CHAPTER II
LITERATURE REVIEW

2.1 Okra

Okra, *Abelmoschus esculentus*, belonging to the family of Malvaceae, is also known as gumbo. It is a tall-growing vegetable and available all year round. The height varies from 40 centimeters to 2 meters, depending on the cultivars. Okra pod is greenish, slightly curved and lanterned-shape. Each pod contains 80-200 seeds inside. The immature seeds are white, but the mature seeds are grey and the color of the pod becomes brown. Okra flowers about 40 days after seed germination. Once flowering starts, pods should be harvested. These juvenile pods, about 4-9 centimeters in length, are desirable for consuming. But, if not harvested, within 10-12 days of flowering, the pods of most cultivars become tough, woody and inedible (Peet, 2001). Okra is grown throughout the tropical and warm temperate regions since the optimum soil temperature for growth is approximately 20 to 30°C. It can be grown in every soil condition except cool or acidic soils. The pH of soil should be between 6.0 to 6.8 (Department of Agriculture, 1998).

Okra is a rich source of nutrients, especially vitamin A, C, and calcium (Department of Agriculture, 1998). These nutrients maintain body function well and lower the risk of many diseases (Wenck, Baren, and Dewan, 1980). For example; vitamin A, a fat soluble and antioxidative vitamin, is important in vision. Vitamin C, an antioxidant, can reduce the risk of fatal heart disease by preventing the clog of blood vessel. Moreover, calcium, known as a bone builder mineral, may also slow down bone loss that can lead to the development of osteoporosis. Potassium, an essential macronutrient, assists in muscle contraction, maintains fluid and electrolyte balance in cells and lowers blood pressure. Beside these nutrients, okra also composes of gum, which is beneficial to health. Gum or mucilage is classified as soluble fiber. It slows down the conversion of carbohydrates to glucose, resulting in the stability of blood glucose level. Moreover, it can reduce blood cholesterol levels which, in turn, reduce cholesterol deposits on arterial walls. Therefore, it helps decrease the risk of heart

diseases (Papazian, 1998). Lastly, since one hundred grams of okra contains only 36 kilocalories and 0.3 g of fat, it is a healthy additive, which maintains texture of a product when preparing a low fat recipe (Department of Agriculture, 1998).

From traditional medicine standpoint, okra fruit is a diuretic and can be used for the treatment of dental diseases (Ndjouenkeu *et al.*, 1996). Its mucilage can be used as a dietary meal for the treatment of gastric irritations. Lengsfeld *et al.* (2004) found that okra has the anti-adhesive quality, which inhibits the adhesion of bacteria to human gastric mucosa. This property is due to a combination of glycoprotein and highly acidic sugar components.

2.2 Chemical properties and characterization of okra mucilage

The unmodified plant gums and mucilages mainly compose of carbohydrate and may contain protein (Stephen and Churms, 1995). They are hydrophilic compounds of high, variable molecular weight which are made up of monosaccharide units linked as glycosides. The extraction methods of mucilage had an effect on chemical compositions (Ibanez and Ferrero, 2003). Ibanez and Ferrero (2003) studied the extraction and characterization of *Prosopis flexuosa* DC seed by extraction the seed in alkaline and neutral media. The results indicated that the mucilage extracted by alkaline medium had lower protein content than the mucilage extracted by neutral medium. However, in the same extraction method, which used water as an extraction medium, extraction temperature, extraction time, and type of alcohol used during precipitation did not affect the extraction yield and chemical composition of *Opuntia ficus indica* mucilage (Sepulveda *et al.*, 2007).

Proximate compositions and yields of okra mucilage are widely different, perhaps due to differences in extraction procedures. Rakdontri, Parnsubsakul, and Muntadilok (2005) studied the extraction conditions of okra mucilage and found that the yields of okra mucilage extracted using two different methods belonging to Wu *et al.* (1995) and Ndjouenkeu *et al.* (1996) were different. For the method of Wu *et al.* (1995), fresh okra was cut and deseeded before macerated with distilled water by using a blender. The insoluble material was removed by centrifugation, and the soluble part

was precipitated with 85% ethanol at a ratio of mucilage to ethanol of 1:4. After precipitating, the insoluble part was collected by centrifugation and then dialyzed against 20 volumes of distilled water, changed 3-4 times daily, for two days. For the method of Ndjouenkeu *et al.* (1996), fresh okra was deseeded and milled using a grinder. Fined deseeded okra (10 g) was mixed with distilled water (250 mL) and heated in a boiling water bath for one hour. The mixture was then centrifuged and the supernatant was collected. The residue was further extracted with two 250 mL aliquots of distilled water, following the same procedure. The final residue was discarded, and the supernatant fractions were combined, filtered through fine-mesh nylon and concentrated to about a quarter of their initial volume on a rotary evaporator. The crude polysaccharide was then precipitated with 85% ethanol (4 times the crude polysaccharide volume). The results showed that the yield of okra mucilage (gram/ 100 gram of dried okra) extracted by the method of Wu *et al.* (1995) and Ndjouenkeu *et al.* (1996) were 11.03 and 1.45, respectively. Since okra mucilage extracted by the method of Wu *et al.* (1995) gave a better yield, the researchers chose this method in extracting the mucilage for further composition analyses. After proximate analysis, they found that okra mucilage contained 95.25±0.39% of moisture. The dry mucilage contained 54.21±2.00 g carbohydrate, 33.56±0.99% g protein, 8.77±1.03 g of ash, 1.93±0.02 g crude fiber, and 1.54±0.03 g fat per 100 g. Okra mucilage contained a low amount of crude fiber and fat because the pod, which contains high amount of insoluble fiber, and seed, which is a good source of oil (Calisir *et al.*, 2003), were removed during the extraction.

