Xanthan gum molecule could sweep more hydrodynamic volume when dissolved in solution and subject to shear, hence giving higher viscosity. This is confirmed by the behavior of xanthan solution as a weak gel and that of guar solution as an entangle solution as presented in section 4.4.2. Since the flow behavior index of okra mucilage solutions was in between that of xanthan and guar gum solutions, it could be assumed that the molecular structure of the polymers presenting in okra mucilage was more flexible than xanthan, but more stiff than that of guar gum.

Table 4.2 Consistency index (K) and flow behavior index (n) of 0.2% w/w okra mucilage at neutral (pH 6.6 ± 0.1) and acidic condition (pH 3.7 ± 0.2).

Concentration (% w/w)	Temperature (°C)	K		n	
		pH 6.6±0.1	pH 3.7±0.2	pH 6.6±0.1	pH 3.7±0.2
0.2	15	0.20^{a1} ± 0.01	0.14^{a1} ± 0.03	0.5^{a1} ± 0.00	0.57^{a1} ± 0.03
	25	0.15^{b1} ± 0.01	0.11^{ab1} ± 0.02	0.55^{ab1} ± 0.01	0.61^{ab1} ± 0.02
	35	0.13^{bc1} ± 0.01	0.08^{b2} ± 0.01	0.55^{ab1} ± 0.00	0.64^{ab2} ± 0.01
	45	0.10^{c1} ± 0.02	0.06^{b1} ± 0.01	0.59^{b1} ± 0.03	0.67^{b1} ± 0.04
0.4	15	0.55^{a1} ± 0.01	0.43^{a1} ± 0.05	0.42^{a1} ± 0.00	0.47^{a2} ± 0.01
	25	0.47^{b1} ± 0.01	0.33^{ab2} ± 0.03	0.43^{a1} ± 0.01	0.49^{ab2} ± 0.01
	35	0.39^{c1} ± 0.01	0.26^{bc2} ± 0.03	0.45^{b1} ± 0.00	0.51^{bc2} ± 0.01
	45	0.33^{d1} ± 0.00	0.21^{c2} ± 0.02	0.47^{c1} ± 0.00	0.53^{c2} ± 0.01
0.6	15	1.43^{a1} ± 0.04	1.22^{a2} ± 0.06	0.34^{a1} ± 0.01	0.37^{a2} ± 0.01
	25	1.15^{b1} ± 0.06	0.92^{b1} ± 0.09	0.36^{a1} ± 0.01	0.39^{b2} ± 0.01
	35	0.95^{c1} ± 0.05	0.74^{bc2} ± 0.05	0.39^{b1} ± 0.01	0.42^{c2} ± 0.01
	45	0.8^{c1} ± 0.06	0.6^{c2} ± 0.005	0.40^{b1} ± 0.01	0.44^{c2} ± 0.01
0.8	15	2.53^{a1} ± 0.08	2.33^{a1} ± 0.10	0.30^{a1} ± 0.01	0.32^{a1} ± 0.01
	25	2.02^{b1} ± 0.01	1.86^{b1} ± 0.15	0.32^{b1} ± 0.01	0.35^{ab2} ± 0.02
	35	1.66^{c1} ± 0.05	1.49^{bc1} ± 0.14	0.34^{c1} ± 0.01	0.38^{ab2} ± 0.02
	45	1.33^{d1} ± 0.04	1.16^{c2} ± 0.17	0.36^{d1} ± 0.00	0.40^{b2} ± 0.03
1.0	15	5.47^{a1} ± 0.52	4.32^{a2} ± 0.02	0.24^{a1} ± 0.00	0.26^{a2} ± 0.00
	25	4.60^{ab1} ± 0.36	3.51^{b2} ± 0.05	0.26^{b1} ± 0.00	0.28^{b2} ± 0.00
	35	3.60^{bc1} ± 0.35	2.96^{c2} ± 0.01	0.29^{c1} ± 0.01	0.30^{c1} ± 0.01
	45	2.90^{c1} ± 0.21	2.30^{d2} ± 0.06	0.31^{d1} ± 0.00	0.33^{d2} ± 0.00

$^{a\text{-}d}$ Different letters in the same column denote significant difference ($p \leq 0.05$).

^{1,2} Different numbers in the same row denote significant difference ($p \leq 0.05$).

Table 4.3 Consistency index (K) and flow behavior index (n) of 0.2% w/w xanthan gum at neutral (pH 6.6 ± 0.1) and acidic condition (pH 3.7 ± 0.2).

Concen-tration (% w/w)	Temp-erature (°C)	K		n	
		pH 6.6±0.1	pH 3.7±0.2	pH 6.6±0.1	pH 3.7±0.2
0.2	15	$0.44^{a1} \pm 0.08$	$0.47^{a1} \pm 0.01$	$0.36^{a1} \pm 0.02$	$0.41^{a1} \pm 0.01$
	25	$0.36^{ab1} \pm 0.07$	$0.36^{b1} \pm 0.01$	$0.40^{ab1} \pm 0.03$	$0.45^{b1} \pm 0.00$
	35	$0.29^{ab1} \pm 0.07$	$0.29^{c1} \pm 0.01$	$0.44^{bc1} \pm 0.04$	$0.48^{c1} \pm 0.00$
	45	$0.24^{b1} \pm 0.03$	$0.24^{d1} \pm 0.00$	$0.49^{c1} \pm 0.01$	$0.52^{d2} \pm 0.00$
0.4	15	$1.92^{a1} \pm 0.09$	$2.13^{a1} \pm 0.18$	$0.17^{a1} \pm 0.01$	$0.24^{a2} \pm 0.01$
	25	$1.72^{b1} \pm 0.07$	$1.89^{ab1} \pm 0.15$	$0.21^{b1} \pm 0.00$	$0.27^{b2} \pm 0.01$
	35	$1.60^{b1} \pm 0.01$	$1.62^{bc1} \pm 0.10$	$0.24^{c1} \pm 0.01$	$0.30^{c2} \pm 0.00$
	45	$1.39^{c1} \pm 0.04$	$1.35^{c1} \pm 0.13$	$0.28^{d1} \pm 0.00$	$0.33^{d2} \pm 0.01$
0.6	15	$4.86^{a1} \pm 0.21$	$5.42^{a1} \pm 0.10$	$0.11^{a1} \pm 0.00$	$0.16^{a2} \pm 0.00$
	25	$4.95^{a1} \pm 0.12$	$4.92^{b1} \pm 0.08$	$0.12^{a1} \pm 0.00$	$0.18^{b2} \pm 0.00$
	35	$4.72^{a1} \pm 0.10$	$4.22^{c1} \pm 0.17$	$0.15^{b1} \pm 0.01$	$0.22^{c2} \pm 0.00$
	45	$3.91^{b1} \pm 0.19$	$3.62^{d1} \pm 0.02$	$0.20^{c1} \pm 0.01$	$0.44^{d2} \pm 0.00$
0.8	15	$6.11^{a1} \pm 0.14$	$9.2^{a2} \pm 0.05$	$0.14^{a1} \pm 0.00$	$0.11^{a2} \pm 0.00$
	25	$7.01^{b1} \pm 0.14$	$9.04^{a2} \pm 0.14$	$0.12^{b1} \pm 0.00$	$0.12^{b1} \pm 0.00$
	35	$7.93^{c1} \pm 0.17$	$8.66^{a1} \pm 0.31$	$0.10^{c1} \pm 0.00$	$0.14^{c2} \pm 0.00$
	45	$7.82^{c1} \pm 0.43$	$7.17^{b1} \pm 0.26$	$0.13^{d1} \pm 0.01$	$0.18^{d2} \pm 0.00$
1.0	15	$7.85^{a1} \pm 0.14$	$13.31^{a2} \pm 0.11$	$0.17^{a1} \pm 0.00$	$0.10^{a2} \pm 0.00$
	25	$8.81^{b1} \pm 0.13$	$14.46^{b2} \pm 0.21$	$0.16^{b1} \pm 0.00$	$0.08^{b2} \pm 0.00$
	35	$10.46^{c1} \pm 0.20$	$14.53^{b2} \pm 0.55$	$0.13^{c1} \pm 0.00$	$0.09^{ab2} \pm 0.00$
	45	$12.23^{d1} \pm 0.07$	$13.69^{ab2} \pm 0.17$	$0.10^{d1} \pm 0.00$	$0.11^{c1} \pm 0.01$

$^{a-d}$ Different letters in the same column denote significant difference ($p \leq 0.05$).

^{1,2} Different numbers in the same row denote significant difference ($p \leq 0.05$).

Table 4.4 Flow test with yield stress of xanthan gum at neutral (pH 6.6 ± 0.1) and acidic condition (pH 3.7± 0.2).

