CHAPTER III

MATERIALS AND METHODS

3.1 Materials and instruments.

3.1.1 Materials.

1. Okra (Local market, Thailand)
2. Xanthan gum (Versagum 80, Cargill, USA)
3. Guar gum (Shree Ram Gum & Chemicals, India)

3.1.2 Chemicals.

1.	Ethyl alcohol	S.R. Lab. Co. Ltd., Thailand	A.R. grade
2.	Petroleum ether	Labscan Asia Co., Ltd., Thailand	A.R. grade
3.	Sulfuric acid	J.T. Baker, USA	A.R. grade
4.	Sodium hydroxide	Labscan Asia Co., Ltd., Thailand	A.R. grade
5.	Boric acid	Fisher scientific, UK	A.R. grade
6.	Selenium reagent mixture	Merck KGaA, Germany	A.R. grade
7.	Methyl red	Merck KGaA, Germany	A.R. grade
8.	Methylene blue	Riedel-de Haën, Germany	A.R. grade
9.	Acetic acid	J.T. Baker, USA	A.R. grade
10.	Sodium acetate trihydrate	Fisher scientific, UK	A.R. grade
11.	Hydrochloric acid	J.T. Baker, USA	A.R. grade

3.1.3 Instruments.

1. Weighing machine (Sartorius A200S, Mettler-Toledo, Switzerland)
2. pH meter (model Cyberscan pH 1100 Bench, RUTECH instruments pte Ltd., Singapore)
3. Centrifuge (IEC multi RF, 220/240 Thermo IEC, USA)
4. Hot air oven (Model 600, Memmert, Gmiott Co. KG, Germany)

5. Magnetic stirrer (Framo ®, Germany)
6. Muffle furnace (Furnance Carbolote, S336RB Parsons Lane, Hope England)
7. Kjedahl distillation unit (Kjedahl and Vapodest, K424 Büchi, Switzerland)
8. Soxhlet apparatus (Soxhlet apparatus, EV16 Gerhardt Bonn, Germany)
9. Rotary evaporator (N-N, Eyela, Japan)
10. Goniometer (FTA200, First ten angstroms, USA).
11. Fourier transform infrared spectrometer (Spectrum one, PerkinElmer, USA)
12. Bohlin rheometer (model C-VOR, Malvern Instruments Ltd., England)
13. Hand-held homogenizer (model x10/25,Ystral, Germany)
14. Colorimeter (ColorFlex , Hunter lab, USA)
15. Microscope (BX 51 TF, Olympus, Japan)
16. Blender (HR 1791, Phillips, Indonesia)

3.2 Methods.

3.2.1 Extraction of okra mucilage.

The extraction of okra was carried out following the method of Wu *et al.* (1995). The deseeded okra fruits were cut into thin slices and blended with 1.2 volumes of double distilled water using a blender. Insoluble material was removed by centrifugation at 15,000×g at 4°C for 1 hr. Next, this soluble fraction is precipitated with 20%, 40%, 60%, 80%, and 95% ethanol. The ratio of crude extract to ethanol is 1:4. After centrifugation, the precipitated raw polysaccharide was centrifuged again at 15,000×g at 4°C for 30 min in order to separate ethanol. The crude polymer is collected and then dialyzed using regenerated cellulose tubular membrane (molecular weight cutoff 12,000-14,000, Membrane filtration products, Inc., USA) against 20 volumes of distilled water for 2 days. The water is changed 3-4 times a day.

3.2.2 Determination of chemical composition and yield of mucilage extracted from okra.

Chemical composition and yield of okra mucilage were determined by the method of AOAC (1995) as described in appendix A. The precipitation condition that gave the highest yield would be selected for further study.

3.2.3 Preparation of gum solutions at various concentrations and pH.

Okra mucilage, xanthan and guar gum were dispersed in distilled water to get the concentrations of 0.2%, 0.4%, 0.6%, 0.8%, and 1.0% w/w. For acidic condition (pH 3.7 ± 0.2), the three gums were dissolved in 0.05 M acetic-acetate buffer at the same concentrations. All gum solutions were kept for 12 hours for fully hydration before measurements.

3.2.4 Determination of properties of okra mucilage and gum solutions.

3.2.4.1 Fourier transform infrared spectrum analysis.

Fourier transform infrared spectrometer (FTIR) analysis of okra mucilage with 99% moisture was determined. An average of 32 scans was recorded at 4 cm^{-1} resolution over the 600-4,000 cm^{-1} range. FTIR spectra of a sample were obtained using a BaF_2 (barium fluoride) liquid cell. The measurements were done in duplicate.

