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APPENDICES

Appendix A

Determination of chemical properties

A1: Determination of yield

$$\text{Yield} = \frac{\text{dried crude polymer (g)}}{\text{dried okra (g)}} \times 100 \qquad \text{(A1)}$$

A2: Moisture determination (AOAC,1995)

Instruments

1. Hot air oven (Model 600, Memmert, Gmiott Co. KG, Germany)
2. Aluminum dish
3. Desiccator

Methods

1. Weigh the aluminium dish, which has been previously dried in a hot air oven at 105^{o}C until the weight of the dish is constant and then cool in a desiccator for 1 hour and weigh accurately the dish again.
2. Weigh accurately 2-3 g of sample into a moisture dish.
3. Place the dish in a hot air oven and dry at 105^{o}C for 5 hours.
4. Remove the dish and cool to room temperature in a desiccator for 1 hour and reweigh the dish.

Calculation

$$\text{Moisture (\%)} = \frac{(W_1 - W_2) \times 100}{W_1} \qquad \text{(A2)}$$

Where, W_1 is weight of the sample before drying (g)

W_2 is weight of the sample after drying (g)

A3: Fat determination (AOAC,1995)

Instrument

1. Soxhlet extraction (Soxhlet apparatus, EV16 Gerhardt Bonn, Germany)
2. Hot air oven (Model 600, Memmert Gmiott Co. KG, Germany)
3. Rotary evaporator (N-N, Eyela, Japan)

Chemicals

1. Petroleum ether

Method

1. Weigh a round bottom flask, which has been previously dried in a hot air oven at 110 ^{o}C for 1 hour and cooled in a desiccator for 1 hour.

2. Weigh accurately 2-3 g of dried sample, wrap with whatman paper no. 1, and place into a Soxhlet thimble. Then, place the thimble into an extraction tube.

3. Add 250 mL of petroleum ether into the known weight round bottom flask.

4. Extract the thimble in the Soxhlet apparatus for 4 hr.

5. Distill off the solvent from the round bottom flask using the rotary evaporator.

6. Dry the round bottom flask in a hot air oven at 60 ^{o}C for 30 min, cool to room temperature in a desiccator for 1 hour and reweigh.

Calculation

$$\text{Fat (\%)} = \frac{\text{weight of crude fat (g)}}{\text{dry weight of sample (g)}} \times 100 \qquad \text{(A3)}$$

A4: Protein determination (AOAC,1995)

Instrument

1. Distillation unit (Kjedahl and Vapodest, K424 Büchi, Switzerland)
2. Kjeldahl flask
3. Conical flask
4. Burette

Chemicals

1. Sulfuric acid (concentrated)
2. 0.1 N sulfuric acid
3. 50% w/v sodium hydroxide
4. 4% w/v boric acid
5. Selenium reagent mixture
6. Methyl red-methylene blue indicator
7. 0.1 N hydrochloric acid

Method

1. Weigh out accurately 0.7-2.2 g of sample on a low ash paper and transfer to a digestion flask.

2. Add 5 g of selenium mixture.

3. Add 30 mL of concentrated H_2SO_4

4. Place the rack and tubes in the digestion apparatus. Connect the exhaust manifold onto the tubes and turn on the water pump. Set the thermostat to 400^{o}C and turn on for 45 min and digest until the solution become clear.

5. After the stated time, lift the rack out of the digestion block and place on the stand to cool. Leave the water pump and manifold connected. Then, remove the manifold when the tubes are cool.

6. Place the tube to the distillation apparatus and add 80 mL of distilled water and 120 mL of 50% w/v sodium hydroxide.

7. Place a conical flask containing 50 mL of 4% w/v boric acid and 4 drops of indicator (0.1% methylene blue + 0.2% methyl red).

8. Run the distillation process.

9. Remove the flask from the apparatus and titrate the ammonia in the flask to the original purplish color with 0.1N HCl.

Calculation

$$\text{Protein (\%)} = \frac{\text{mL of titer} \times \text{N of HCl} \times 14 \times 6.25}{\text{Weight of sample (g)} \times 10} \qquad \text{(A4)}$$

A5: Ash determination (AOAC, 1995)

Instrument

1. Muffle furnace (Furnace Carbolote, S336RB Parsons Lane, Hope England)

2. Hot plate

3. Crucible

4. Fume hood

5. Desiccator

Method

1. Weigh a crucible, which has been previously dried in a muffle furnace at 500 ^{o}C and cooled in a desiccator for 2 hours.