Concen-tration (% w/w)	Temp-erature (°C)	Yield stress		K		n	
		pH 6.6±0.1	pH 3.7±0.2	pH 6.6±0.1	pH 3.7±0.2	pH 6.6±0.1	pH 3.7±0.2
0.8	15	$2.74^{a} \pm 0.47$	$6.60^{a} \pm 0.04$	$2.64^{a} \pm 0.12$	$2.94^{a} \pm 0.01$	$0.24^{a} \pm 0.01$	$0.24^{a} \pm 0.00$
	25	$4.03^{ab} \pm 0.36$	$6.49^{a} \pm 0.11$	$2.47^{a} \pm 0.05$	$2.96^{a} \pm 0.02$	$0.25^{a} \pm 0.01$	$0.25^{a} \pm 0.00$
	35	$5.49^{b} \pm 0.72$	$5.18^{a} \pm 1.3$	$2.67^{a} \pm 0.33$	$4.00^{a} \pm 1.54$	$0.22^{a} \pm 0.02$	$0.24^{a} \pm 0.26$
	45	$5.30^{b} \pm 0.66$	$2.34^{b} \pm 1.19$	$3.06^{a} \pm 1.03$	$5.17^{a} \pm 1.37$	$0.21^{a} \pm 0.05$	$0.23^{a} \pm 0.04$
1.0%	15	$1.51^{a} \pm 1.24$	$7.26^{a} \pm 0.28$	$5.23^{a} \pm 1.22$	$6.36^{a} \pm 0.32$	$0.21^{a} \pm 0.03$	$0.17^{a} \pm 0.00$
	25	$4.90^{ab} \pm 0.00$	$12.24^{b} \pm 0.5$	$2.73^{b} \pm 0.05$	$2.88^{b} \pm 0.21$	$0.32^{b} \pm 0.01$	$0.26^{b} \pm 0.01$
	35	$3.91^{ab} \pm 0.39$	$12.26^{b} \pm 0.43$	$5.12^{a} \pm 0.03$	$3.12^{b} \pm 0.09$	$0.22^{a} \pm 0.00$	$0.26^{b} \pm 0.01$
	45	$6.60^{b} \pm 2.07$	$7.91^{a} \pm 0.69$	$4.74^{a} \pm 1.82$	$6.34^{a} \pm 0.47$	$0.21^{a} \pm 0.05$	$0.19^{c} \pm 0.00$

[a-d] Different letters in the same column denote significant difference ($p \leq 0.05$).

Table 4.5 Consistency index (K) and flow behavior index (n) of 0.2% w/w guar gum at neutral (pH 6.6 ± 0.1) and acidic condition (pH 3.7 ± 0.2).

Concentration (% w/w)	Temperature (°C)	K		n	
		pH 6.6±0.1	pH 3.7±0.2	pH 6.6±0.1	pH 3.7±0.2
0.2*	15	0.02^{a1} ± 0.00	0.02^{a1} ± 0.00	1	1
	25	0.01^{b1} ± 0.00	0.02^{b1} ± 0.00	1	1
	35	0.01^{b1} ± 0.00	0.01^{b1} ± 0.00	1	1
	45	0.01^{b1} ± 0.00	0.01^{b1} ± 0.00	1	1
0.4	15	0.25^{a1} ± 0.01	0.21^{a1} ± 0.02	0.72^{a1} ± 0.02	0.75^{a1} ± 0.02
	25	0.15^{b1} ± 0.03	0.15^{ab1} ± 0.04	0.78^{b1} ± 0.04	0.80^{ab1} ± 0.06
	35	0.10^{bc1} ± 0.03	0.08^{b1} ± 0.02	0.93^{c1} ± 0.08	0.90^{b1} ± 0.05
	45	0.08^{c1} ± 0.00	0.08^{b1} ± 0.02	0.86^{bc1} ± 0.00	0.86^{ab1} ± 0.04
0.6	15	1.02^{a1} ± 0.08	1.04^{a1} ± 0.06	0.57^{a1} ± 0.02	0.57^{a1} ± 0.01
	25	0.77^{b1} ± 0.07	0.74^{b1} ± 0.01	0.61^{b1} ± 0.01	0.62^{b1} ± 0.01
	35	0.56^{c1} ± 0.07	0.57^{c1} ± 0.05	0.67^{c1} ± 0.02	0.66^{bc1} ± 0.02
	45	0.43^{c1} ± 0.05	0.45^{c1} ± 0.03	0.70^{c1} ± 0.02	0.69^{c1} ± 0.02
0.8	15	3.83^{a1} ± 0.16	3.43^{a1} ± 0.34	0.4^{a1} ± 0.01	0.43^{a1} ± 0.01
	25	2.95^{b1} ± 0.34	2.42^{b1} ± 0.14	0.46^{ab1} ± 0.02	0.48^{b1} ± 0.02
	35	2.37^{bc1} ± 0.23	1.99^{bc1} ± 0.22	0.49^{bc1} ± 0.02	0.52^{c1} ± 0.02
	45	1.92^{c1} ± 0.18	1.68^{c1} ± 0.06	0.53^{c1} ± 0.02	0.54^{c1} ± 0.00
1.0	15	9.82^{a1} ± 0.13	10.64^{a1} ± 0.64	0.33^{a1} ± 0.01	0.31^{a1} ± 0.01
	25	7.80^{b1} ± 0.06	8.74^{b1} ± 0.49	0.37^{b1} ± 0.01	0.35^{b1} ± 0.01
	35	6.64^{c1} ± 0.05	7.08^{c1} ± 0.45	0.40^{c1} ± 0.01	0.38^{c1} ± 0.01
	45	5.32^{d1} ± 0.25	6.00^{c1} ± 0.59	0.43^{d1} ± 0.00	0.41^{d1} ± 0.01

[a-d] Different letters in the same column denote significant difference ($p \leq 0.05$).

[1,2] Different numbers in the same row denote significant difference ($p \leq 0.05$).

* Using a Newtonian model.

Table 4.6 Activation energy (E_a) and Consistency index (K) reference of okra mucilage, xanthan gum, and guar gum at neutral (pH 6.6 ± 0.1) and acidic condition (pH 3.7 ± 0.2).

	Ea		K_{ref}	
	pH 6.6±0.1	pH 3.7±0.2	pH 6.6±0.1	pH 3.7±0.2
Okra mucilage				
0.2% w/w	-1.77	-2.38	0.24	0.19
0.4% w/w	-1.43	-1.99	0.66	0.54
0.6% w/w	-1.61	-1.95	1.71	1.51
0.8% w/w	-1.77	-1.92	3.12	2.95
1.0% w/w	-1.79	-1.71	6.89	5.34
Xanthan gum				
0.2% w/w	-1.69	-1.86	0.54	0.58
0.4% w/w	-0.87	-1.27	2.14	2.52
0.6% w/w	-0.58	-1.13	5.47	6.32
0.8% w/w	0.72	-0.66	5.79	10.33
1.0% w/w	1.25	0.07	6.66	13.68
Guar gum				
0.2% w/w	-1.73	-2.31	0.02	0.03
0.4% w/w	-3.18	-2.93	0.34	0.29
0.6% w/w	-2.42	-2.31	1.37	1.33
0.8% w/w	-1.91	-1.94	4.75	4.12
1.0% w/w	-1.66	-1.60	11.89	12.84

