Effect of xanthan-guar mixtures on
stability of sterilized coconut milk

BY
Ms. Parichart Yamalee
ID: 501-8992

A special project submitted to the Faculty of Biotechnology,
Assumption University in part of fulfillment of the requirement for the
degree of Bachelor of Science in Biotechnology
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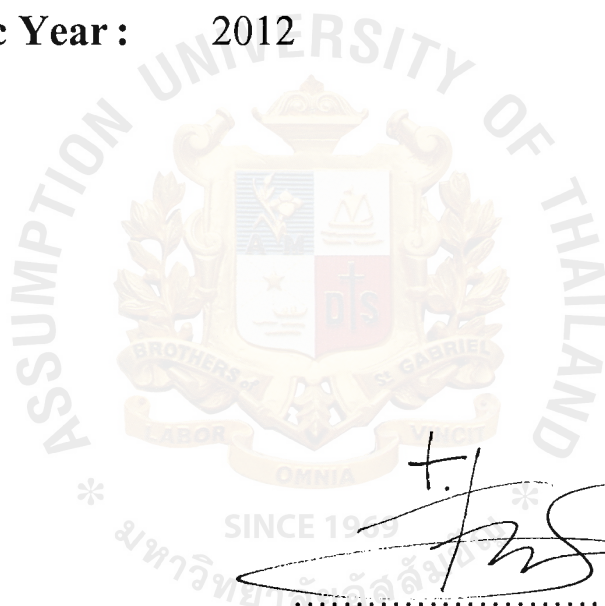
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By	:	Ms. Parichart Yamalee
Advisor	:	Dr.-Ing. Tatsawan Tipvarakarnkoon
Level of study	:	Bachelor of Science
Department	:	Agro-Industry
Faculty	:	Biotechnology
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Dr.-Ing. Tatsawan Tipvarakarnkoon

(Advisor)

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ABSTRACT

Coconut milk is one of the ten important exported organic agricultural products of Thailand (Press release THAIFEX-World of Food Asia, 2009). However, problems still arise among canned coconut milk products due to the instability of its emulsion. To delay creaming instability, the addition of mixing of xanthan:guar (0:100, 15:85, 50:50, 85:15, 100:0) at (0.05, 0.10 and 0.15wt%) has been used and investigated their effect onto stability of coconut milk emulsions. Fresh coconut milk (32 ± 1.59% fat content) was purchased from local market and immediately preheated at 80°C before use. Stabilized coconut milks prepared by homogenized at 15,500 rpm for 2 min using rotor/stator homogenizer before adding xanthan gum/guar gum (Bronson and Jacobs International Co., Ltd.) and gently stirred for 15 min using magnetic stirrer. The rheological properties, coarse oil droplet size, particle size determination, and creaming index were determined for non-sterilized and sterilized coconut milk emulsions. From the results, increasing concentrations of xanthan gum/guar gum gave high viscosity, decreasing coarse oil droplet diameter (μm) which induced higher stability by delaying creaming index of coconut milk emulsions. It was also found in this experiment that creaming index (%) of mixing gum of 50:50 and 85:15 at 0.10% and 0.15% gave lowest creaming index at day 7. As well, for amplitude sweep and frequency sweep of mixing gum showed the solid-like property ($G' > G''$). After sterilization, the creaming index was unexpectedly lower than non-sterilized samples after stored for 7 days even if coarse oil droplet size of the sterilized samples were bigger than those of non-sterilized. The reason might be due to the bridging flocculation by xanthan gum/guar gum has been occurred and induced the stability in coconut milk emulsions.

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1. INTRODUCTION

Coconut milk is not the juice found inside a coconut, but the diluted cream pressed out from the thick, white flesh of a well-matured coconut. Coconut milk is the natural oil-in-water emulsion extracted from the endosperm of mature coconut (*Cocos nucifera* L.) either with or without the addition of water (Seow & Gwee, 1997). The white, opaque emulsion has been used widely as an important food ingredient, especially in Asia and Pacific regions. The emulsion is known to be naturally stabilized by coconut proteins: globulins and albumins, and phospholipids (Birosel, Gonzales, & Santos, 1963).Coconut milk contains about 54% moisture, 35% fat, 4% protein and 11% solid non-fat (Simuang et al., 2004; Tansakul and Chaisawang, 2006). However, the coconut milk emulsion is unstable, and readily separates into two distinct phases of a heavy aqueous phase and a lighter cream phase (Cancel, 1979; Gonzalez, 1990). The reason for the instability is that the protein content and quality in coconut milk is not sufficient to stabilize the fat globules (Monera & del Rosario, 1982). Therefore, stabilizer and/or emulsifier are needed to add into coconut milk. Coconut milk cannot sterilize with the same temperatures as other products. This is caused by coagulation process of coconut milk if heated above 100ºC and can reduce almost all of flavor in coconut milk itself. Therefore, long time preservation is needed to stabilize with emulsifier or stabilizer and hydrocolloid followed by homogenization or agitation method to reduce the particle size of fat globule. The emulsion is also enhanced upon the reduction in droplet size. Small of the particle size diameter is better than big because it give better stability.

In this experiment, xanthan gum and guar gum were used as hydrocolloids to control the viscosity of emulsion and improve the stability of coconut milk emulsions. Xanthan and guar gum were mixed for 0:100, 15:85, 50:50, 85:15 and 100:0 to delay creaming in coconut milk emulsion. To achieve these goals, a sophisticated rheometer was used. Shear rate examinations and dynamic viscoelastic measurements were carried out. Other physical examinations including % creaming index, particle size distribution and microscopic measurements were also determined.

OBJECTIVES

1. To study the rheological properties of xanthan and guar gum solutions.

2. To study the effect of different mixing methods of gum onto sterilized coconut milk.

3. To study the effect of ratio of xanthan-guar mixtures on stability of sterilized coconut milk.

2. LITERATURE REVIEW

2.1 Coconut milk

The coconut is an important fruit tree in the world, providing food for millions of people, especially in the tropical and subtropical regions and with its many uses it is often called the "tree of life". Coconut (*C. nucifera*) belongs to the family of the *Arecaceae* (Palmae), the subfamily *Cocoideae*. There are mainly two distinct of coconut *i.e.* tall and the dwarf. The tall varieties grow slow and bear fruits 6 to 10 years after planting. Coconut milk finds a special place in many tropical cuisines, especially Thai, Singaporean, and Malaysian. It acts as a source of a wide range of vitamins, minerals, potassium, folate and other vital nutrients.[1]Coconut milk is an oil-in-water emulsion formed from the aqueous extract of coconut solid endosperm. The emulsion is relatively unstable because of the large droplet size (Tangsuphoom & Coupland, 2008) and the poor emulsifying properties of coconut proteins adsorbed at the oil–water interface (Monera & del Rosario, 1982; Onsaard, Vittayanont, Sringam, & McClements, 2006).

For coconut milk compositions; the chemical composition of fresh coconut milk shows very wide variation of geographical sources, maturity of nuts and methods of extraction. The compositions of the emulsion directly determine from coconut kernel without water reported by different workers are given in Table2.1.