As stated earlier, okra mucilage is a naturally occurring plant polymer. It could possibly compose of polysaccharides and proteins. Wu *et al.* (1995), who characterized polysaccharides of okra mucilage, indicated that okra mucilage contained approximately 40% galactose, 27% rhamnose, 24% galacturonic acid, and less than 4% protein, as estimated by the Lowry method. Lengsfeld *et al.* (2004) stated that the treatments of extracted mucilage caused differences in polysaccharide composition. In their work, after the extraction, the fresh extract was further processed by fractionated precipitation with ethanol to final volumes of 35, 45, and 60 %v/v. Precipitations using higher ethanol concentrations led to badly resolvable products. In their work, the raw

polysaccharide of the 45% ethanol precipitate (RPS) was chosen for further study because it provided the best yield with high molecular polysaccharides and best solubility properties. As presented in table 2.1, the total carbohydrates content of fresh extract (FE) was significantly lower ($p \leq 0.01$), but the protein content was two times higher than that of raw polysaccharide (RPS). Moreover, three acidic polysaccharide fractions (AF I-III) contained a high uronic acid content than RPS and were free of protein. Comparing to previous studies of Tomada *et al.* (1980) and Bhat and Tharanathan (1985) who found that galacturonic acid was the only uronic acid, Lengsfeld *et al.* (2004) suggested that the main structural elements contained more galacturonans than rhamnogalacturonans, and both of glucuronic and galacturonic acid were present.

Table 2.1 Composition of okra polysaccharide fractions (percent) and sugar composition (mole percent) after Trifluoroacetic acid (TFA) hydrolysis, acetylation, and GC- MS analysis.

	FE	RPS	NF	AF I	AF II	AF III
Rhamnose	13.8	19.9	21.2	13.9	15.0	7.2
Arabinose	1.5	2.1	14.4	1.2	2.3	0.3
Xylose	0.8	1.3	2.9	0.5	0.4	0.5
Mannose	0.9	0.5	3.6	0.9	1.5	0.3
Galactose	24.7	18.2	43.6	30.1	20.6	15.3
Glucose	6.0	3.7	14.3	3.2	6.7	1.0
Total uronic acids	28.5	32.9	-	50.1	53.3	75.2
Residual protein	23.7	11.9	-	<0.1	<0.1	<0.1
Average molecular weight (kDa)	1380	1380	30.2	692	977	1380
Yield	1.9[b]	1.3[b]	<1.0[c]	34.4[c]	8.0[c]	16.1[c]

[a]All values were determined from the dry weight of the respective lyophilized fraction referred to [b] the fresh weight of the extracted okra fruits and [c] the amount of RPS used in

AEX chromatography. FE, fresh extract; RPS, raw polysaccharide; NF, neutral AEX fraction; AF I-III, acidic AEX fractions I-III.

Source: Lengsfeld *et al.* (2004)

2.3 Hydrocolloids

The term hydrocolloid may include polysaccharides from plant, seaweed, microorganism, gelatin, and biopolymer modified by chemical or enzyme reaction from starch or cellulose. However, generally, hydrocolloid refers to a group of water-soluble naturally occurring polymers (Williams and Phillips, 2003). They consist of hydrophilic, long chain, high molecular weight molecules that increase viscosity of solution and form gel. Besides, the second functional properties of hydrocolloid are emulsifying, whipping, suspending, and encapsulating (Williams and Phillips, 2003).

Due to their functional properties, hydrocolloids can be divided into two types, which are thickening agent and gelling agent. For thickening characteristics, hydrocolloids increase the viscosity of the solutions. The viscosity is significantly influenced by the polymer hydrodynamic volume, which increases with radius of gyration (R_g). R_g increases with molecular mass, chain rigidity, and electrostatic charge density. For gelling characteristics, hydrocolloids are able to form gel by physical association of their polymer chains through, for example, hydrogen bonding (Hill, Ledward, and Mitchell, 1998)

The potential food application of hydrocolloids is tremendous. They can be used in a variety of foods, which include jam and jellies, salad dressing, ice cream, and beverage. They have been used in food products in order to control texture and organoleptic properties mainly by enhancing the viscosity and gel characteristics. Each hydrocolloid has its own unique functional characteristic, which is due to its chemical structure, molecular size, and shapes. Thus, it is necessary to choose suitable type of hydrocolloid when applying in food products.

The commercially important hydrocolloids, their origins, their functional properties, and their food application areas are shown in table 2.2.

Table 2.2 Source, function, and main applications of hydrocolloids.

Hydrocolloid	Source	Function	Application area
Botanical			
Carboxymethyl cellulose	Trees and cotton	Thickener	Dairy and desserts, ready to eat meal, bakery products, meat products
Pectin	Citrus peel and apple pomace	Gelling agent	Dairy and desserts, bakery products, soups, sugar confectionary
Guar gum	Seed endosperm	Thickener	Dairy and desserts, bakery, sauces and dressing, ready to eat meal
Locust bean gum	Seed endosperm	Thickener	Dairy and desserts
Gum arabic	Tree gum exudate	Produces low viscosity solutions at high gum concentrations, emulsifier	Sugar confectionary, beverages
Gum tragacanth	Tree gum exudate	Thickener	Dressing and sauces, icings
Algal			
Agar	Gelidium	Gelling agent	Confectionary, dairy and desserts
Carrageenan	*Euchema cottonii*	Gelling agent	Dairy and desserts, meat products, sugar confectionary
Microbial			
Xanthan gum	*Xanthaomonas campestris*	Thickener	Dairy and desserts, ready to eat meals, sauces and dressings
Gellan gum	*Sphingomonas elodea*	Gelling agent	Sugar confectionary, dessert and jellies, fruit preparations
Animal			
Gelatin	Cattle, pig, fish	Gelling agent	Sugar confectionary, meat products, dairy and desserts

Source: Williams and Phillips (2003)

2.3.1 Guar gum

Guar gum is a non- ionic plant hydrocolloid extracted from the seeds of *Cyamopsis tetragonolobus* and *C. psoraloides*, which is found in northwest India and Pakistan (Imeson, 1992). Since it is non-ionic, it is susceptible to strong acids, organic acids like citric, acetic, and ascorbic acids, alkali in the presence of air and strong oxidizing agents. The guar pod is used as an ingredient for animal feed and human consumption. The gum powder is available in a wide range of particle sizes, which differ in their solubilization rates. It is regarded as generally recognized as safe (GRAS) by the USFDA and by other worldwide regulatory agencies (Nussinovitch, 1997).