4.5 Emulsion stability.

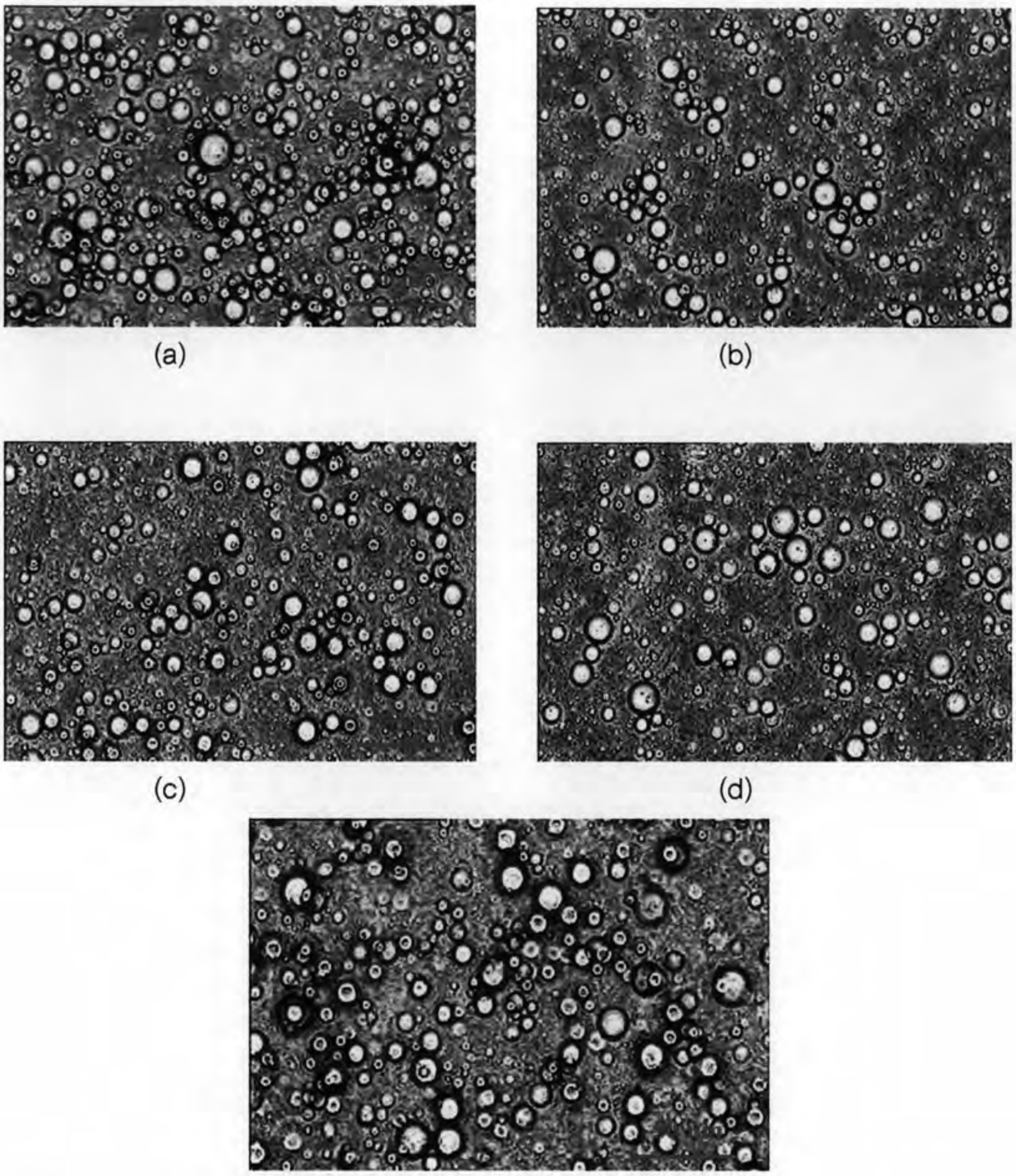
4.5.1 Oil droplets morphology and size analysis.

Microstructure of the emulsions stabilized by various gum solutions, which are solutions of okra mucilage, xanthan, and guar gum, are shown in figures 4.10, 4.11, and 4.12, respectively. Size distribution of oil droplets in emulsions are shown in figure 4.13 and table 4.7. For microstructure of the emulsions, it could be observed that an increase of concentration from 0.2 to 0.4% w/w of okra mucilage and xanthan gum solutions led to a decrease of oil droplets size. On the contrary, at higher concentrations (0.6, 0.8 and 1.0% w/w OM and XG), oil droplets size in the emulsions became larger. In case of guar gum, the size of oil droplets increased as concentration increased. When considering types of gum solution stabilized emulsions, at every gum solution, the size of oil droplets of emulsion stabilized by guar gum was larger than that stabilized by xanthan gum, and okra mucilage, respectively. For the okra mucilage stabilized emulsion (figure 4.10), there was a wide distribution of particle sizes at every concentration. The beginning of coalescence between smaller droplets and larger droplets could be observed. In case of xanthan gum stabilized emulsion (figure 4.11), individual, well distributed, droplets with no flocculation were observed. However, the droplets were densely packed. In contrast, a wide distribution of particle sizes and flocculation were clearly observed in the emulsion stabilized by guar gum (figure 4.12).

The particle size distribution of oil droplets was determined by using an image J® analyzer. The results were expressed as size distribution. The size of oil droplets in emulsions stabilized by OM, XG, and GG ranged from 3-18, 2-28, and 5-29 μm, respectively. According to figure 4.13 and table 4.7, the gum concentrations of 0.6% and 0.8% had the narrowest oil droplets size distribution, for okra mucilage and xanthan gum stabilized emulsion, respectively. However, the oil droplet size distributions of guar gum stabilized emulsion were evenly distributed at every concentration.

As the concentrations of OM and XG solutions increased from 0.2 to 0.4% w/w, the viscosity of gum solution stabilized emulsions was increased and they could restrict the movement of oil droplets, resulting in the small oil droplet size. When the concentration of gum solution increased, the viscosity of the gum solution was higher. An increase in the viscosity of the emulsion, then, had an effect on the homogenization process because the higher viscosity lessened the break up of oil droplets (Huang *et al.*, 2001). As a result, a size of oil droplets was larger. This was evidenced by, for okra mucilage stabilized emulsion, the average oil droplet size was decreased from 9.28 to 8.22 μm and was increased from 8.59 to 9.9 μm, when the gum concentration in emulsion increased from 0.2-0.4% w/w and from 0.6-1.0% w/w, respectively.

When considering type of gum solution used to stabilize emulsions, the results showed that, at every concentration, the oil droplets size of okra mucilage solution stabilized emulsion was the smallest, followed by those stabilized by xanthan gum solution and guar gum solution, respectively. This is due the properties of protein presented in okra mucilage. Proteins can reduce interfacial tension of oil in water emulsion, and also form a thin film layer covering the oil droplets (Fennerma, 1996). As a result, flocculation and coalescence of the dispersed phase are prevented. In addition, the flow behavior and structure of gum solution also involved with stabilization. For okra mucilage and xanthan gum solutions, from the viscoelastic behavior, they tended to form macromolecular network with elastic properties, as evidenced by the higher G' over G" along the tested frequency range (figure 4.4 and 4.6). On the contrary, for guar gum, there were a cross over between G' and G", indicating the weak network formation (figure 4.8). Therefore, due to the stronger network present in okra mucilage and xanthan gum solutions, they could prevent flocculation of oil droplets better than guar gum solutions. Since the complex viscosity of xanthan gum was higher than that of okra mucilage (figure 4.5 and 4.7), when the force was applied, the higher viscosity of xanthan gum might lessen the movement of oil droplets, resulting in a larger droplet size.

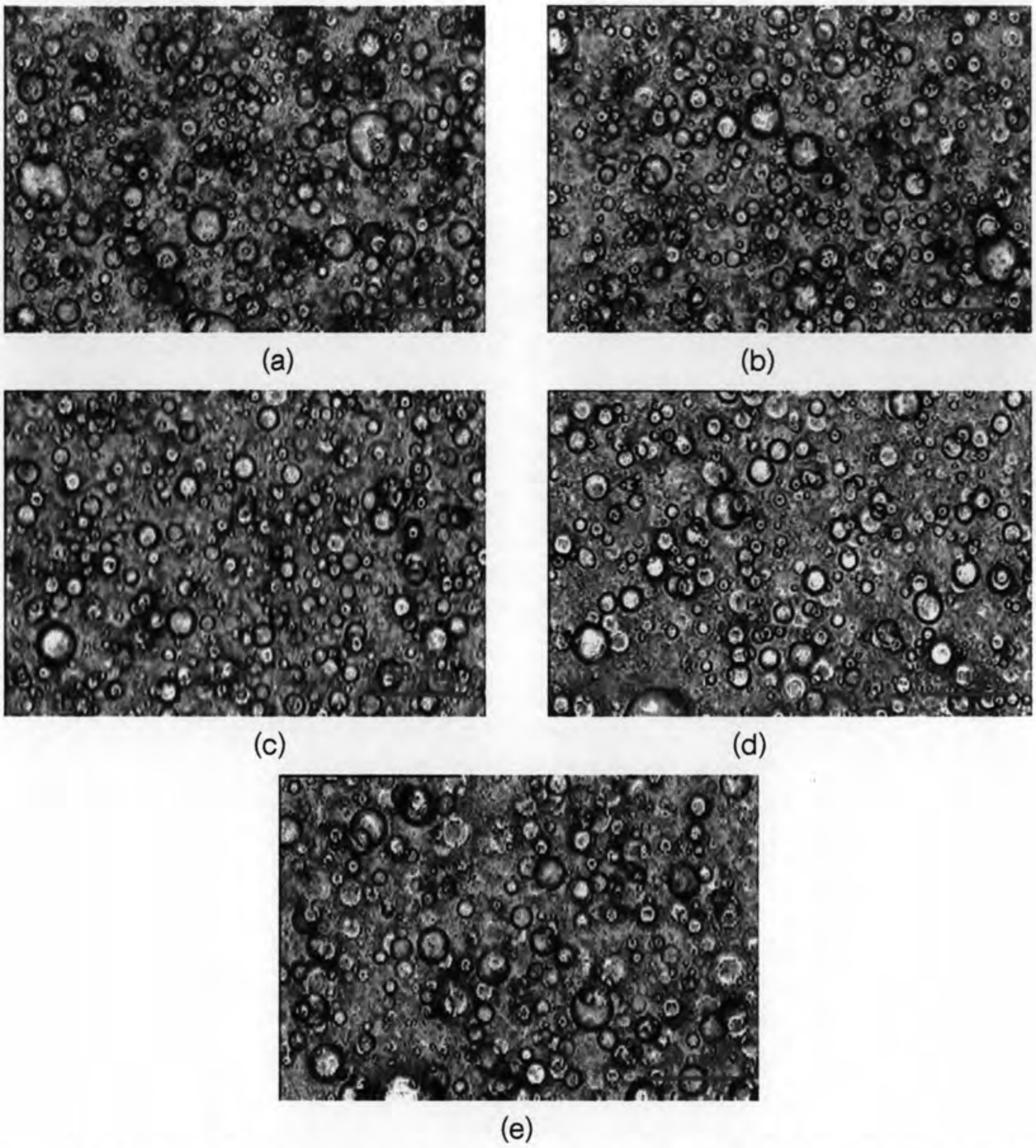


(a) (b)

(c) (d)

(e)

Figure 4.10 Effect of concentration; 0.2% (a), 0.4% (b), 0.6% (c), 0.8% (d), 1.0% (e) on microstructure of emulsion containing okra mucilage at acidic condition (pH 3.7± 0.2). The pictures were taken at 10×magnification.