Table 2.1 Proximate composition of undiluted whole coconut milk as reported by different sources (modified from Seow and Gwee, 1997)

Constituent (%)	Nathanael (1954)	Popper et al. (1966)	Jaganathan (1970)	Anon. (1984)	Gonzalez (1990)	Tansakul & Chaisawang (2006)
Moisture	50.0	54.1	50.0	53.9	50	55.3
Fat	39.8	32.2	40.0	34.7	34	35
Protein(N x 6.25)	2.8	4.4	3.0	3.6*	3.5	4.02
Ash	1.2	1.0	1.5	1.2	2.2	1.02
Carbohydrates (by differences)	6.2	8.3	5.5	6.6	7.3	4.70**

* N X 5.30; ** contains solid non-fat about 9.74 %

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2.2 Food Emulsion

Emulsions are thermodynamically unstable systems that tend to break down over time due to a variety of physicochemical mechanisms, e.g., gravitational separation, creaming, flocculation, and Ostwald ripening. A great deal of research has been carried out to establish the role of aqueous phase composition on the stability of oil-in-water emulsions, e.g., pH, mineral ions, polymers, and surface-active molecules. On the other hand, much less is known about the influence of droplet composition on emulsion stability. The density of an oil droplet depends on the type and concentration of non polar molecules contained within it, and therefore the creaming stability of emulsions depends on droplet composition.

2.3 Tween60

Polysorbate-60. (Polyoxethylene sorbitan fatty acid ester). This is an effective emulsifier/solubiser and is another ingredient in Emulsifying Wax NF. On it's own it comes as a semi-solid of yellowish colour with a texture similar to Vaseline and needs warming before use. It is a food-safe emulsifier and within the toiletry indusry is recommended mainly for rendering essentail oils and fragrance oils soluble in water. On it's own it is not generally suitable for emulsifying oils and water into lotions and creams. It is very useful in getting fragrances into water before emulsifying into lotions and creams using a suitable emulsifying wax. It is generally recommended as a simple solubiser in the manufacture of room sprays and skin cleansers although the resultant mixture loses clarity. Polysorbates also come in other varieties, i.e. Polysorbate-20 or Polysorbate-80, which can also be used in various emulsifying applications either on their own or in combination.
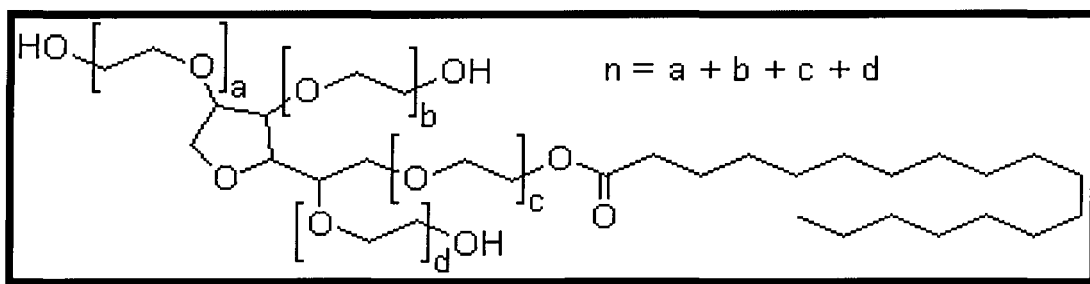
Figure 2.1 Chemical structure of Tween 60 or Polyoxyethylene (20) sorbitan monostearate
Ref: http://www.chemical-buyers.com/cas-900/9005-67-8.html

2.4 Food Hydrocolloid (Chaplin, 2012)

Hydrocolloids, often called gums, are hydrophilic polymers, of vegetable, animal, microbial or synthetic origin, that generally contain many hydroxyl groups and may be polyelectrolytes. They are naturally present or added to control the functional properties of aqueous foodstuffs. Most important amongst these properties are viscosity (including thickening and gelling) and water binding but also significant are many others including emulsion stabilization, prevention of ice recrystallization and organoleptic properties. The degree with which the hydrocolloid solutions mix with saliva, determined by their degree of chain entanglement, determines flavor perception. The commercially important hydrocolloids and their origins are given in Table2.2 .

Table 2.2 Source of commercially important hydrocolloids (Phillips and Williams, 2000)

Source		Hydrocolloid
Botanical	- Trees	cellulose
- Tree gum extrudates		gum Arabic, gum karaya, gum ghatti, gum tragacanth
- Plants		starch, pectin, cellulose
- Seeds		Guar gum, locust bean gum, tara gum, tamarind gum
- Tubers		Konjac mannan
Algal	- Red seaweeds	Agar, carrageenan
- Brown seaweeds		alginate
Microbial		Xanthan gum, curdlan, dextran, gellan gum, cellulose
Animal		Gelatin, caseinate, whey protein, chitosan

2.5 Xanthan gum

2.5.1 Source

Xanthan gum is a microbial desiccation-resistant polymer prepared commercially on a large scale (>30,000 tons per year) by aerobic submerged fermentation from *Xanthomonas campestris*. It is naturally produced to stick the bacteria to the leaves of cabbage-like plants. It is relatively expensive by weight but becoming rather less so. As the media used to grow the *Xanthomonas* may contain corn, soy or other plant material, manufacturers should make clear if any residues may remain.

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2.5.2 Structural unit

Xanthan gum is an anionic polyelectrolyte with a β-(1→4)-D-glucopyranose glucan (as cellulose) backbone with side chains of -(3→1)-α-linked D-mannopyranose-(2→1)-β-D-glucuronic acid-(4→1)-β-D-mannopyranose on alternating residues. Slightly less than half (~40%) of the terminal mannose residues are 4,6-pyruvated and the inner mannose is mostly 6-acetylated (that is, the side chains are mainly β-D-mannopyranosyl-(1→4)-(α-D-glucuronopyranosyl)-(1→2)-β-D-mannopyranoside-6-acetate-(1→3)-. Some side chains may be missing.
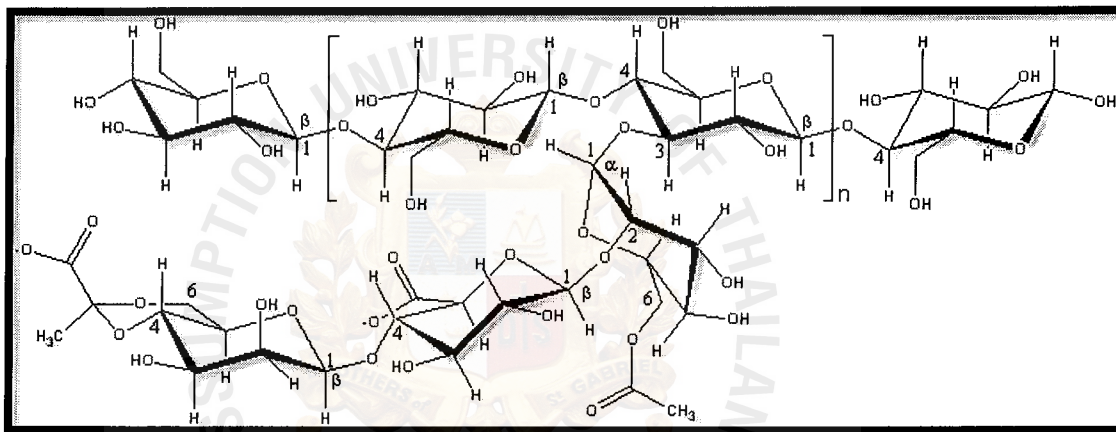
Figure 2.2 Chemical structure of xanthan gum
Ref: http://www.lsbu.ac.uk/water/hyxan.html

2.5.3 Molecular structure

Xanthan gum has a relatively reproducible specification as it is produced by fermentation. Each molecule consists of about 7000 pentamers and the gum is less polydisperse than most hydrocolloids. Its natural state has been proposed to be bimolecular antiparallel double helices. It may form a very stiff intramolecular (single molecule hairpin) double stranded helical conformation by the annealing of the less stiff 'natural' denatured elongated single stranded chains. The glucan backbone is protected by the side chains which lie alongside, making it relatively stable to acids, alkalis and enzymes (this is particularly important as preparations can contain cellulase). Use of

different strains or fermentation conditions may give rise to differing degrees of acetylation and pyruvylation, which moderates the functionality.