2. Weigh accurately 3-5 g of sample into a crucible.

3. Char the sample on a hot plate in the fume hood.

4. Transfer the crucible to a muffle furnace heated to 550 ^{o}C.

5. Leave the crucible in the muffle furnace for 4 hr. until the ash is white or grayish-white.

6. After incineration, cool in a desiccator for 2 hour and reweigh.

Calculation

$$\text{Ash (\%)} = \frac{(A-B) \times 100}{C} \qquad \text{(A5)}$$

Where, A = weight of crucible + weight of sample after incineration (g)

B = weight of crucible (g)

C = weight of sample before incineration (g)

A6: Carbohydrate determination (Aluko, McIntosh, and Reaney, 2001)

$$\text{Carbohydrate (\%)} = 100 - (\text{protein} + \text{fat} + \text{ash}) \qquad \text{(A6)}$$

Appendix B

Determination of functional properties of okra mucilage and gum solutions

B1: Determination of surface tension by a goniometer

1. Place the sample in a 3 mL. syringe (try to remove all air bubbles in the syringe).

2. Attach the needle, which the inner and outer diameters are 0.483 and 0.711 mm., respectively, to the syringe.

3. Connect the syringe with the stand by using a syringe adapter and a gauge as holders.

4. Connect the wire to the camera at video out.

5. Open the FTA program and set the pump rate to 12 μL/ second.

6. Run the machine and press IF tension to show the result.

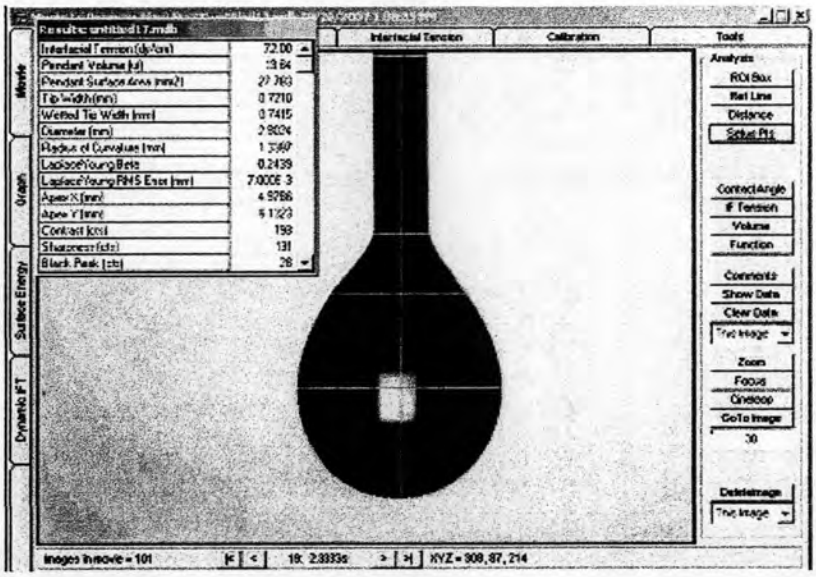


Figure B1 Determination of surface tension of de-ionized water using goniometer.

B2: Determination of flow behavior using a rheometer

Instrument

1. Bohlin rheometer (model C-VOR, Malvern instruments Ltd., UK.) with Peltier controller.

2. The cone and plate geometry (CP 4/40, cone and plate, 4^{o} angle, and 40 mm. diameter).

Method

1. Open the pump, cooler, Peltier controller, and Bohlin rheometer. Place the solvent trap on top of the probe. Connect the probe to the rheometer. Set zero and a gap of 150 μm.

2. Turn on the program, select the mode for testing.

For Amplitude sweep test, the following parameters are set.

- Pre-shear off
- Auto- tension off
- Sweep type AMP sweep

Range	Log
Frequency	1 Hz
Minimum strain	0.01%
Maximum strain	1000%

- Isothermal 25°C

For frequency sweep test, the following parameters are set.

- Oscillation test parameters: frequency sweep

Minimum frequency	0.1 Hz
Maximum frequency	30 Hz
Range	Log
Strain	1%

- Isothermal 25°C

For steady shear test, the following parameters are set.

- Pre conditioning: controlled rate

Shear rate	0.1 s^{-1}
Apply time	20 s

Equilibrium time 5 s

- Shear profile

Up and down

Start shear $0.1\ s^{-1}$

End shear $50\ s^{-1}$

Range linear

4. Place the sample at the center of a fixed lower plate and lower the geometry in order to contact with the sample.

5. Remove the excess sample and unlock the rotating upper plate.

6. Press start button for running the machine.

B3: Oil droplets morphology and size analysis of emulsion by using an image J analyzer program

1. Capture a stage micrometer and the sample with a microscope at the same magnification using a PCTV USB2 vision program.

2. Draw a line over a known distance on the micrometer. Select analyze and, then, set scale.

3. Convert the image to grayscale: Image -> Type -> 8 bit.

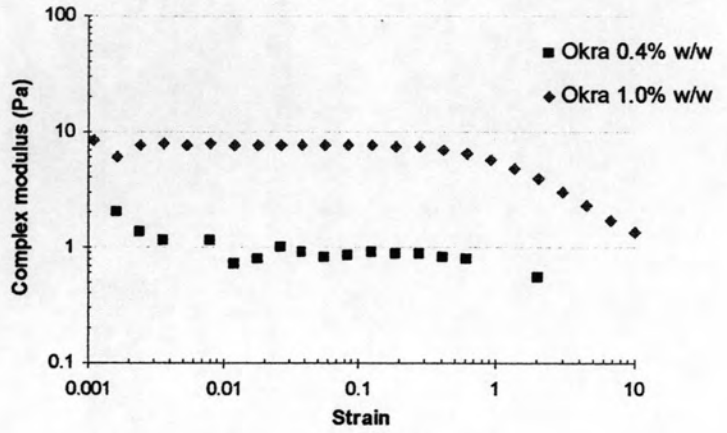
4. Threshold the image using the automated routine: Process -> Binary -> Threshold.