2.3.1.1 Structure of guar gum

As shown in figure 2.1, the structure of guar gum is galactomannan, which its linear chain is based on a backbone of β(1->4)-linked D-mannose residues. To this chain, single α-D-galactose residues are linked by C-1 through a glycosidic bond to C-6-mannose. The average galactose to mannose ration of guar gum is 1:2. The unsubstituted D-mannopyranosyl units represent the so-called smooth side; meanwhile the substituted D-galactopyranosyl units constitute the hairy side (Imeson, 1992).

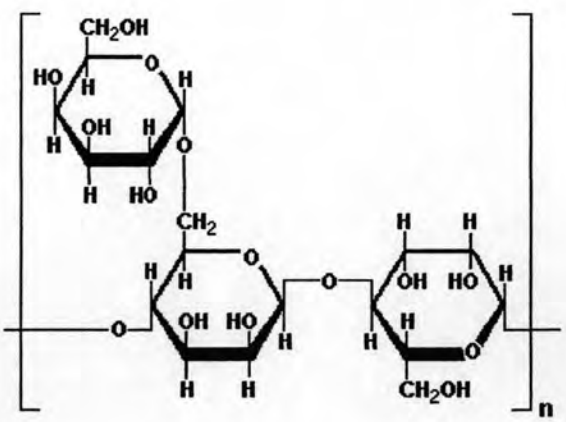

Figure 2.1 Chemical structure of guar gum.

Source: Zamora (2005)

2.3.1.2 Gum solution properties

Since guar gum has high substitution ratios, it tends to hydrate fully in cold water. This is because the presence of side chains which can interfere with the conformation of stable crystalline regions and promotes water penetration. Guar gum can hydrate fully and yields its maximum potential viscosity at 25^{o}C (Nussinovitch, 1997).

When guar gum is dissolved in water, the long mannan chain unfolds to form an open, flexible, non-ordered conformation, a random coil. As concentration increases, the polysaccharide chains will come into contact, resulting in their mutual entanglement. Therefore, the viscosity increases with an increase in concentration. If the molecule is longer, the conformation is more extended and the entanglement is stronger. This also relates to the intrinsic viscosity, which is the spatial volume occupied by the molecule when it is allowed to rotate freely about its center of gravity. Comparing to other galactomannans, guar gum has the highest intrinsic viscosity, followed by tara gum and locust bean gum, respectively. This is due to an increase in the degree of polymerization (Nussinovitch, 1997).

Guar gum exhibits pseudoplastic flow behavior, which its viscosity decreases with shear rate. It is a result of orientation of the extended molecules under the shear gradient as they align themselves parallel to the direction of flow (Casas, Mohedano, and Garci a-Ochoa, 2000).

2.3.1.3 Food applications of guar gum

Guar gum is widely used in many food products since it is able to thicken aqueous solution, and control or prevent syneresis. For example, guar gum can control the growth of ice crystal in ice cream (Sutton and Wilcox, 1998). In oil-in-water emulsion foods, like mayonnaise, guar gum is used as a stabilizer to prevent phase separation and also gives the cling effect. Lastly, it is also used to modify the texture of the gel and prevent syneresis in aerated and non-aerated gelated desserts (Nussinovitch, 1997).

2.3.2 Xanthan gum

Xanthan gum is an anionic hydrocolloid produced by the bacteria *Xanthomonas campestris*. Commercially xanthan is produced by an aerobic fermentation by *X. campestris* in a batch culture. It has been accepted as food additive in the USA and Europe, with an E number E415 (Imeson, 1992).

2.3.2.1 Chemical structure of xanthan gum

As shown in figure 2.2, xanthan gum is an anionic hydrocolloid with a β-(1⟶4)-D-glucopyranose glucan backbone with side chains containing two mannoses and one glucuronic acid. Slightly less than half (~40%) of the terminal mannose residues are 4,6-pyruvated and the inner mannose is mostly 6-acetylated. Due to the side chain, the xanthan polymer can completely hydrate. The molecular formation of xanthan is considered as a helix, which is stabilized by hydrogen bonds. The conformation of xanthan can be classified as a rigid ordered state and a more flexible disordered state. The conversion between the rigid ordered state and the more flexible disordered state may take place with increasing temperature and/ or shear (figure 2.3). The structure changes to random coil form as temperature increases and returns to its original state, which is more viscous, upon cooling. The transition temperature depends on many factors such as gum concentration and ionic strength. The transition temperature increases as salt concentration increases. In distilled water, the thermal transition temperature of xanthan gum at low concentrations (0.1-0.3%) is approximately 40°C (Nussinovitch, 1997).

2.3.2.2 Functionality of xanthan gum

Xanthan gum is soluble in cold water and it exhibits shear thinning behavior (Casas, Mohedano, and Garci a-Ochoa, 2000). This characteristic is due to its semi-rigid conformation. Since, xanthan has a very high viscosity at low shear rates and a high yield value comparing to other gums, it has a good ability in stabilizing emulsions and dispersions. At high shear rates, it has a relatively low viscosity, which makes the products become easy to pour, mix or pump. Xanthan gum is able to form thermo-

reversible gels when it is mixed with galactomannans such as locust bean gum or tara gum (Nussinovitch, 1997). Mixture of xanthan gum with other galactomannans such as guar gum results in an enhanced viscosity (Casas, Mohedano, and Garci a-Ochoa, 2000). The differentiation between increased viscosity and gelation is due to the mannose-galactose ratio of the galactomannan and the distribution of galactose side chains along the mannan backbone.