The conversion between the ordered double helical conformation and the single more-flexible extended chain may take place over hours of annealing (equilibrating) at between 40°C - 80°C. The weakly bound network formed is highly pseudoplastic, viscosity reducing considerably with shear increase and returning in full immediately on release. High viscosity solutions (~1%) appear gel-like but still shear-thin. The rationale for this strange behavior is the hydrogen-bonded and entangled association between the side chains of the highly extended molecules, which resists dissociation. Shear thinning with greater strain is mainly due to the conformation of the side chains flattening against the backbone under shear, so reducing the intermolecular interactions.

2.5.4 Functionality

Xanthan gum is mainly considered to be non-gelling and used for the control of viscosity due to the tenuous associations endowing it with weak-gel shear-thinning properties. It hydrates rapidly in cold water without lumping to give a reliable viscosity, encouraging its use as thickener, stabilizer, emulsifier and foaming agent. The consistent water holding ability may be used for the control of syneresis and to retard ice recrystallization (ice crystal growth) in freeze-thaw situations; xanthan gel strength being improved on freeze-thaw. Its most important property being its very high low-shear viscosity coupled with its strongly shear-thinning character. The relatively low viscosity at high shear means that it is easy to mix, pour and swallow but its high viscosity at low shear gives good suspension and coating properties and lends stability to colloidal suspensions. Being relatively unaffected by ionic strength, pH (1 - 13), shear or temperature it may be used in such products as salad dressings.

Xanthan gum is capable of synergistic interactions with galactomannans and glucomannans (for example, konjac mannan,; a non-ionic relatively rigid gelling, naturally partially acetylated, polysaccharide possessing a mixed $(1 \rightarrow 4)$-linked β-D-mannopyranose/β-D-glucopyranose backbone with about 8% β-$(1 \rightarrow 6)$-glucosyl branchpoints). It synergistically forms thermoreversible soft elastic gels with locust bean gum on cooling mixtures; locust bean gum being preferred over guar gum as it has fewer galactose side chains and the interaction (here) concerning the smooth $(1 \rightarrow 4)$-linked β-D-mannopyranose backbone regions. A greater proportion of guar gum (80:20) is

required for optimal synergy compared to locust bean gum (50:50) with the associating complex not requiring segments of unsubstituted backbone. The synergy is best at high xanthan extension and is thus reduced by high salt and low pH. Xanthan gums may contain cellulase, which prevents their use with cellulose derivatives.

2.6 Guar gum

2.6.1 Source

Guar gum (also called guaran) is a plant hydrocolloid extracted from two leguminous (guar) plants, *Cyamopsis tetragonolobus* and *C. psoraloides*, found in northwest India and Pakistan. where it acts as a food and water store. Guar gum is widely used as a guar gum emulsifying, guar gum for food that is food ingredients, guar gum thickening, pet food additives and gelling additives. Plantago ovata as a plantago ovata for food and guar gum manufacturer provide guar gum for food nutraceuticals guar gum for textile.

2.6.2 Structural unit

Guar gum is a galactomannan similar to locust bean gum consisting of a (1 →4)-linked β-D-mannopyranose backbone with branchpoints from their 6-positions linked to α-D-galactose (that is, 1→6-linked-α-D-galactopyranose). There are between 1.5 - 2 mannose residues for every galactose residue.
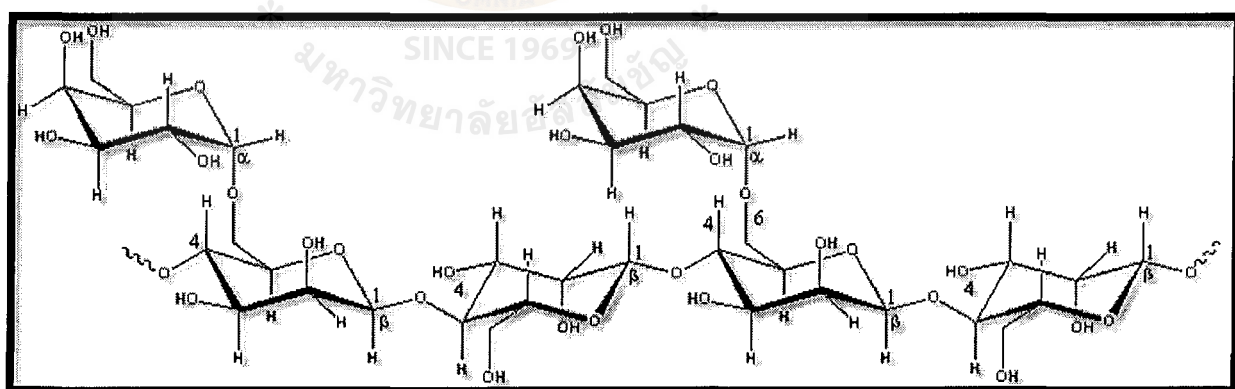
Figure 2.3 Chemical structure of guar gum
Ref: http://www.lsbu.ac.uk/water/hygua.html

2.6.3 Molecular structure

Guar gum is made up of non-ionic polydisperse rod-shaped polymers consisting of molecules (longer than found in locust bean gum) made up of about 10,000 residues. Higher galactose substitution also increases the stiffness (that is, decreases the

flexibility) but reduces the overall extensibility and radius of gyration of the isolated chains. The galactose residues prevent strong chain interactions as few unsubstituted clear areas have the minimum number (about 6) required for the formation of junction zones. Of the different possible galactose substitution patterns, the extremes of block substitution and alternating substitution give rise to the stiffer, with greater radius of gyration, and most flexible conformations respectively (random substitution being intermediate). Its persistence length is greater than that for locust bean gum at about 10 nm. If the galactose residues were perfectly randomized, it unlikely that molecules would have more than one such area capable of acting as a junction zone, so disallowing gel formation. A block substitution pattern, for which there is some experimental evidence, would allow junction zone formation if the blocks were of sufficient length. Use of endo-1,4-β-D-mannanase and α-D-galactosidase have shown that shorter chain lengths and lower degrees of substitution lead to the formation of large, but soluble, assemblies while longer galactomannans have reduced solubility. Enzymatic hydrolysis of some of the galactose side chains may allow guar gum to be used to replace a dwindling locust bean gum supply.

2.6.4 Functionality

Guar gum is an economical thickener and stabilizer. It hydrates fairly rapidly in cold water to give highly viscous pseudoplastic solutions of generally greater low-shear viscosity when compared with other hydrocolloids and much greater than that of locust bean gum. High concentrations (~ 1%) are very thixotropic but lower concentrations (~ 0.3%) are far less so. Guar gum is more soluble than locust bean gum and a better emulsifier as it has more galactose branch points. Unlike locust bean gum, it does not form gels but does show good stability to freeze-thaw cycles. Guar gum shows high low-shear viscosity but is strongly shear-thinning. Being non-ionic, it is not affected by ionic strength or pH but will degrade at pH extremes at temperature (for example, pH 3 at

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50°C). It shows viscosity synergy with xanthan gum. With casein, it becomes slightly thixotropic forming a biphasic system containing casein micelles.

Guar gum retards ice crystal growth non-specifically by slowing mass transfer across solid/liquid interface.Unfortunately for the food industry, guar gum has been found to be ideal for use by the oil and gas industry to extract gas and oil from source rocks using

pressurized fluid (hydraulic fracturing, or 'fracking'). This has increased both the demand for guar and its price several-fold.

2.7 Rheology of solutions

2.7.1 Definitions

Rheology is the science of deformation and flow of matter under controlled testing conditions and concerned with mechanical properties of various solid-like, liquid-like, and intermediate technological and natural products (materials). It accomplishes its goals by means of models representing principle peculiarities of behavior of these materials. The behavior of a material is arelationship between forces and deformations (or changes of shape) and a model gives a mathematical formulation of the relationship, rheological properties being expressed by the structure of a model (i.e., its mathematical image) and values of constants included in a model characteristic for a material.