5. Analyze Particles: Analyze -> Analyze particles. Then, an analysis of particle size distribution is also shown in a new window.

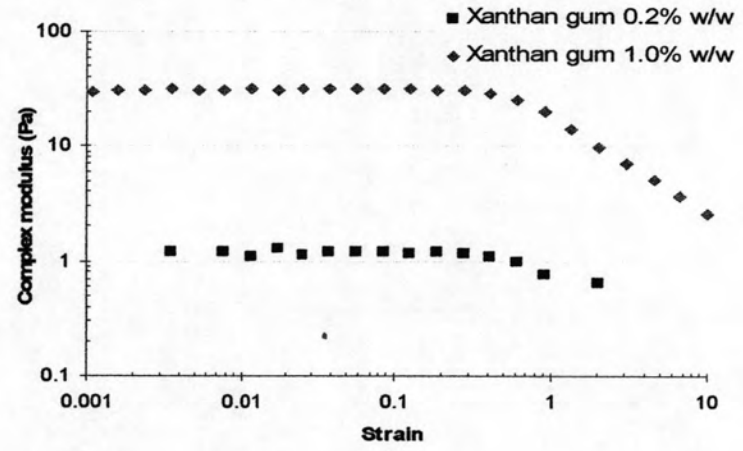
Appendix C

Linear viscoelastic range

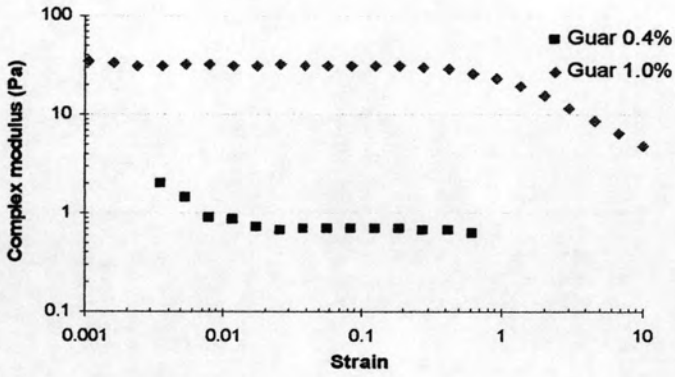
C1: Linear viscoelastic range of emulsion containing gum solutions.



(a)



(b)



(c)

Figure C1 Linear viscoelastic range of emulsion containing okra mucilage (a), xanthan gum (b), and guar gum (c).

C2: Linear viscoelastic range of salad dressing.

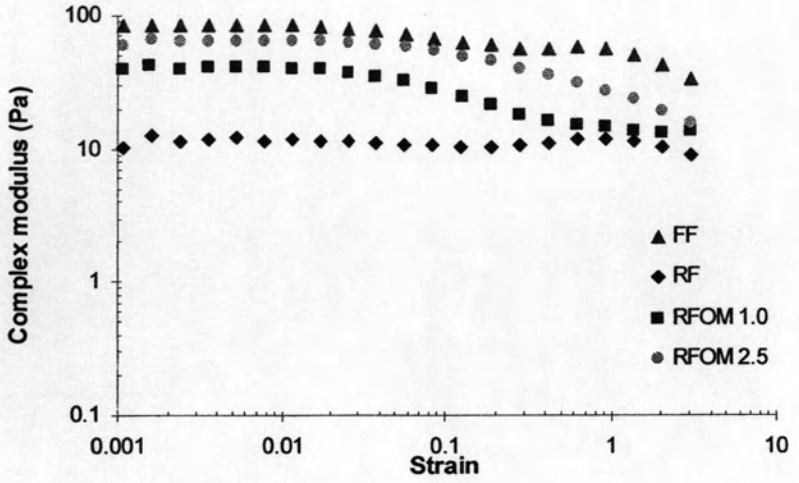


Figure C2 Linear viscoelastic range of the control full fat salad dressing (FF), the salad dressing with 50% reduced fat (RF), the 50% reduced fat with 1.0% w/w okra mucilage (RFOM 1.0), and the 50% reduced fat with 2.5% w/w okra mucilage (RFOM 2.5) at the frequency range of 0.01-30 Hz.

VITA

Ms. Thirada Patipatpaopong was born on 23 November, 1983, in Bangkok, Thailand. She studied at Mahidol University International College (MUIC), majoring in Food Science and Technology, and received Bachelor of Science with second class honors in 2005. In 2005, she enrolled in the master degree program in Food Science and Technology (English program) at Department of Food Technology, Faculty of Science, Chulalongkorn University. She completed her master degree program in the 2007 academic year. Her present address is 123/24 Prachachuen road, Bangsue, Bangkok, 10800.