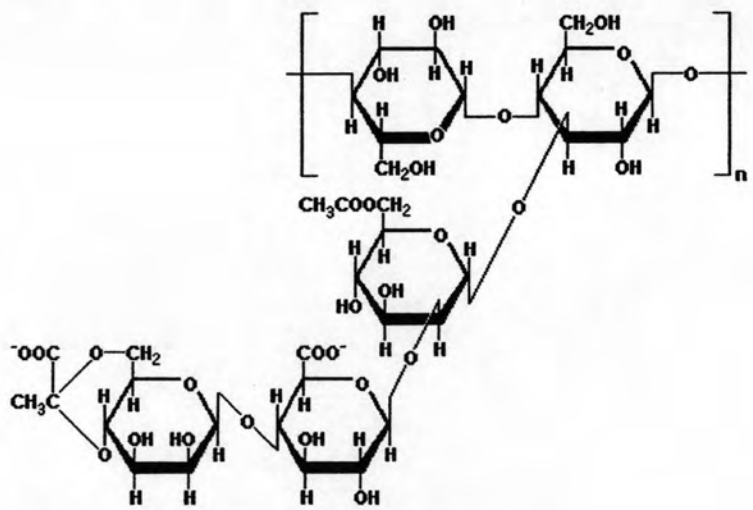

Figure 2.2 Chemical structure of xanthan gum.

Source: Zamora (2005)

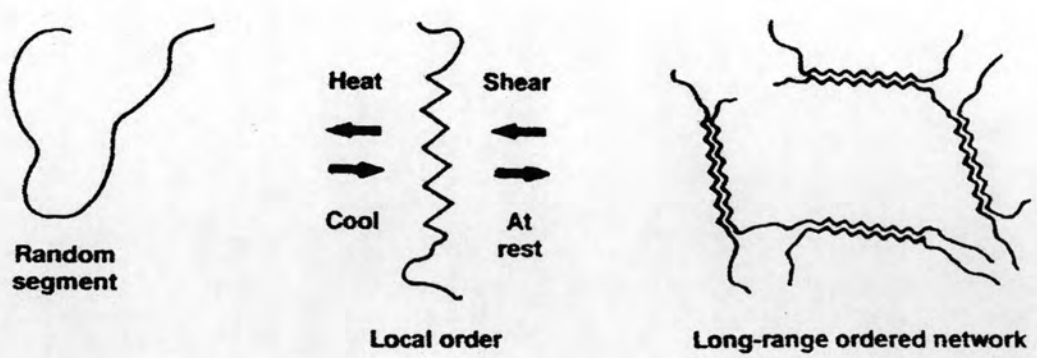

Figure 2.3 Conformational ordering in xanthan gum polysaccharide.

Souce: Nussinovitch (1997)

2.3.2.3 Food applications of xanthan gum

Xanthan gum has a wide variety of applications in foods. Xanthan is used in products such as sauces, gravies, desserts which can be heated or refrigerated without losing their desirable characteristics. It is also used to stabilize salad dressing, to control the rheological properties of mayonnaise, to improve the mouthfeel of citrus- and fruit flavored beverages, to provide good stabilization of the air cells in whipped products, and to prevent lump formation during kneading of baked goods (Nussinovitch, 1997).

2.4 Fourier Transform Infrared Spectrometry (FTIR)

Fourier Transform Infrared Spectrometry (FTIR) is a measurement technique which uses infrared (IR) radiation to identify unknown materials and determine the quality and consistency of a sample. IR radiation is passed through a sample, in which some of the infrared radiation is absorbed by the sample and some of it is passed through. When infrared radiation interacts with the sample and is absorbed, the chemical bonds in the sample vibrate. The presence of chemical bonds in a sample is necessary for infrared absorbance to occur. Each functional group, chemical structural fragments within molecules, tends to absorb infrared radiation at different wave number range and the resulting spectrum represents the molecular absorption and transmission, which is unique for each sample. For example, the C=O stretch of a carbonyl group occurs at ~1,700 cm^{-1} in ketones, aldehydes, and carboxylic acids (Smith, 1996). Table 2.3 shows FTIR spectrum with corresponding functional properties. Since, the structure of unknown molecules can be identified from the infrared spectrum, FTIR is a useful chemical analysis tool. Besides it is very sensitive and easy to use, the analysis procedure is very fast and there is no need for external calibration (Smith, 1996).

FTIR mainly consists of the source of radiation, interferometer, and detector (figure 2.4). The radiation beam is emitted from the radiation source and enters the interferometer. The beam-splitter in the interferometer divides the radiation into two optical beams. The first beam reflects off of a flat mirror, which is fixed in place, and then goes back to the splitter. Thus, the path length of this beam is a fixed distance.

The other beam reflects off a moveable mirror, which is allowed to move a very short distance away from the beam-splitter. Therefore, the path length of this beam is variable. Then, the two beams recombine when they meet back at the beam-spiltter. Because the paths of these two beams are different, the signal which exits the interferometer is the result of these two beams interfering with each other. The resulting signal is called an interferogram signal. The interferogram signal then exits the interferometer and enters the sample compartment where it is transmitted through or reflected off of the surface of the sample. This is where specific frequencies of energy, which are uniquely characteristic of the sample, are absorbed. Finally, the beam passes through the detector for final measurement and the measured signal is interpreted by a computer (Leelapojanaporn, 2003).

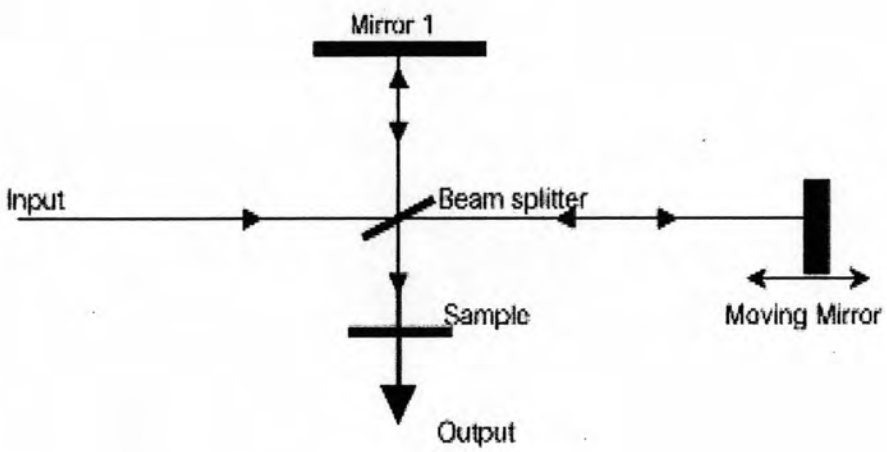

Figure 2.4 Schematic drawing of the Michelson interferometer.
Source: Leelapojanaporn (2003)

Table 2.3 FTIR spectrum with corresponding functional groups.