2.7.2 Viscosity

Movement of all fluids and liquid-like materials is based on the model of sliding of near layers compare to each other when any external force applied to a body induces either to movement as a whole or to change its initial shape (Malkin and Isayev, 2006). With a small deformation, the angle of shear is equal to shear strain, $\gamma = dx/dy$ (Rao, 1999).

Viscosity is the internal friction of a fluid or its tendency to resist flow. It is noted by the symbol η for Newtonian fluids whose viscosity does not depend on shear rate. It is defined as the proportional between shear stress and shear rate in units of Pas (Eq.3.1)

$$\tau = \eta . \gamma = \eta \frac{dv}{dr} = \eta \frac{dv}{dn} = \frac{F}{A} \qquad ; \text{ for Newtonian fluids in pipes (3.1)}$$

Newtonian flow behavior, the shear rate is directly proportional to the shear stress pass through the origin point.

Non-Newtonian flow behavior, the shear rate not pass through the origin point on arithmetic coordinates of shear stress and shear rate or no linear curve.

2.7.3 Fluid flow behavior

The main types of fluid flow behavior can described by means of function between shear rate and shear stress as shown in Table 2.8.From Table 2.8, by fitting the regression parameters in these given equations, the effective or apparent viscosities can calculated in the dependent on shear rate. The fluid that is independ on shear rate can be explained by Newton's equation. For the group of non-Newtonian fluids can be explained by Ostwald-de Waele was used for more than 95 % of cases. On the contrary, the hypothesis of Bingham-, Casson- and Herschel-Bulkley equations were used for the non-Newtonian materials (solid-like behavior). The parameters in these equations can be used to describe the structure degradation and also the flow behavior changing in processing. The appropriate equations were selected according to the number of parameters and the successful regression of flow curves. The less number of parameters and the high correlation between experimental data and model equation, i.e., the high correlation coefficient (r) and standard deviation (SD), would be the criteria of selections.

Table 2.3 Standard rheological models with defined viscosity

Model	Equation		Effective viscosity		Number of model parameters
	$\tau = f(\dot\gamma)$ in Pa		$\eta_{eff} = f(\dot\gamma)$ in Pas		
Newton	$\tau = \eta \cdot \dot\gamma^1$	(3.4)	$\eta_{dyn} \neq f(\dot\gamma)$		1
Ostwald-de Waele	$\tau = K \cdot \dot\gamma^n$	(3.5)	$\eta_{eff} = f(\dot\gamma) = K \cdot \dot\gamma^{n-1}$	(3.6)	2
			$\eta_{proz} = f(\dot\gamma) = n \cdot K \cdot \dot\gamma^{n-1}$	(3.7)	2
Herschel-Bulkley	$\tau = \tau_0 + K \cdot \dot\gamma^n$	(3.8)	$\eta_{eff} = \dfrac{\tau_0}{\dot\gamma} + K \cdot \dot\gamma^{n-1}$	(3.9)	3 Non-linear plastic
Bingham	$\tau = \tau_0 + \eta_{BH} \cdot \dot\gamma$	(3.10)	$\eta_{eff} = \dfrac{\tau_0}{\dot\gamma} + \eta_{BH}$	(3.11)	2 Linear plastic
Casson	$\tau = \sqrt{\tau_0} + \sqrt{\eta_{CA} \cdot \dot\gamma}$	(3.12)	$\eta_{eff} = \dfrac{\tau_0}{\dot\gamma} + \eta_{CA} + 2\sqrt{\tau_{0CA} \cdot \eta_{CA} / \dot\gamma}$	(3.13)	2 Non-linear plastic

Ref: Tipvarakarnkoon (2009)

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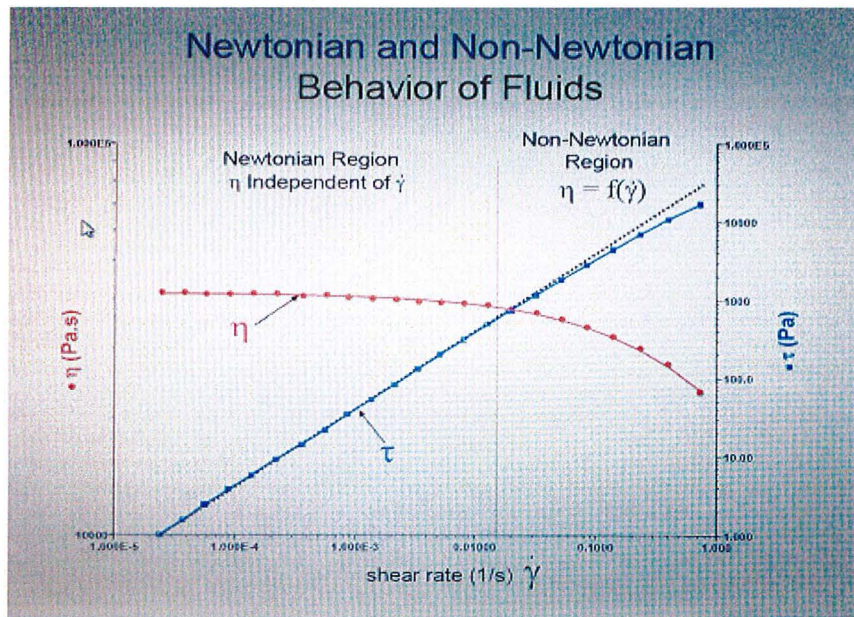
Figure 2.4 Newtonian and Non-Newtonian behavior of fluids

Ref: Somwangthanaroj (2010)

2.7.4 Viscoelasticity

Rheological behavior related to viscoelasticity is the most relevant for the description of a majority of real materials. Viscoelastic effects exist in Newtonian liquids (e.g., water) under special conditions of measurement, such as very high frequencies. The other extreme of viscoelastic effects is offered by the example of vibration damping in bells, due to "viscous" losses in metal. All material has viscoelastic properties. Viscoelastic behavior of many foods can be determined by means of oscillatory testing. Small amplitude oscillatory shear, also called dynamic rheological experiment, can be used (Rao, 1999). In the small amplitude oscillatory shear experiment, a sinusoidal oscillating stress or strain with a frequency f is applied to the material, and the phase different between the oscillating stress and strain, as well as the amplitude ratio, is measured. The information obtained should be equivalent to data from a transient experiment at time t = f^{-1} (Rao, 1999).

In an oscillatory test a food sample is subjected to a small sinusoidal oscillating strain or deformation γ (t) at time t according to Eq. 3.2 for evaluating the elastic component. Differentiation yields the next equation of Eq. 3.3, which shows the strain rate γ(t) for evaluating the viscous component to be - $\pi/2$ radians out of phase with the strain.

$$\tau = G \cdot \gamma + \eta \cdot \dot{\gamma} \qquad\qquad \text{; dominant in elastic component (3.14)}$$

$$\dot{\gamma} = \frac{\tau}{G} + \frac{\eta}{\dot{\gamma}} \qquad\qquad \text{; dominant in viscous component (3.15)}$$

The Elastic (Storage) Modulus: Measure of elasticity of material. The ability of the material to store energy. $G' = (stress^*/strain)\cos\Theta$

The Viscous (loss) Modulus: The ability of the material to dissipate energy. Energy lost as heat. $G'' = (stress^*/strain)\sin\Theta$

The storage modulus G' expresses the magnitude of the energy that is stored in the material or recoverable per cycle of deformation. G'' is a measure of the energy that is lost as viscous dissipation per cycle of deformation. It is noted that if G' is much greater than G'' the material will behave as solid-like or elastic. If the G'' is much greater than G' the material will behave as liquid-like. The dynamic oscillation tests can be used to obtain useful properties of viscoelastic foods as described as follows.