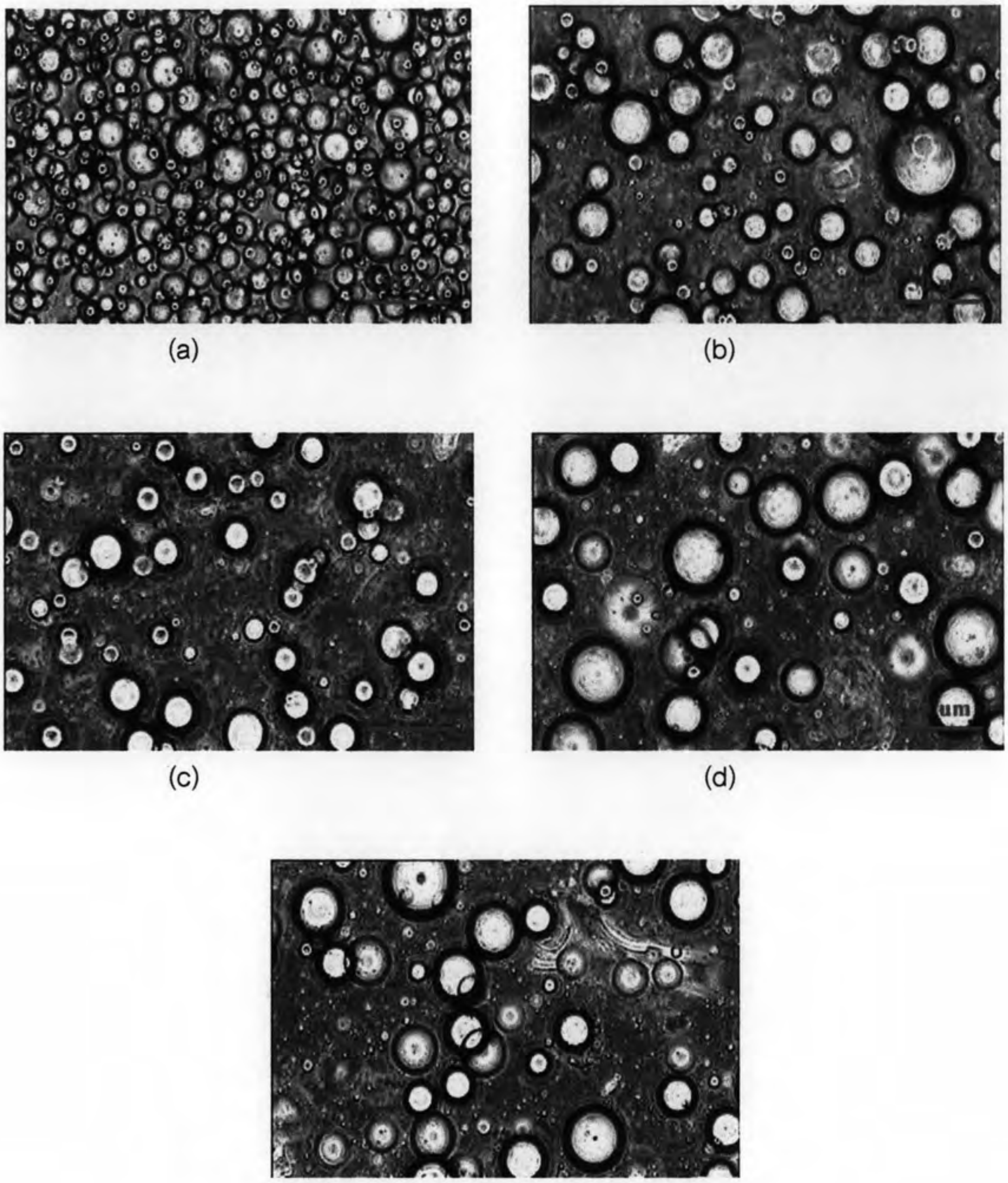


(a) (b)

(c) (d)

(e)

Figure 4.11 Effect of concentration; 0.2% (a), 0.4% (b), 0.6% (c), 0.8% (d), 1.0 %(e) on microstructure of emulsion containing xanthan gum at acidic condition (pH 3.7 ± 0.2). The pictures were taken at 10×magnification.



(a) (b)

(c) (d)

(e)

Figure 4.12 Effect of concentration; 0.2% (a), 0.4% (b), 0.6% (c), 0.8% (d), 1.0%(e) on microstructure of emulsion containing guar gum at acidic condition (pH3.7 ± 0.2). The pictures were taken at 10×magnification.

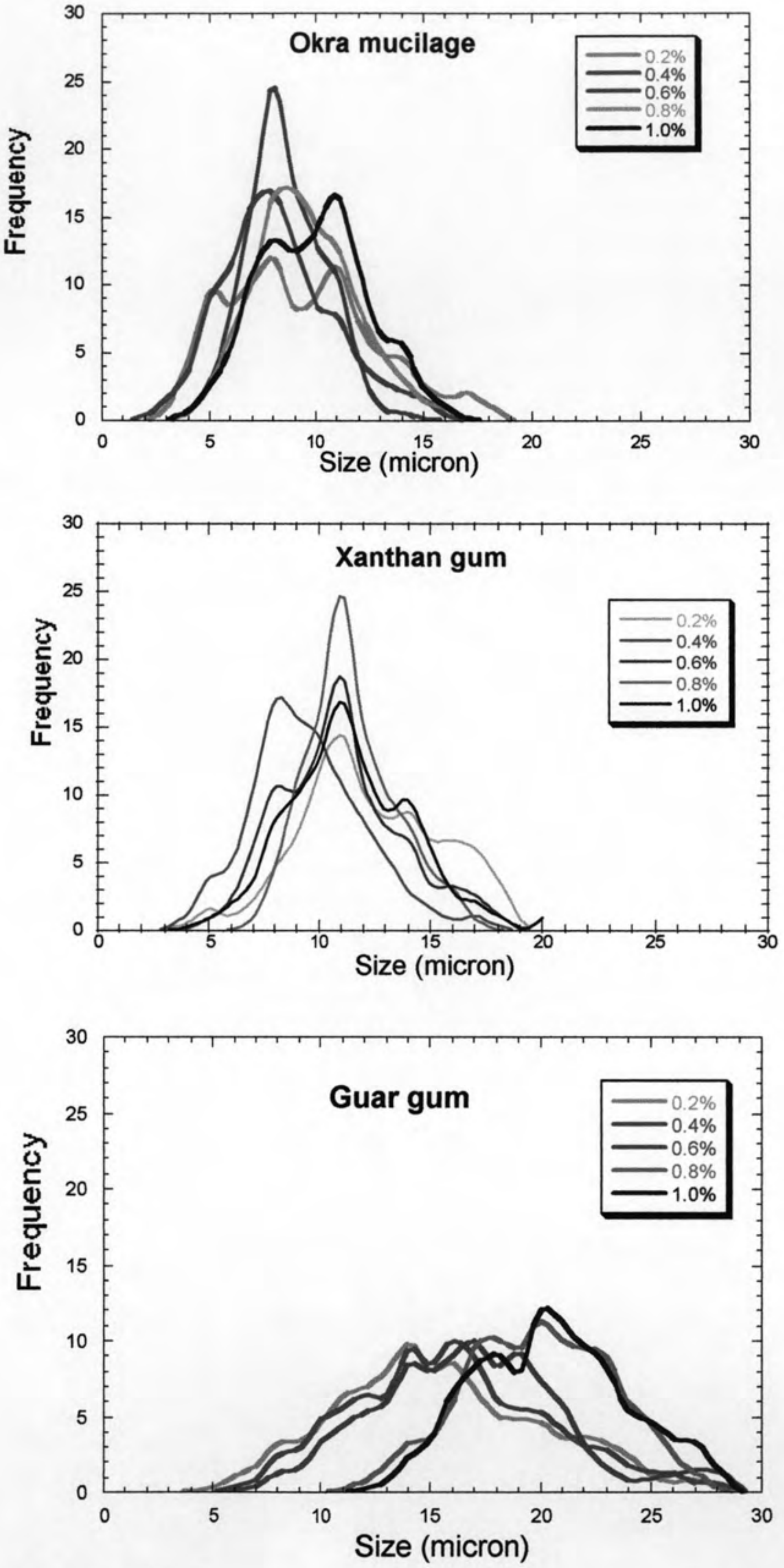


Figure 4.13 Effect of concentration (0.2-1.0% w/w) of gum solutions in emulsion at acidic condition (pH 3.7 ± 0.2) on size distribution of oil droplets.