Bond	Compound Type	Frequency range, cm^{-1}
C-H	Alkanes	2960-2850 stretch
		1470-1350scissoring and bending
	CH_3 Umbrella Deformation	1380 -Doublet - isopropyl, *t*-butyl
C-H	Alkenes	3080-3020 stretch
		1000-675 bend
C-H	Aromatic Rings	3100-3000 stretch
	Phenyl Ring Substitution Bands	870-675 bend
	Phenyl Ring Substitution Overtones	2000-1600 - fingerprint region
C-H	Alkynes	3333-3267 stretch
		700-610 bend
C=C	Alkenes	1680-1640 stretch
C≡C	Alkynes	2260-2100 stretch
C=C	Aromatic Rings	1600, 1500 stretch
C-O	Alcohols, Ethers, Carboxylic acids, Esters	1260-1000 stretch
C=O	Aldehydes, Ketones, Carboxylic acids, Esters	1760-1670 stretch
O-H	Monomeric -- Alcohols, Phenols	3640-3160 stretch
	Hydrogen-bonded -- Alcohols, Phenols	3600-3200 stretch
	Carboxylic acids	3000-2500 stretch
N-H	Amines	3500-3300 stretch
		1650-1580 bend
C-N	Amines	1340-1020 stretch
C≡N	Nitriles	2260-2220 stretch
NO_2	Nitro Compounds	1660-1500 asymmetrical stretch
		1390-1260 symmetrical stretch

Source: Calfornia State University, 1998.

2.5 Rheology

Rheology is the study of deformation and flow of matters (Rao, 1999). It has many applications in the field of food quality assurance, food processing, and handling. For example, a food engineer needs information on flow behavior of the fluids and semi-fluid food during manufacturing (mixing and pumping) to design the right process.

2.5.1 Types of fluid flow behavior

2.5.1.1 Newtonian behavior

The shear stress of a Newtonian fluid is directly proportion to the shear rate and the plot begins at the origin. The viscosity is independent of shear rate within the laminar flow range. Examples of Newtonian foods are water, sugar syrups, and most honey (Bourne, 2002). The model of a Newtonian fluid is described by equation 2.1.

$$\sigma = \eta\dot{\gamma} \qquad (2.1)$$

where σ = shear stress (N/m^2)

η = viscosity ($N.s/m^2$)

$\dot{\gamma}$ = shear rate (s^{-1})

2.5.1.2 Non-Newtonian behavior

Most fluid foods are non-Newtonian. The plot between shear stress-shear rate is not linear and/or it does not begin at the origin. Flow behavior of non-Newtonian fluids depends on shear rate, and either on the duration of shear (time-dependent) or not on the duration of shear (time-independent) (Bourne, 2002).

Time-independent non-Newtonian fluids can be divided into shear thinning and shear thickening types. For shear thinning (pseudoplastic) behavior, the curve begins at the origin of the shear stress-shear rate plot and is concave downward, and an increase in shear rate gives a less than proportional increase in shear stress. The apparent viscosity of a shear thinning fluid is dependent on shear rate, which an increase in shear rate leads to a reduction of viscosity. Salad dressing is a good example of shear thinning foods. In case of shear thickening behavior, the shear stress-shear rate plot begins at the origin but is concave upward in which an increase in shear

rate gives an increase in shear stress. Shear thickening fluid exhibits an increase in apparent viscosity as shear rate is increased. Examples are partially gelatinized starch dispersions and some chocolate syrup (Barnes, Hutton, and Walters, 1993).

Shear thinning and shear thickening fluids can be described by using the power law model (equation 2.2).

$$\sigma = K\dot{\gamma}^{n} \tag{2.2}$$

where K = consistency index (Pa.s)

n = flow behavior index (dimensionless)

The exponent n is dimensionless that reflects the closeness to Newtonian flow. When the magnitude of n is less than 1, the fluid is shear thinning, and when n is more than 1, the fluid is shear thickening. However, the fluid is Newtonian when n equals to 1 and the consistency index is identically equal to the viscosity of the fluid (Rao, 1999).

Bingham plastic fluid exhibits a yield stress (σ_0), which is the minimum shear stress that must be exceeded to make the flow begin. Viscosity of this fluid does not depend on shear rate (Bourne, 2002). The model describing such behavior is shown in equation 2.3.

$$\sigma = \sigma_0 + \eta\dot{\gamma} \tag{2.3}$$

where σ_0 = yield stress (N/m^2)

Shear thinning with yield stress is often found in foods such as tomato ketchup and mayonnaise.

Other flow behaviors such as shear-thinning or shear thickening with yield stress can also be observed in some food (Bourne, 2002). In such case, the power law model with yield stress (equation 2.4) can be used to explain the behavior.

$$\sigma = \sigma_0 + k\dot{\gamma}^{n} \tag{2.4}$$

The flow behaviors mentioned above can be depicted by figure 2.5.