Amplitude sweeps. This study is to determine G' and G'' as a function of amplitude at a fixed frequency and temperature (in appendix A). The linear curve in a small oscillating regime expresses the microstructure of material which has no structural change within the studied range.

Frequency sweeps. This study is to determine G' and G'' as a function of frequency (f) at a fixed amplitude, especially in the linear regime, and temperature. Based on frequency sweeps, one can designate solid-like and liquid-like properties. For example, for dilute biopolymer solution, G'' is usually larger than G' which presents a liquid-like behavior. For concentrated biopolymer solution, G'' is usually larger than G' which presents a solid-like behavior.

Temperature sweeps. This study is to determine G' and G'' as a function of temperature at a fixed frequency and amplitude. This test is well suited for studying phase change transition or gelation during heating and cooling process.

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2.7.5 Yield stress

Yield stress (τ_0) is the minimum stress that must be applied to a sample in order to induce flow. This yield stress parameter refers to the material that exhibits particle micro-gel dispersion. This parameter is the criteria of the energy input of the mechanical forces in process. It describes the structural characteristic of the dispersion phase and the viscosity in each model as follows.

$\tau < \tau_0$ elastic behavior, $\tau = \tau_0$ solid-liquid transition, $\tau > \tau_0$ viscous behavior

3. MATERIALS AND METHODS

3.1 Preparation of emulsifies

3.1.1 Xanthan gum and Guar gum

Xanthan gum and Guar gum (Bronson and Jacobs International Co., Ltd.) was diluted to obtain 0.1, 0.3 and 0.5% wt/wt by distilled water before use. The solution was dispersed using magnetic stirrer for 2 hours before kept in refrigerator for overnight.

For gum mixtures, Xanthan and Guar gum powder were mixed before dispersing into distilled water at room temperature with continuous stirring using magnetic stirrer for 2 hours. The solution was then kept in refrigerator for overnight before use.

3.1.2 Tween 60

Tween 60 (Merck schuchardt OHG, Germany) was diluted to obtain 6% wt/wt by distilled water. Heated and stirred with stirring rod until it was changed to clear solution.

3.1.3 Coconut milk preparation

Fresh coconut milk without added water was purchased from a local market (Bangkapi market, Bangkok, Thailand). The fat content of coconut milk (32 ± 1.59%) was determined by Babcock method (adapted from AOAC Official Method 989.04, Fat in Raw Milk, 2006) and Fresh coconut milk was immediately preheated at temperature 80°-85°C for 5 minutes. Heat fresh coconut milk until 80°C before percolated preheat coconut milk using stainless steel sieve.

3.2 Preparation of stabilize coconut milk emulsion

Preheated coconut milks were homogenized using the rotor-stator homogenizer (IKA ULTRA-TURRAX® T18 basic, Germany) at 15,500 rpm for 2 minutes. Solution was also diluted by distilled water to obtain the fat concentrations of 20% respectively. 0.15% Tween60, 0.3% Sodium metabisulfite and 0.05% Sodium azide were added as emulsifier, antimicrobial and antibrowning effect prior homogenization. Homogenized samples were then mixed with xanthan or guar gum solutions at 0.05%, 0.1% and 0.15% then stirrer coconut milk emulsion by using magnetic stirrer for 15 min.

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To study the effect of mixing process, the mixtures were then homogenized at 15,500 rpm for 2 min by using rotor-stator homogenizer. After that, pour xanthan or guar gum solutions at 0.05%, 0.1% and 0.15% then mixtures were gently stirred for 15 min by using magnetic stirrer. Each sample was prepared for the same total amount of 200 g in 400 ml tall glass beaker. The effect of mixing method onto the stability of coconut milk emulsions was then investigated. All samples were then measured rheological properties, creaming index, optical microscopy and coarse oil droplet size determination. All samples were in at least duplicates.

To study the effect of gum mixtures in combination with heat treatment, gum mixtures (xanthan:guar) was used to stabilize coconut milk at various combinations (0:100, 15:85, 50:50, 85:15, 100:0) and at different concentrations (0.05%, 0.1% and 0.15%). 80 ml of homogenized coconut milk were poured into 100 ml Duran bottles and sterilized at 121°C for 30 minutes by auto-clave sterilization. After sterilization, all samples were immediately cooled down to approximately 50°C by shaking in room temperature water. Both non-sterilized and sterilized coconut milks were then measured creaming index, optical microscopy and coarse oil droplet size determination. Only sterilized coconut milks were also investigated on rheological properties and particle size distribution. All samples were in at least 3 replicates. The determination of creaming index, rheological properties and optical microscopy were done.

(a)

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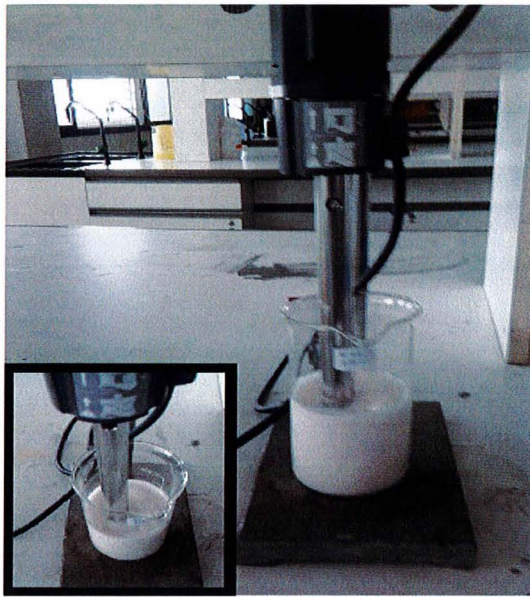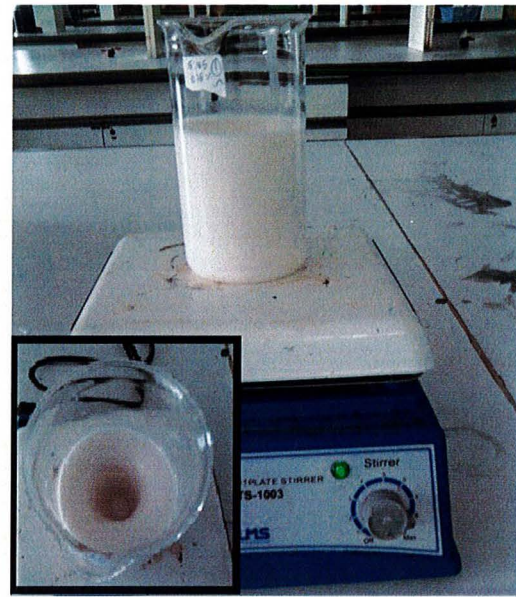
<center>(b)   (c)</center>

Figure 3.1 Prepared coconut milk emulsion; (a) pre-heat coconut milk in water-bath, (b) homogenizing coconut milk emulsion by using the rotor-stator homogenizer, and (c) stirring coconut milk emulsion by using magnetic stirrer.

3.3 Creaming Stability Measurements

Transferred 10 ml of emulsion sample into a test tube (with 15 mm internal diameter and 125 mm height), tightly sealed with a plastic cap. Stored emulsion sample in temperature; 30°C, 20°C, and 5°C for 7 days. After day pass, a number of emulsions separated into two parts were an opaque (cream) layer at the top and a turbid or transparent (serum) layer at the bottom. Total height of the emulsions in the tubes (Ho) and the height of the serum layer ($H1$) were measured in everyday for 7 days. The extent of creaming was characterized by a creaming index from formula percentage of creaming index = 100* ($H1/Ho$). Creaming index provided indirect information about the extent of droplet aggregation in an emulsion. For example, higher creaming index, faster the droplets move, therefore more droplet aggregation has occurred.