Table 4.7 The average size of oil droplets of the emulsion stabilized by okra mucilage, xanthan gum, and guar gum at acidic condition (pH 3.7 ± 0.2).

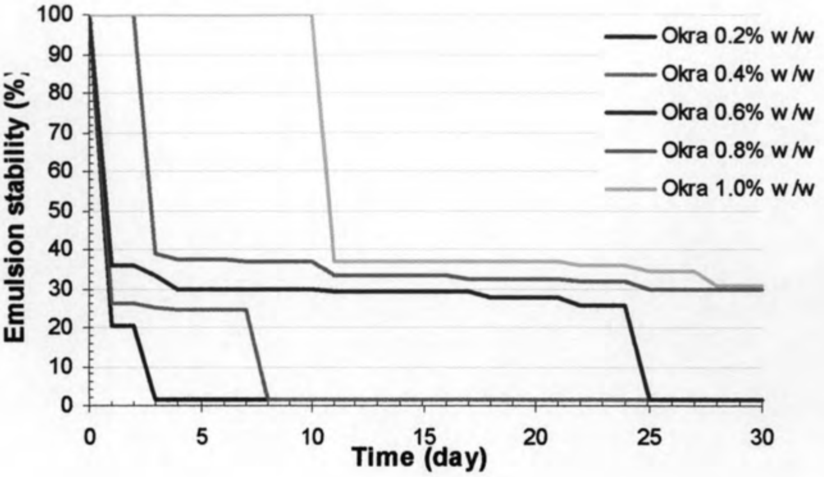
	Average size of oil droplets (μm.)		
Gum concentraton (% w/w)	Okra mucilage stabilized emulsion	Xanthan gum stabilized emulsion	Guar gum stabilized emulsion
0.2	9.28 ± 3.37	12.68 ± 3.91	15.45 ± 4.62
0.4	8.22 ± 2.55	9.50 ± 2.51	15.94 ± 4.39
0.6	8.59 ± 1.68	10.92 ± 2.68	16.79 ± 4.22
0.8	9.47 ± 2.25	11.41 ± 1.89	19.88 ± 3.19
1.0	9.90 ± 2.46	11.41 ± 2.76	20.50 ± 3.33

4.5.2 Emulsion stability (ES) analysis.

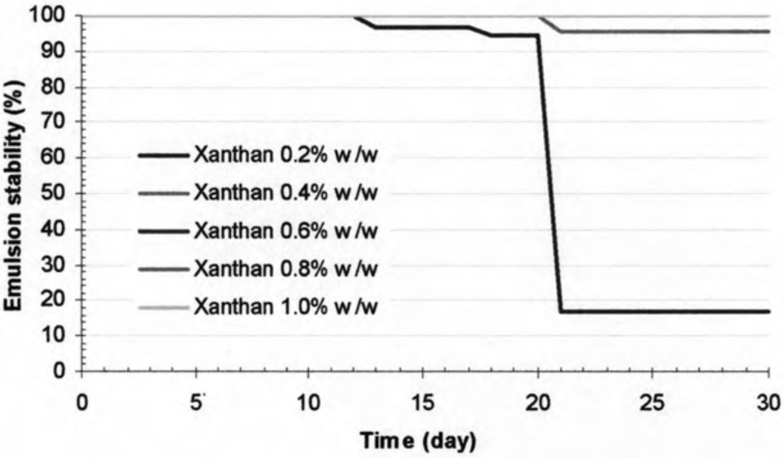
Figure 4.14 shows emulsion stability of emulsions stabilized by okra mucilage, xanthan gum, and guar gum solutions. From the results, okra mucilage stabilized emulsion (OMSE) and guar gum stabilized emulsion (GGSE) were not as stable as xanthan gum stabilized emulsion (XGSE). For OMSE, at the gum concentrations of 0.2-0.6% w/w, the phase separation occurred at day 1, whereas 0.8, and 1.0%, the phase separation occurred at day 3 (39% ES) and day 11 (38% ES), respectively. In case of XGSE, at 0.2 and 0.4% w/w, the phase separation occurred at day 13 (96% ES) and 21 (95% ES), respectively. No phase separation was observed at 0.6, 0.8, and 1.0% w/w of XGSE at 30 days of storage. For GGSE, at 0.2-0.6% w/w, the phase separation occurred at day 0. At 0.8 and 1.0% w/w, emulsion stability were reduced from 100% to 36% at day 4 and from 100% to 39% at day 13, respectively.

For OMSE, XGSE, and GGSE, an increase in the gum concentrations from 0.2% to 1.0% w/w increased emulsion stability. This was because of an increase in consistency index (table 4.8) as concentration increased. According to the power law model, if shear rate is 1 s^{-1}, K is the viscosity or stress at that point. Therefore, an increase in the viscosity of the continuous phase retarded the movement of the oil droplets, resulting in better emulsion stabilization (Huang *et al.*, 2001).

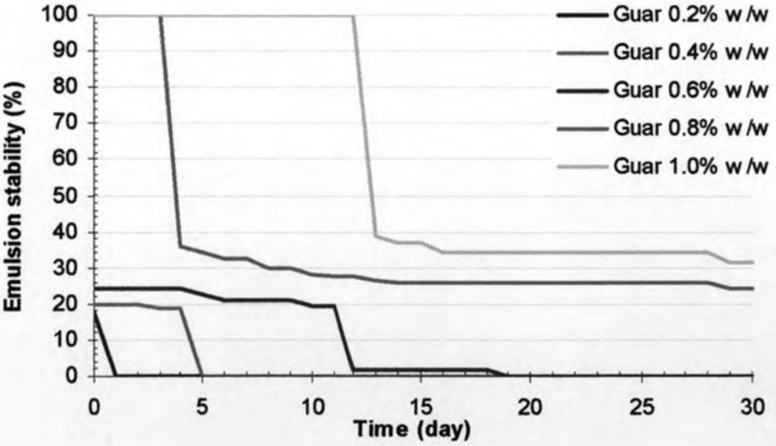
At a fixed concentration, the emulsion stabilized by xanthan gum had the highest emulsion stability (figure 4.14). The result was in agreement with viscoelastic properties and consistency index of gum solutions. As discussed in section 4.4.2, okra mucilage and xanthan gum solutions exhibited a gel-like behavior, whereas guar gum solutions exhibited entangle polymer solution that could flow. According to tables 4.2-4.5, consistency index of XG was the highest among the three gum solutions at every concentration. This suggested that the movement of oil droplets of emulsion stabilized by xanthan gum solutions was retarded by the structure and the viscosity of xanthan gum. It could be observed that, at 0.8-1.0% w/w, emulsions stabilized by GG had better emulsion stability that those stabilized by OM. This might be due to the higher K of 0.8-1.0% w/w GG than that of 0.8-1.0% w/w OM.



(a)



(b)



(c)

Figure 4.14 Effect of concentration (0.2-1.0%) of okra mucilage (a), xanthan (b), and guar gum (c) at acidic condition (pH 3.7 ± 0.2) on emulsion stability.

4.5.3 Flow behavior of emulsion containing gum solutions.

4.5.3.1 Viscoelastic behavior of emulsion containing gum solutions.

Figures 4.15 to 4.17 show viscoelastic behavior of emulsion stabilized by okra mucilage, xanthan gum, and guar gum solutions at various concentrations. Viscoelastic behavior of OSME and GGSE were similar (figure 4.15 and 4.17). At every concentration, they exhibited viscous flow at low frequency and then exhibited an elastic, gel like behavior at higher frequency. This indicated a unique characteristic of concentrated polymer solution, which was due to the entanglement of flexible polymer. An increase in gum concentrations of okra mucilage and guar gum in emulsions caused a shift of crossover from higher frequency to lower frequency. This was due to an addition of molecule of polymer, which led to the more entanglement of polymer-polymer interaction. In case of XGSE, at the gum concentration of 0.2% w/w, it exhibited a viscous flow behavior similar to viscoelastic behavior of OMSE and GGSE; G'' higher than G' over the tested frequency range. At higher xanthan gum concentrations (0.4-1.0% w/w), G' dominated over G'', indicating a gel-like behavior. As a result of an increase in gum concentration, an increased in entangled polymer molecule of xanthan gum form a relatively strong network, exhibiting an elastic, gel-like consistency behavior. From the results in viscoelastic characterization, it could be concluded that emulsion stabilized by okra mucilage and guar gum tended to be pourable, while emulsion stabilized by xanthan gum tended to be spoonable.