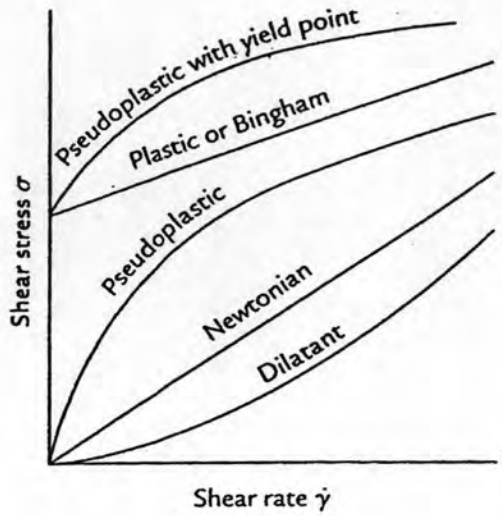

Figure 2.5 General flow behaviors of fluids.

Source: Bourne (2002)

For time-dependent Newtonian fluid, the viscosity of this type of fluids depends on the duration of shear, which can be divided to thixotropic and rheopectic flow behaviors. For thixotropic, time-dependent shear-thinning behavior, the apparent viscosity decreases with time of shearing, but the change is reversible. It means that the viscosity will revert to its original viscosity on standing. The shear stress values in ascending order of shear rate are higher than those in descending order. This is because, at rest condition, there is a linkage between the particles or molecules. But, when the shear is high enough, the linkage is broken resulting in a decrease in flow resistance. Thixotropic behavior is normally found in foods like salad dressing and soft cheese. Rheopectic or antithixotropic is time-dependent shear-thickening behavior. The apparent viscosity increases with time of shearing and the change is reversible. Opposite to thixotropic, the shear stress values in descending order of shear rate are higher than those in ascending order. The loop between the up curve and the down curve is called hysteresis loop and can be used to quantify the magnitude of time-dependency granted that some affecting factors are well controlled (Rao, 1999).

2.5.2 Viscoelasticity

Many hydrocolloids are capable of forming gels of various strengths, depending on their structures, concentration and environment factors such as ionic strength, pH and temperature. Viscoelasticity refers to the material that exhibits some of the elastic properties of an ideal solid and some of the flow properties of an ideal liquid. Small amplitude oscillatory shear (SAOS) can be used to determine viscoelastic properties of foods by determining the effect that an oscillating force has on the movement of the material (Bourne, 2002). In this nondestructive test, a sinusoidal oscillating stress or strain is applied to the material, and the phase difference between the oscillating stress and strain, as well the amplitude ratio, is measured. The information obtained is shown in the form of G', G'', G^*, and η^*. The storage modulus (G') represents a perfectly elastic solid component, whereas the viscous (or loss) modulus (G'') represents a viscous liquid component. If G' is higher than G'', the material will behave more like a solid and the deformation will be essentially elastic or recoverable. In contrast, if G'' is higher than G', the material will behave like liquid. Tan δ, the ratio of loss modulus and storage modulus, can also quantify the balance between storage and loss modulus. If a value of tan δ is greater than unity, it indicates that a sample is more liquid. If it is lower than unity, it means that a sample is more solid, regardless of the viscosity. Complex modulus (G^*) describes the total resistance of the sample to oscillatory shear, regardless of whether that deformation is recoverable (elastic) or non-recoverable (viscous). In amplitude sweep test, a decrease in complex modulus indicates the break down of the structure of a sample. Complex viscosity (η^*), complex modulus divided by angular frequency, is a frequency dependent viscosity function determined during forced harmonic oscillation of shear stress. It describes the flow resistance of a sample in the structured state. A high value for the complex viscosity the greater is the resistance to flow in the structured state (Rao, 1999).

Dynamic rheological test is quite suitable because it is nondestructive and does not interfere with gel formation and soften the structure of the material. There are three types of dynamic rheological tests, which are frequency sweep test,

temperature sweep test, and time sweep test. For the frequency sweep test, G' and G" are determined as a function of frequency at a fixed temperature (Rao, 1999).

Generally, the viscoelastic spectrum of polymer can be divided into four frequency areas (figure 2.6). The behavior of polymer at each frequency area depends on size and structure of the polymer and its concentration. The first area is terminal zone, which has a low experimental frequencies or a long time scale of measurement. It can be observed that G' is much lower than G" and the crossover occurs at the end of this zone. This is due to the complete energy dissipation and chain relaxation of the polymer subject to shear force. The second part is the plateau zone. In this area, the storage modulus becomes higher than the loss modulus. The storage modulus is parallel to the x axis, resulting from the network that supports the applied stress. For the glass transition zone; zone III, there is a segment relaxation of the polymer network. As a result, a crossover between G' and G" occurs, in which G' is lower than G". The last zone; zone IV, is the glassy zone, where time scale of measurement is very short. Chemical bonds of polymers are only allowed for stretching and bending. Therefore, G' is higher than G" and G" decreases as frequency increases (Kasapis, 1998).

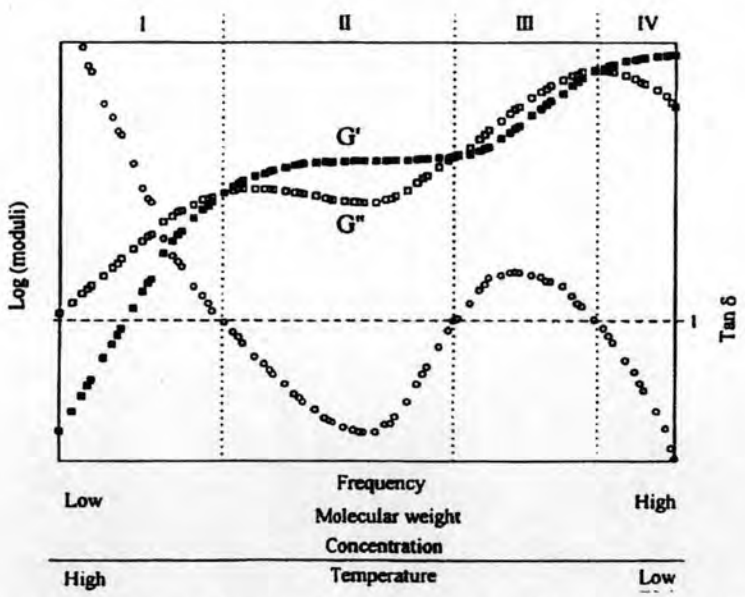

Figure 2.6 Moduli spectrum of polymer in dynamic test (Zone I: the terminal zone, zone II: the plateau zone, zone III: the glass transition zone, and zone IV: the glassy zone).