3.4 Optical microscopy

Coconut milk emulsions were diluted by distilled water at ratio 1:20 and gently agitated in a glass test tube by vortex mixer immediately before each analysis to prevent too much crowed of oil droplets. Drop of dilution into a microscope glass slide then cover it with cover slip. The emulsion structure was observed and taken the photo by using a

<center>17</center>

microscope Eye-piece camera (AM 423X, ANMO Electronic Corporation, Taiwan) at connected with a digital microscope (Nikon microscope YS2-H, Nikon Corporation, Japan). The magnification is at 1200x (30x from eyepiece magnification with a 40x objective lens). The images of the emulsion were then analyzed by a Dino Capture 2.0 Software (DinoCapture 2.0, Version 1.2.7) installed on a computer. At least 5 photos of oil droplets were taken from each coconut milk emulsion samples. All samples were done at least in duplicates. For each photo, 10 individual oil droplets were then measured coarse oil droplet size by randomly selection.

3.5 Particle Size Determination

To determine the particle size, the emulsions were examined with a laser diffraction particle analyzer (Mastersizer 2000; Malvern instruments, Malvern, UK) using the known refractive index of 1.333 for water and of 1.46 for coconut oil. In process sample were gently dispersed in de-ionized water untilled the obscuration to 20%. The specific volume mean diameter $d_{4,3}$, specific surface mean diameter $d_{3,2}$ and specific surface area (SSA) were calculated by the internal Mavern software with known density of fresh coconut milk was 0.98305 g/cm^3. The reproducibility of the droplet size determination was tested by measuring the particle size of three equally prepared emulsions.
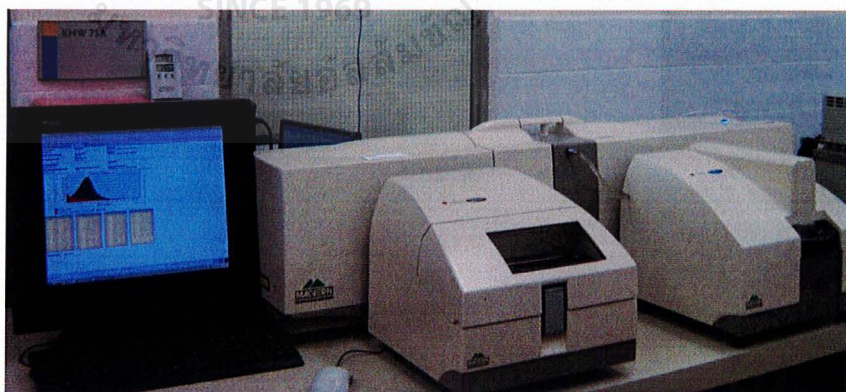
Figure 3.2 Malvern Laser diffraction particle size analyzer – Mastersizer 2000
Ref: www.ryerson.ca (2011)

3.6 Rheological measurement of coconut oil samples: Steady state shear examination

3.6.1 Steady state shear examination of Xanthan gum solution, Guar gum solution and Tween solution

Viscosity was measured as a function of shear rate using a MCR301 Rheometer (Physica®, Anton Paar GmbH, Graz, Austria-Europe) equipped with a double gap rotational cylinder DG26.7 and Peltier Cylinder temperature system TEZ150P. 8 ml sample were taken with the controlling temperature at 20°C. Shear rate examination profile was given. The data was collected every 4 sec at each state measurement.

Upward curve; $100 < \dot{\gamma} < 500$ /s $\quad$ t = 120 s 30 points

Downward curve; $500 < \dot{\gamma} < 100$ /s $\quad$ t = 120 s 30 points

All data from the last state were calculated using a linear model. The flow behavior of studied solutions was explained according to Newton's equation.

3.6.2 Steady state shear examination of fresh coconut milk and coconut milk emulsions

Viscosity was measured as a function of shear rate using a MCR301 Rheometer (Physica®, Anton Paar GmbH, Graz, Austria-Europe) equipped with a equipped with a Z3 DIN rotational cylinder and Peltier Cylinder temperature system TEZ150P. 8 ml sample were taken with the controlling temperature at 30°C. Shear rate examination profile was given. The data was collected every 4 sec at each state measurement.

Upward curve; $\quad 0.1 < \dot{\gamma} < 100$ /s $\quad$ t = 60 s $\quad$ 20 points

Constant; $\quad \dot{\gamma} = 100$ /s $\quad$ t = 60 s $\quad$ 20 points

Downward curve; $\quad 100 < \dot{\gamma} < 0.1$ /s $\quad$ t = 60 s $\quad$ 20 points

All data from the last state were calculated using the effective viscosity using a linear and power law model at shear rate of 100 /s. The flow behavior of studied solutions was explained according to Ostwald de-Wale and Herschel-Bulkley's equations.

3.6.3 Oscillation test of fresh coconut milk and coconut milk emulsions

Viscoelastic properties were measured by determining the storage (G′) and loss (G″) moduli as functions of amplitude or frequency. All gum solutions were measured by controlling temperature at 30 °C for 5 min before each measurement. All measurements were done at least in duplicate. Amplitude sweep was done by varying the strain rate or deformation from 0.001 to 1 at a constant frequency, $f = 1$ Hz. The storage (G′) and loss (G″) moduli and tan δ, were plotted against strain rate. Frequency sweep was done at a constant strain rate of 0.001 in the frequency range from 0.1 to 50 Hz. The storage (G′) and loss (G″) moduli, as well as tan δ, were plotted against frequency (Hz).

Figure 3.3 The MCR 301 Rheometer for shear rate examination profile

3.7 Analysis of fat content by Babcock Method (adapted from AOAC Official Method 989.04, Fat in Raw Milk, 2006)

The temperature of fresh coconut milk was adjusted in immediately in water bath to 38±1˚C. Then transfer 8.8±0.05 ml warm coconut milk into glass bottle. Ammonia solution was added into prepare coconut milk for 3 ml and then added 4 ml of N-Butyl alcohol and mix it well. Slowly added 8.8 ±0.05 ml Conc. Sulfuric acid into prepared coconut milk and mix it then, centrifuged the bottle at 500 rpm for 5 minutes.

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Add distilled water at room temperature into Babcock bottle to the first level of neck and then transferred serum/top layer (oil/golden yellow color) into Babcock bottle by using glass dropper.

4. RESULTS AND DISCUSSION

4.1 Rheological properties of gum solutions

In comparison, guar gum solution gave lower consistency index (k), higher flow index (n) and lower effective viscosities. Distinctively, xanthan gum solutions used in this studied gave markedly low flow index which related to high structural stability under shear, compared to other literature (Tipvarakarnkoon, 2009). When mixed at 50:50, a gum mixture induced relatively high viscosities with high k and low n values. The synergistic effect occurred to give the result of high value on viscosity (Table 4.1 and Figure. 4.1).