The decrease of the complex viscosity with frequency of OMSE, XGSE, and GGSE at various gum concentrations could be observed. The gum concentration used to stabilize emulsions did affect the complex viscosity. As stated previously, higher gum concentration led to more entangled polymer. Hence, an increase in concentration resulted in an increase in complex viscosity. The complex viscosity of the emulsion containing xanthan gum was the highest, whereas that of emulsion containing guar gum and emulsion containing okra mucilage was quite similar. This corresponded to the results on emulsion stability presented earlier, which emulsion containing xanthan gum had the best stability over the observation period.

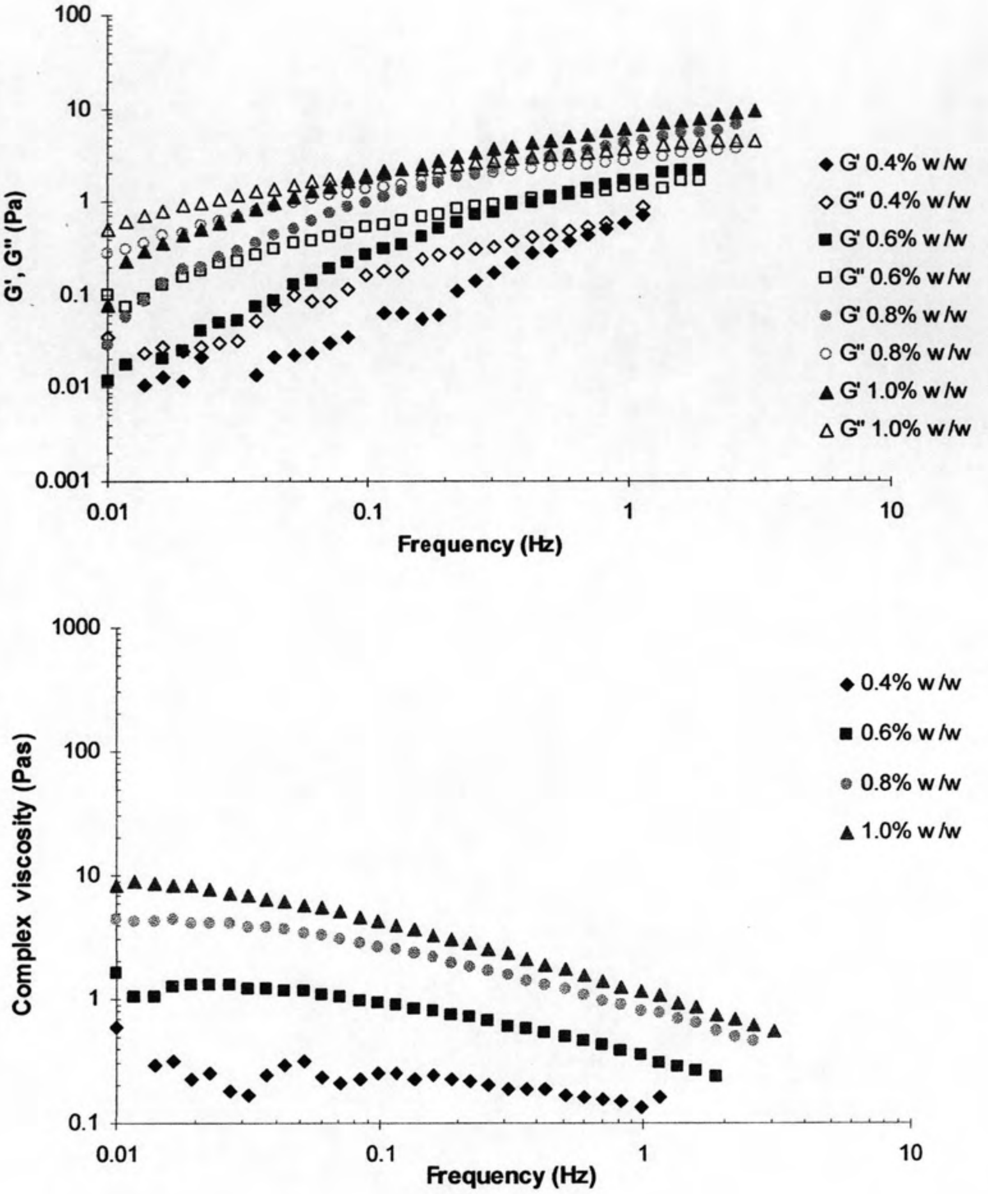


Figure 4.15 The effect of okra mucilage concentration (0.4-1.0% w/w) on viscoelastic behavior of okra mucilage stabilized emulsion.

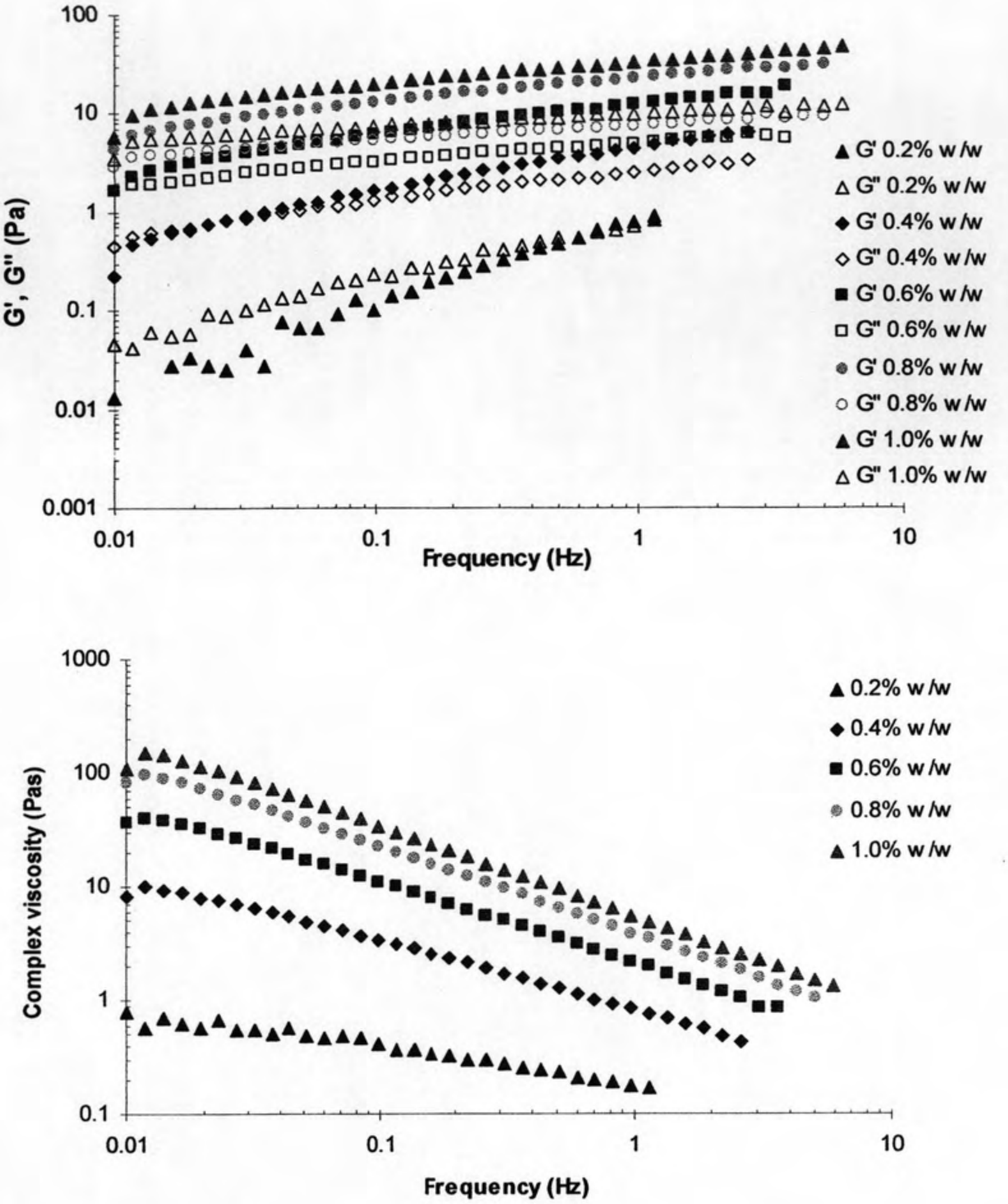


Figure 4.16 The effect of xanthan gum concentration (0.2-1.0% w/w) on viscoelastic behavior of xanthan gum stabilized emulsion.