Source: Kasapis (1998)

2.5.3 Flow behavior of okra mucilage

According to Ndjouenkeu *et al.* (1996), and Rakdontri *et al.* (2005), okra mucilage possesses non-Newtonian shear thinning behavior, which its viscosity decreases and shear stress increases with an increase in shear rate. Factors affecting flow behavior of okra mucilage include temperature, concentration of solute, and molecular weight and conformation of solute.

Temperature has an effect either on apparent viscosity at a specified shear rate or the consistency index (K) of the power law model. According to Rakdontri *et al.* (2005), who studied rheological properties of okra mucilage, temperature has an effect on the viscosity of okra mucilage. The viscosity and consistency index of okra mucilage decreases as temperature increases (table 2.4).

Table 2.4 Viscosity of 4.75% w/v or w/w okra mucilage at various temperatures.

Temperature (^{o}C)	n	K (Pa.s)	$\eta_{a,\ 100\ s^{-1}}$ (Pa.s)	$\eta_{a,\ 10\ s^{-1}}$ (Pa.s)
20	0.26 $\pm$ 0.01	10.59 $\pm$ 0.04	0.35	1.93
30	0.26 $\pm$ 0.00	9.34 $\pm$ 0.28	0.31	1.70
40	0.27 $\pm$ 0.00	8.16 $\pm$ 0.21	0.28	1.52
50	0.29 $\pm$ 0.00	6.34 $\pm$ 0.11	0.24	1.24
60	0.29 $\pm$ 0.00	5.60 $\pm$ 0.17	0.21	1.09

Source: Rakdontri *et al.* (2005)

The change in viscosity as a function of temperature can be described by the Arrhenius equation (equation 2.5) (Rao, 1999).

$$\eta_a = \eta_{\alpha A} \exp (E_A/RT) \qquad (2.5)$$

where, η_a is the apparent viscosity at a specific shear rate, $\eta_{\alpha A}$ is the frequency factor, E_A is the activation energy (J mol^{-1}), R is the gas constant (J mol^{-1} K^{-1}), and T is temperature (K).

E_A is the energy barrier that must be overcome to make the flow occur, or in the other words, the activation energy for viscous flow. The higher the absolute

activation energy, the higher the effect that temperature imposes on the viscosity. Lower absolute activation energy means that the viscosity is less sensitive to changing temperature.

The second factor is the concentration of polymer, at a constant temperature, there is a direct nonlinear relationship between the concentration of polymer and viscosity, in which the viscosity increases as the concentration of polymer increases. According to the result of Rakdontri *et al.* (2005), at the shear rate of 10 s^{-1}, the viscosity of okra mucilage increases from 0.01 to 1 Pa.s when the polymer concentration increases from 0.50 to 4.75% (w/w).

The last factor is molecular weight (MW) and conformation of polymer. At equal concentration, there is a nonlinear relationship between the molecular weight of the solute and the viscosity of the solution. The polymer which has higher MW normally gives higher viscosity than the polymer with lower MW (Bourne, 2002).

There is a relationship between the structure and conformation of polymer and its viscosity. In dilute solution, there is no interaction between molecules and its molecule is extended. Thus, the viscosity depends on its hydrodynamic volume. A sample with more extended molecules has higher viscosity than that with more compact molecules with similar molecular weight. Generally, less flexible polymeric chains give rise to more extended structures than a flexible polymeric chain. For a sample with negatively charged, the repulsion between similar charges increases molecular extension. But, this can be reduced at higher ionic strength or below the pK_a of the anionic groups. This reduction is particularly noticeable for polymers with high molecular weight (Bourne, 2002).

The research of Ndjouenkeu *et al.* (1996) showed the effect of polymer conformation on the viscosity. The researchers prepared a few moderate concentrations within the range of 0.096-0.96 %w/v of the okra extractions in water and in 0.1 M NaCl. The rheological measurement was performed at 25°C. The viscosity of okra gum in salt solution was lower than that in water and the differences increased with decreasing polymer concentration. This might be because of the presence of a substantial proportion of charged (galacturonate) residues in the structure of okra gum. In water, the individual coils were expanded by intra-molecular electrostatic repulsion.

When salt was added, the repulsion was limited. The coils became more contracted and this led to a more compact form and a reduction in viscosity.

2.6 Emulsifying property of okra mucilage

Sausage, milk, and salad dressing are examples of food emulsion. Although they contain oil and water, there is no phase separation or any coagulation of lipid during storage and distribution. Emulsion is a mixture of at least two immiscible liquids, one of which is dispersed in the other in the form of fine droplets. The liquid in droplets is called the dispersed or discontinuous phase, whereas the surrounding liquid is called the continuous phase. Emulsion is thermodynamically unstable systems because the contact between oil and water molecules is unfavorable. They tend to break down with time, and then the phase separation is occurred. Therefore, an emulsifier is required to form and a stable food emulsion. Emulsifier is a surface active substance that absorbs on the surface of emulsion droplets to form a protective coating that prevents the droplets from aggregating with one another. Both polysaccharide and protein have emulsifying properties, but their mechanisms that facilitate emulsion formation and stability are different (Fennema, 1996).

In food emulsion, protein and polysaccharide form and stabilize the emulsion by different mechanisms. When polysaccharides or hydrocolloid are added to an emulsion, they swell in the aqueous phase by absorbing water in their molecules. This increases the viscosity and density of the continuous phase, and also enhances gel characteristics. As a result, the movement of dispersed phase, particle sedimentation, and droplet creaming are retarded. Moreover, hydrocolloids may also absorb onto the surface of particles or droplets and inhibit aggregation by steric and electrostatic force. Therefore, hydrocolloid can function as suspension and emulsion stabilizer (Williams and Phillips, 2003).