Table 4.1 Rheological parameters of gum solutions

Sample (xanthan:guar)	Ostwald-de Waele				Herschel-Bulkley					A_{TH}
	K	n	r	η_{eff} (100/s)	τ_0	K	n	r	η_{eff} (100/s)	Pa/s
	Pas^n	-	-	mPas	Pa	Pas^n	-	-	mPas	
Guar 0.1%	0.007	0.880	0.975	3.90	0.005	0.004	1.027	0.998	4.32	0.00
Guar 0.3 %	0.058	0.846	0.987	26.81	0.000	0.068	0.792	0.997	25.79	1.13
Xanthan 0.1%	0.184	0.458	1.000	15.05	0.004	0.183	0.458	1.000	15.09	0.00
Xanthan 0.3%	1.635	0.258	1.000	51.26	0.031	1.655	0.244	1.000	51.27	0.00
0.1% (50:50)	0.088	0.561	0.999	11.34	0.011	0.074	0.597	1.000	11.61	0.05
0.3% (50:50)	0.943	0.375	1.000	52.47	0.042	0.896	0.384	1.000	52.70	6.85

For valid parameters, their standard deviations (4 replicates) are ±0.005-0.2 (K), ±0.01-0.2 (n), and ± 0.9-6.80($\eta_{eff\,(100/s)}$)
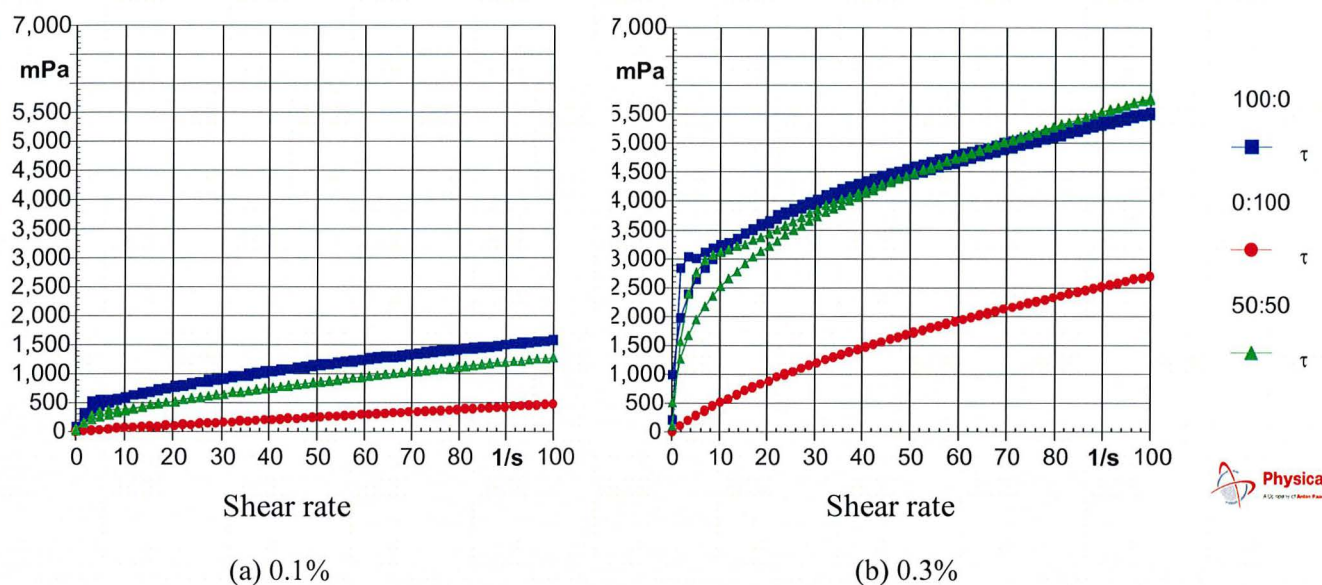
Figure 4.1 Flow curve of gum solutions (xanthan:guar) at (a) 0.1% w/w and (b) 0.3% w/w

The viscosity is related to shear rate and shear stress, so it is possible to operate a viscometer in one or two modes (Bell, 1993). Firstly, by applying a rotation and measuring the torque generated (controlled stain), or secondly, by applying a fixed torque and measuring the speed generated (controlled stress). Due to its exceptional rheological properties, xanthan gum is widely used as an effective stabilizer or a suitable thickener for various kinds of water-based systems. Its numerous areas of application cover a broad range including food, pharmaceutical, cosmetic, agricultural, textile, ceramic, and petroleum industries (Kang and Pettit, 1993; Schott, 1990; Garcia-Ochoa et al., 2000). The most important rheological properties of xanthan gum are high viscosity at low shear rates, pronounced shear-thinning nature, and good resistance to shear degradation. Xanthan gave the lowest flow index (n) value therefore induced high structural stability. It results from its rigid rod-like conformation in solution and its side chain. The side chains of xanthan wrap around the cellulose backbone thereby protecting the unstable β - (1→4) linkages from attack, in the solution. At 25 °C of distilled water, the backbone of xanthan was disordered or partly ordered (helical) in the form of a randomly broken helix but highly extended due to the electrostatic repulsions from the charged groups on the side chains. At high viscous solution under non-covalent bonding, principally hydrogen bond was found. Furthermore, xanthan was able to particulate or form intermolecular associations in solutions that resulted in the formation of a complex network of weakly bound molecules and able to form time independent interactions. Therefore, this

formation of a weak network resulted in yield-point values which induced the ability to stabilize emulsions and dispersions.

From Table 2.1, rheological parameters of gum solutions, it suggested that guar gum had high n value than xanhan gum. Guar gum exhibited high viscous non-Newtonian behavior and (Casas, Mohedano, García-Ochoa, (2000)) showed pseudoplastic behavior. More side chain in guar gum which conform a disordered more extended random coil after dissolution in water (Malkin, 1994). In aqueous solution, guar immobilizes water and forms the artificial network which causes the strong hydrogen bonding within. For xanthan gum and guar gum mixing gave low flow behavior and also high effective viscosity. Therefore, xanthan gum and guar gum mixing gave properties tend to xanthan gum.

As shown in Fig. 4.2, xanthan gum solution showed semi-solid behavior with high value of G′ (storage modulus), On the other hand, guar gum solution showed semi-liquid behavior with lower G′ and high G″ (loss modulus). The xanthan gum and guar gum mixture (50:50) showed properties closed to xanthan gum solution which was tend to have high G′. High value of G′ stated the strong structure under shear which would gave a good tendency of emulsion stability when added to coconut milk emulsion.

(a)

(b)
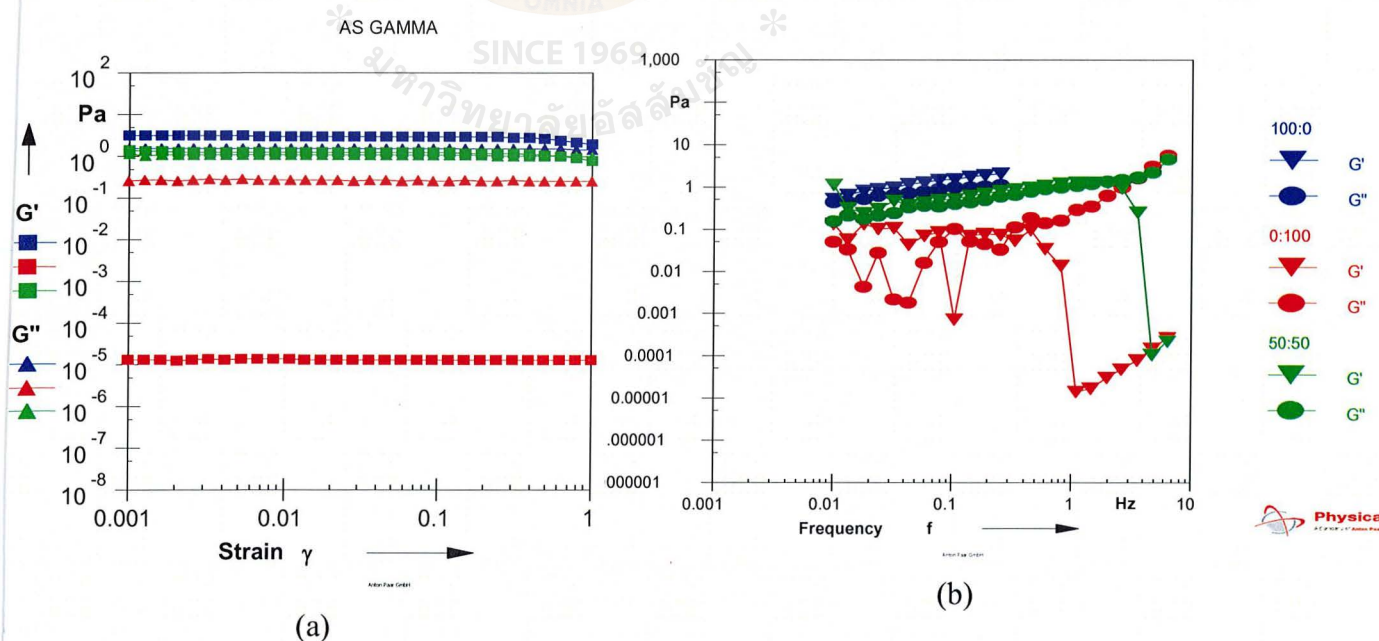
Figure 4.2: Amplitude sweep (a) and frequency sweep (b) curve of gum solutions (xanthan:guar) at 0.3% w/w