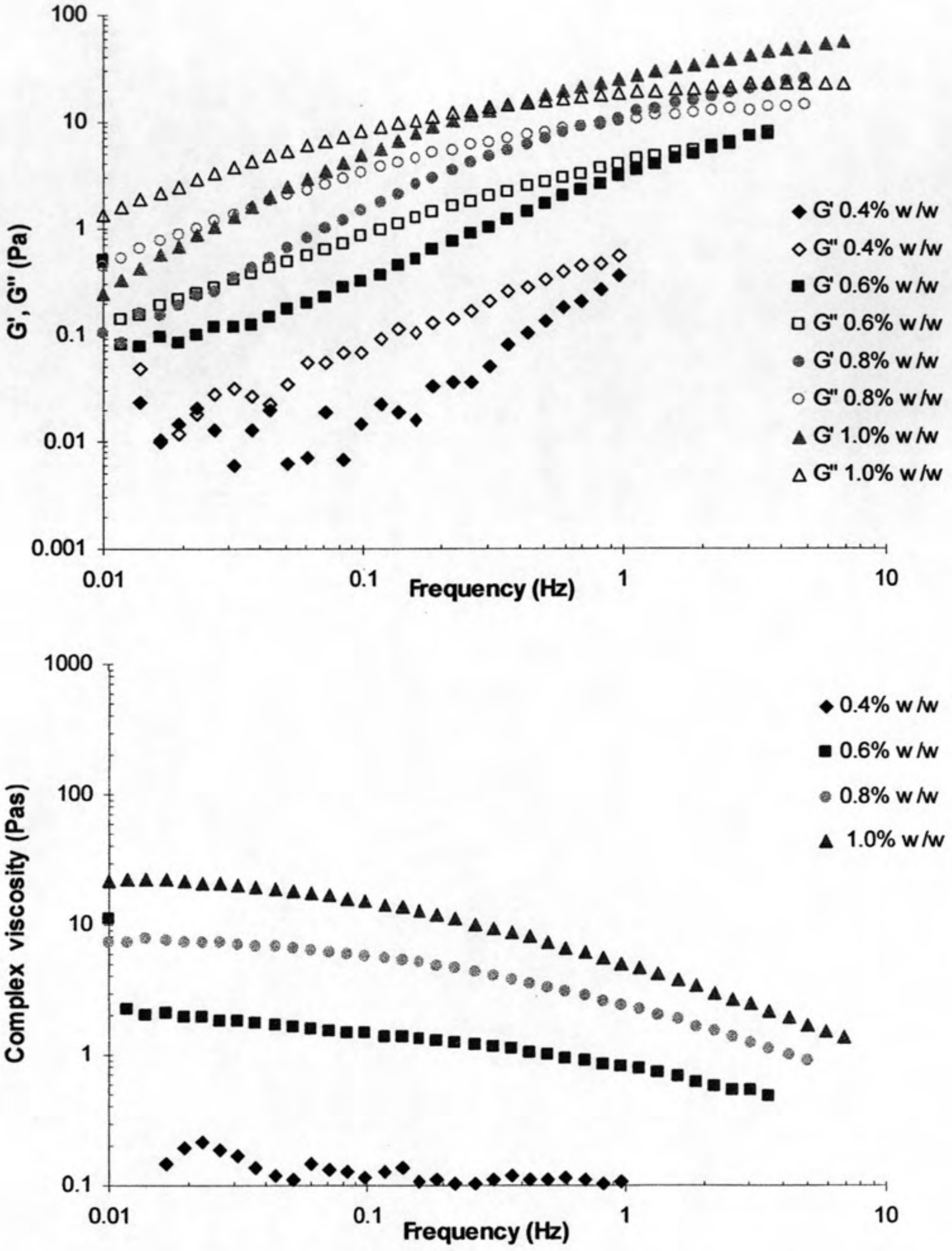


Figure 4.17 The effect of guar gum concentration (0.4-1.0% w/w) on viscoelastic behavior of guar gum stabilized emulsion.

4.5.3.2 Flow behavior of emulsion stabilized by gum solutions.

Table 4.8 shows K and n of emulsion stabilized by okra mucilage, xanthan gum, and guar gum solutions. All OMSE, XGSE, and GGSE exhibited shear shinning behavior, since n is less than 1. According to the results, the gum concentration affected flow behavior of the emulsions, in which K significantly increased and n significantly decreased as concentration increased ($p \leq 0.05$), suggesting the emulsions exhibited more shear thinning behavior. The result was in agreement with the results of dynamic test, which G', G'', and complex viscosity increased with increasing gum concentrations. It can be concluded that an increased in gum concentrations caused an increases in entanglement and polymer-polymer interaction, and retarded the mobility of polymer, resulting in an increase in viscosity and pseudoplasticity.

Among these three gum solutions, at same concentration, n of XGSE was the lowest and K of which was the highest. Therefore, XGSE was more viscous and more shear thinning than OMSE and GGSE

Table 4.8 Consistency index (K) and flow behavior index (n) of emulsions containing okra mucilage, xanthan, and guar gum at an acidic condition (pH 3.7 ± 0.2), 25°C.

Concen-tration (% w/w)	Okra mucilage		Xanthan gum		Guar gum	
	K	n	K	n	K	n
0.2	$0.08^{a} \pm 0.00$	$0.71^{a} \pm 0.02$	$0.36^{a} \pm 0.12$	$0.44^{a} \pm 0.00$	$0.02^{a} \pm 0.00$	$0.97^{a} \pm 0.00$
0.4	$0.27^{b} \pm 0.02$	$0.56^{b} \pm 0.00$	$1.92^{b} \pm 0.04$	$0.27^{b} \pm 0.00$	$0.15^{b} \pm 0.00$	$0.74^{b} \pm 0.01$
0.6	$0.86^{c} \pm 0.03$	$0.41^{c} \pm 0.01$	$4.71^{c} \pm 0.06$	$0.19^{c} \pm 0.00$	$0.56^{c} \pm 0.04$	$0.64^{c} \pm 0.00$
0.8	$1.87^{d} \pm 0.05$	$0.35^{d} \pm 0.01$	$8.78^{d} \pm 0.41$	$0.14^{d} \pm 0.00$	$4.12^{d} \pm 0.23$	$0.43^{d} \pm 0.01$
1.0	$3.77^{e} \pm 0.01$	$0.28^{e} \pm 0.00$	$12.70^{e} \pm 0.90$	$0.12^{e} \pm 0.00$	$10.14^{e} \pm 0.14$	$0.34^{e} \pm 0.01$

$^{a\text{-}d}$ Different letters in the same column denote significant difference ($p \leq 0.05$).

4.6 Flow behavior and physical properties of salad dressing containing okra mucilage.

From section 4.5.2, the emulsion containing 1.0% w/w okra mucilage could be stabilized for up to 13 days without an addition of other emulsifiers. It was also observed that an addition of okra mucilage at increasing concentration caused an increase in emulsion viscosity. Therefore, the addition of okra mucilage can be useful in improving the consistency of reduced fat emulsified food, which usually encounters problem with altered consistency. The study in this section involved an application of okra mucilage in salad dressing that was replaced 50% fat. The flow behavior and physical properties of salad dressing containing okra mucilage were investigated, in comparison with the control full fat salad dressing and the 50% reduced fat salad dressing.

4.6.1 Rheological properties of salad dressing.

Figure 18 shows viscoelastic behavior of the control full fat salad dressing (FF), the salad dressing with 50% reduced fat (RF), the 50% reduced fat with 1.0% w/w okra mucilage (RFOM 1.0), and the 50% reduced fat with 2.5% w/w okra mucilage (RFOM 2.5). It could be observed that every sample exhibited a gel like behavior with G' values well above those of G'' at all frequencies in the measurement range (0.01-10 Hz). G', G'', and complex viscosity of FF was higher than RFOM 2.5, RFOM 1.0, and RF, respectively. G' and G'' of the FF was the highest and they were less dependent on the frequency compared with others. This might be due to the network formed by protein-protein interaction that establishes the pseudo-gel network structure (Diftis, Biliaderis, and Kiosseoglou, 2005). As the fat was reduced to 50% its original content, the dependency of G' and G'' of RF on frequency was similar to the FF, but, the magnitudes of G', G'' and complex viscosity were lower. Thus, the amount of fat affected the viscoelastic behavior of salad dressing, in which the salad dressing with higher fat content exhibited more gel-like characteristics than at lower fat content. The higher moduli and viscosity were due to more packing of fat droplets in higher fat content emulsion than in lower fat content emulsion (Ma and Barbosa-Canovas, 1995). As fat concentration decreased, the mean distance between droplets was greater. Therefore, a lower complex viscosity was observed. When the 1.0% okra mucilage was added in the RF, producing RFOM 1.0, G' and G'' were higher comparing to the RF. This indicated that the addition of okra mucilage strengthened the gel-like structure of salad dressing. This might be due to the network formation of the polymers in okra mucilage that filled in the gaps between fat droplets and supported them. Although the G' and G'' values of RFOM 2.5 were higher than the RF and RFOM 1.0, their dependency on frequency was the highest. This suggested that RFOM 2.5 exhibited more shear dependent flow, which is the nature of the okra mucilage. From this result, it can be concluded that RFOM 2.5 was more slimy than the others.