Protein comprises amino acids, which the side chains contain a wide variety of chemical groups, ranging from hydrocarbon to polar and ionisable forms. Protein can help forming and stabilizing emulsion because it possesses both hydrophilic and hydrophobic, or, in the other word, amphiphilic property (Hasenhuettl and Hartel, 1997).

The interfacial tension between two immiscible phases can be lowered due to the attachment of protein to the droplets. This is because, at the interface, non polar side of amino acids tends to orient toward fat or oil. On the other hand, polar side chains are oriented toward the aqueous phase.

Electrostatic charge and steric hindrance of protein film around the lipid globules prevent flocculation. Electrostatic effect occurs because of the ionic side chains of protein. There is a repulsive force between protein containing same charges, so that the attraction between droplets is prevented. Steric hindrance is caused by hairs of protein (Walstra, 1996). When protein is denatured, its configuration changes to the unfolded form that has flexible molecular chains or hairs. After these hairs are attached to the droplets, these droplets cannot coalesce because there are physical entanglements of hairs protruding into the continuous phase. Moreover, soluble proteins increase the viscosity of the continuous phase, resulting in reducing the rate of coalescence (Sikorski, 2002). Hence, it can be concluded that the efficiency of proteins as emulsifiers depends on their surface hydrophobicity, electrostatic charge, steric effects, and viscosity in solution.

There are many researches that study emulsifying properties of gums and mucilage (Akhtar *et al.*, 2002; Carvajal-Millan *et al.*,2007; Garti *et al.*,1997; Garti and Reichamn, 1994; Huang, Kakuda, and Cui., 2001; Ndjouenkeu *et al.*, 1997). Several food gums and mucilage exhibit interfacial property and emulsifying activity. These properties have been attributed to the protein that is covalently linked with highly branched polysaccharide structures, whereas the emulsion stabilization is attributed predominantly to the carbohydrate portion, contributing viscosity (Dickinson, 1995). However, some hydrocolloids, which are rigid and very hydrophilic, were found to migrate slowly to air-water and oil-water interfaces, and exhibit some surface and interfacial activities (Garti *et al.*, 1997). The researchers suggested that these hydrocolloids can absorb onto oil droplets and sterically stabilize emulsions against flocculation and coalescence. Huang *et al.* (2001) studied the emulsifying activity of fenugreek gum comparing to mucilage and commercial hydrocolloids such as xanthan gum and locust bean gum. The results showed that, at the gum concentration of 1.0% w/w, only fenugreek gum had an emulsion stability of 100% over 90 days period,

whereas xanthan and locust bean gum had an emulsion stability of 65% and 40%, respectively. This may be due to the property of fenugreek gum which retards the movement of oil droplets by increasing the viscosity of the continuous phase and reduces the interfacial tension by absorbing at the oil-water interfaces. Carvajal-Millan *et al.* (2007) also investigated emulsion activity of maize bran gum, and found that emulsion stability index increased from 0.01% to 0.20% as the concentration of maize bran gum increased from 0% to 1%.

Ndjouenkeu *et al.* (1997) studied emulsifying properties of okra mucilage. As mentioned previously, okra mucilage contains glycoprotein, an organic compound comprising proteins that are bound with carbohydrate in the form of polysaccharide with covalent interactions. Therefore, it should stabilize emulsion by decreasing interfacial tension between oil-water interface, and increasing the viscosity of the continuous phase. According to their results, the ability of okra mucilage to stabilize emulsion depended on concentration. To stabilize emulsion, the interfacial protein layer covering oil droplets should be as thick as possible. The thickness of the interfacial protein layer could be inferred from the volume fraction (Φ_e) of oil in the emulsion at equilibrium. The low Φ_e values related to thicker film. Figure 2.7 showed that, at okra gum concentration above 10 g/L, the interfacial gum layer increased, inferring an increase in emulsion stability, with an increase in concentration. This may link to water oil absorption index (WOAI) of okra, which is the ratio of water absorption capacity to oil absorption capacity. At low concentrations, okra polysaccharide molecules, which had relatively low WOAI (1.8), were not enough to absorb water. Hence, the interfacial okra gum layer was quite thin. But, as the concentration increased, there were more molecules. Therefore, the layer was thicker and the emulsion was more stable. To conclude, the absorbed okra gum showed an emulsifying capability for stabilizing oil-in-water emulsions.

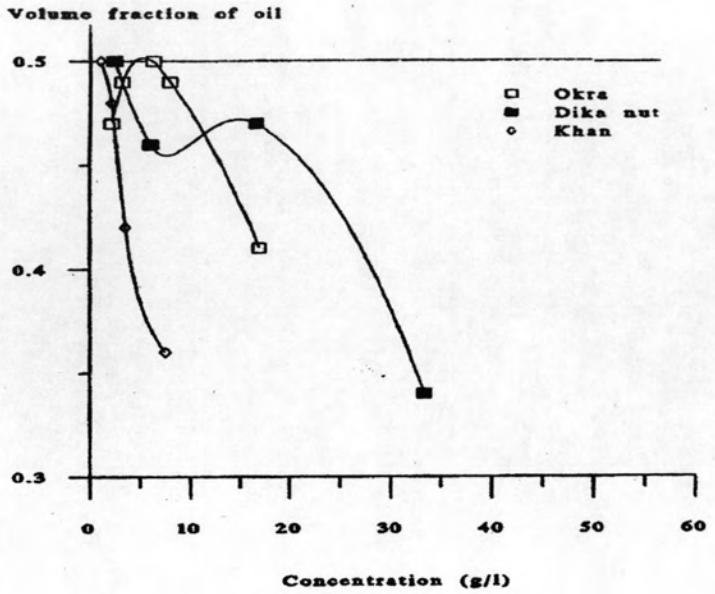

Figure 2.7 Volume fraction of oil in emulsion at equilibrium as a function of gum concentration.

Source: Ndouenkeu *et al.* (1997)