3.2.4.2 Surface tension.

Surface tension of all gum solutions (at every concentration) were determined by using an automated goniometer equipped with the FTA 32 V 2.0 software. The procedure is shown in appendix B1. The measurements were done in triplicate.

3.2.4.3 Rheological characterization.

1. Determination of Linear Viscoelastic Range (LVR).

The linear viscoelastic range of okra mucilage, xanthan, and guar gum at 0.2%, 0.4%, and 1.0% w/w in neutral condition were determined with amplitude sweep test over 0.01 to 1000% strain range by using the cone and plate geometry (4^{o} angle, 40 mm) at a fixed frequency of 1 Hz and at $25^{o}C$. The measurements were done in triplicate. The procedure is shown in appendix B2.

2. Determination of flow behavior.

All gum solutions at various concentrations and pH were studied. The cone and plate geometry (4°, 40 mm) was used for testing. A frequency sweep test was conducted by applying a constant strain of 1% which was within the LVR region. The test frequency was varied in the range of 0.01-30 Hz and the temperature was fixed at 25°C. Steady flow test for all gum solutions were performed at 15, 25, 35, and 45°C. The shear rate was ranged from 0.1 to 50 s^{-1} in 3 minutes and from 50 to 0.1 s^{-1} in 3 minutes. The measurements were done in triplicate

3.2.5 Determination of emulsion stability.

3.2.5.1 Emulsion preparation.

The emulsions were prepared by mixing 1 part of soybean oil with 9 parts of gum solution (0.2-1.0% w/w, pH 3.7 ± 0.2). The mixtures were then homogenized using a hand homogenizer at 24,000 rpm for 4 min. All rheological tests at 25°C following 3.2.4.3 were immediately conducted after preparing the emulsion.

3.2.5.2 Oil droplets morphology and size analysis.

Microstructure of the emulsions was analyzed by using a microscope at 10x magnifications. The picture was captured using PCTV USB2 Vision (Pinnacle systems GmbH, Braunschweig, Germany). Size distribution of at least 50 oil droplets was determined for each sample using an image J analyzer program (National Institute of Health, USA). The procedure is shown in appendix B3. The measurements were done in triplicate.

3.2.5.3 Emulsion stability analysis.

Emulsion stability (ES) was determined according to the procedure described by Huang *et al.* (2001). Twelve mL of each of emulsion was placed into capped vials and stored at room temperature (25°C). The total height, along with heights of the emulsion layer and the bottom aqueous layer were recorded to the nearest 1 mm. for 30 days. ES was calculated as the percentage of the emulsion layer remaining following equation 3.1.

$$ES\ (\%) = H_2 / H_1 \times 100 \qquad (3.1)$$

where H_1 is initial emulsion height

H_2 is remaining emulsion height

3.2.6 Application of okra mucilage as a stabilizer in salad dressing.

3.2.6.1 Preparation of salad dressing containing okra mucilage.

Salad dressings were prepared by combining the ingredients shown in table 3.1. Sugar and salt were dissolved in vinegar, and then all ingredients except soybean oil were mixed together using a blender at low speed. The oil was later slowly added to the mixture and mixed until it was homogenous. The four dressings, which were the control sample with 100% oil, the 50% reduced oil sample, the 50% reduced oil with 1.0% w/w okra mucilage, and, the 50% reduced oil with 2.5% w/w okra mucilage based on the total weight of water were then subject to tests following the procedures in 3.2.4.3 and 3.2.5.3.

Table 3.1 Ingredients for the preparation of salad dressings.

	Amount of ingredients (g)			
Ingredient	control with 100% oil	50% reduced oil sample	50% reduced oil with 1.0% w/w okra mucilage	50% reduced oil with 2.5% w/w okra mucilage
Whole egg	25	25	25	25
Sugar	59.5	59.5	59.5	59.5
Salt	4.5	4.5	4.5	4.5
Vinegar	33	33	33	33
Sweet condensed milk	9.5	9.5	9.5	9.5
Mustard	3.75	3.75	3.75	3.75
Soybean oil	82	41	41	41
Okra mucilage (96% Moisture content)	0	0	18.25	86.5

3.2.6.2 Color determination.

Color of salad dressings were measured by a colorimeter in the CIE LAB color system (L*, a*, and b*), using D65 as the light source. L* is the lightness coordinate, a* is the red/green coordinate, with +a* indicating red, and -a* indicating green, and b* - the yellow/blue coordinate, with +b* indicating yellow, and -b* indicating blue. Six measurements at six different positions were done for each sample.

3.2.7 Statistical analysis.

The experiment was designed as a completely randomized design (CRD). The statistical analysis of the results was conducted by the analysis of variance (ANOVA) and Duncan's New Multiple Range Test to evaluate the difference between means at the 95% confidence interval.