According to table 4.9, K of FF and RFOM 2.5, which were higher than that of RF and RFOM 1.0, were not significantly difference. RFOM 2.5 had the lowest n, followed by FF, RF, and RFOM1.0, respectively, indicating that RFOM 2.5 exhibited more pseudoplasticity than the others.

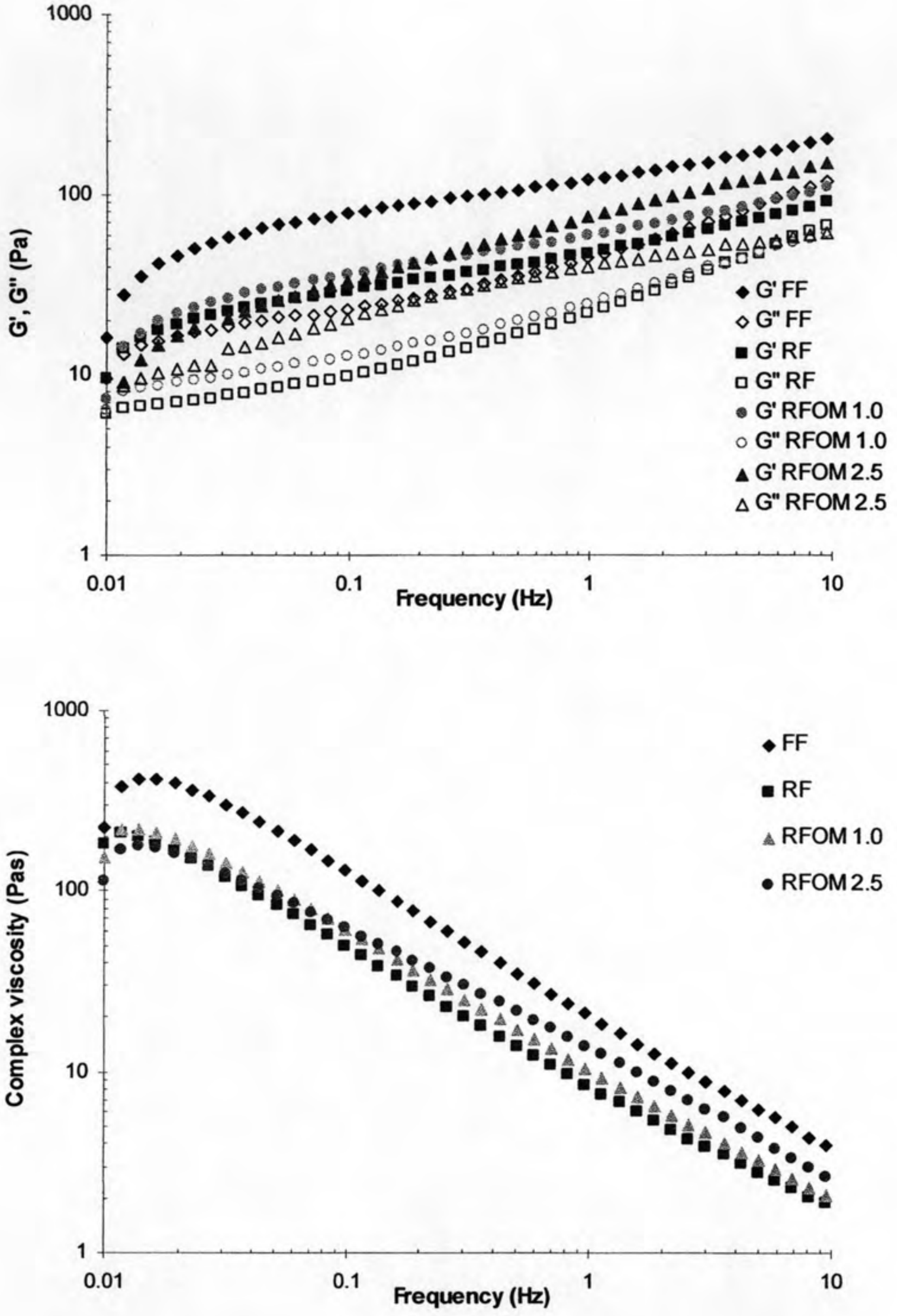


Figure 4.18 Viscoelatic behavior of the control full fat salad dressing (FF), the salad dressing with 50% reduced fat (RF), the 50% reduced fat with 1.0% w/w okra mucilage (RFOM 1.0), and the 50% reduced fat with 2.5% w/w okra mucilage (RFOM 2.5) at the frequency range of 0.01-30 Hz.

Table 4.9 Consistency index (K) and flow behavior index (n) of the control full fat salad dressing (FF), the salad dressing with 50% reduced fat (RF), the 50% reduced fat with 1.0% w/w okra mucilage (RFOM 1.0), and the 50% reduced fat with 2.5% w/w okra mucilage (RFOM 2.5).

Salad dressing	K	n
The control full fat salad dressing (FF)	$10.44^{a} \pm 0.38$	$0.57^{a} \pm 0.01$
50% reduced fat (RF)	$2.46^{b} \pm 0.76$	$0.69^{b} \pm 0.03$
50% reduced fat + 1.0% w/w okra mucilage (RFOM 1.0)	$2.25^{b} \pm 0.07$	$0.78^{c} \pm 0.00$
50% reduced fat + 2.5% w/w okra mucilage (RFOM 2.5)	$9.87^{a} \pm 0.23$	$0.41^{d} \pm 0.00$

$^{a\text{-}d}$ Different letters in the same column denote significant difference ($p \leq 0.05$).

4.6.2 Color determination.

As shown in table 4.20, the color of samples was significantly different. The lightness (L-value) of salad dressing had the major impact on the perceived of the product. The L-value of RFOM 2.5 was lower than other samples, whereas the L-value of FF and RFOM 1.0 were not significantly different ($P \leq 0.05$). The color of okra mucilage ($L^* = 43.01$, $a^* = 1.92$, and $b^* = 23.88$) slightly influenced the color of reduced fat salad dressing. The a-value (redness) and b-value (yellowness) increased with increasing the level of okra mucilage substitution, or in the other words, the reduced fat salad dressing containing okra mucilage appeared to be more red and yellow. However, as observed by bare eyes under daylight, figure 4.19, there was no difference in color between these samples.

Table 4.10 Color of the control full fat salad dressing (FF), the salad dressing with 50% reduced fat (RF), the 50% reduced fat with 1.0% w/w okra mucilage (RFOM 1.0), and the 50% reduced fat with 2.5% w/w okra mucilage (RFOM 2.5).

Salad dressing	L*	a*	b*
The control full fat salad dressing (FF)	$74.58^{a} \pm 0.65$	$-1.80^{a} \pm 0.07$	$19.25^{a} \pm 0.18$
50% reduced fat (RF)	$73.19^{b} \pm 0.33$	$-1.78^{a} \pm 0.13$	$20.07^{b} \pm 0.26$
50% reduced fat + 1.0% w/w okra mucilage (RFOM 1.0)	$74.34^{a} \pm 0.16$	$-1.23^{b} \pm 0.06$	$21.41^{c} \pm 0.63$
50% reduced fat + 2.5% w/w okra mucilage (RFOM 2.5)	$71.49^{c} \pm 0.33$	$0.09^{c} \pm 0.34$	$22.06^{c} \pm 0.32$

[a-d] Different letters in the same column denote significant difference ($p \leq 0.05$).

Figure 4.19 Color of the control full fat salad dressing (FF), the salad dressing with 50% reduced fat (RF), the 50% reduced fat with 1.0% w/w okra mucilage (RFOM 1.0), and the 50% reduced fat with 2.5% w/w okra mucilage (RFOM 2.5) at day 0.

4.6.3 Emulstion stability of salad dressing.

The results showed that, for every sample, no separation was observed over the tested period of 30 days. This was due to egg, which acted as an emulsifier that gave the stability. In the emulsion, oil droplets are dispersed in the continuous phase, and they tend to flocculate due to the attractive forces. Egg, which contains protein, an amphiphilic molecule, is a good emulsifier, which reduces the interfacial tension between two phases and also forms a protective steric barrier around oil droplets. In conclusion, the control full fat salad dressing (FF), the salad dressing with 50% reduced fat (RF), the 50% reduced fat with 1.0% w/w okra mucilage (RFOM 1.0), and the 50% reduced fat with 2.5% w/w okra mucilage (RFOM 2.5) were stable over the tested period of 30 days.