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4.2 Comparison between mixing methods on emulsion stability of stabilized coconut milks

To study the effect of mixing process, homogenized sample were then added the gum solution by two different methods of (A) homogenization and (B) agitation using magnetic stirrer. The results showed that using agitation method, coconut milk emulsions containing gum higher than 0.1% showed slightly increased effective viscosities and lower n values (Table 4.2). This indicated that agitation method would give better result in creaming behavior. However, the increasing was too diminishing. Nevertheless, agitation method would make lower power consumption.

Table 4.2 Rheological parameters of coconut milk emulsions containing gums prepared by different mixing methods of gums

Mixing methods	Ostwald-de Waele				Herschel-Bulkley					A_{TH}
	K	n	r	η_{eff}	τ_0	K	n	r	η_{eff}	Pa/s
	Pas^n	-	-	(100/s)	Pa	Pas^n	-	-	(100/s)	
				mPas					mPas	
Homogenization										
0.05%XG	0.733	0.493	0.999	71.57	0.080	0.637	0.526	1.000	72.878	19.00
0.10% XG	0.946	0.470	1.000	77.48	0.00	0.923	0.469	1.000	77.351	16.65
0.15%XG	1.848	0.401	0.999	113.80	0.064	2.035	0.393	1.000	124.392	43.44
Agitation (stir)										
0.05%XG	0.537	0.504	0.998	50.80	0.082	0.442	0.542	1.000	52.013	17.91
0.10% XG	1.000	0.462	1.000	78.93	0.046	0.992	0.458	1.000	79.233	14.90
0.15%XG	2.062	0.383	1.000	119.02	0.00	2.115	0.681	1.000	928.289	32.13
Homogenization										
0.05%GG	0.140	0.663	0.992	26.69	0.025	0.107	0.776	1.000	28.184	11.79
0.10% GG	0.124	0.729	0.998	30.88	0.014	0.103	0.775	1.000	31.884	7.92
0.15%GG	0.242	0.733	0.996	57.95	0.036	0.202	0.742	1.000	58.508	19.10
Agitation (stir)										
0.05%GG	0.198	0.597	0.991	26.30	0.058	0.129	0.699	1.000	27.985	10.03
0.10% GG	0.346	0.606	0.992	46.16	0.096	0.232	0.689	0.999	48.755	46.59
0.15%GG	0.315	0.649	0.995	57.44	0.064	0.233	0.710	0.999	59.827	26.16

For valid parameters, their standard deviations (4 replicates) are ±0.005-0.2 (K), ±0.01-0.2 (n), and ± 0.9-6.80(η_{eff} (100/s))

For microstructural point of view, coconut milk emulsions prepared by agitation method induced higher G′, which is desired for the emulsion tends to be more stabilize (Fig. 4.3). Therefore, the agitation method for mixing gum solution onto coconut milk emulsion would be suggested to be more appropriate than homogenization method.
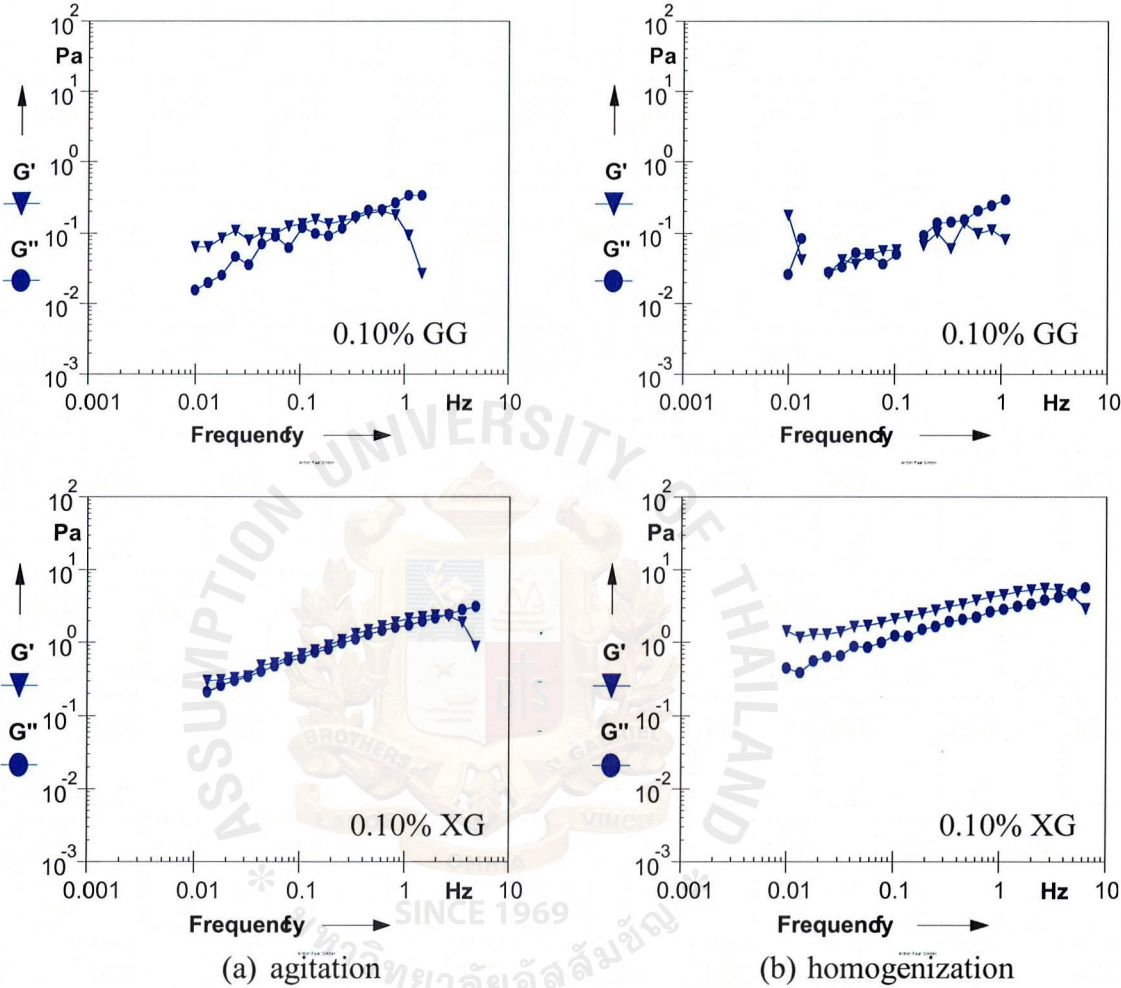
Figure 4.3 Frequency sweep curves of coconut milk emulsions containing guar (GG) or xanthan gum (XG) by different mixing methods of gums: (a) agitation and (b) homogenization

For non-sterilized coconut milk emulsions, samples prepared by agitation method seem to have smaller coarse oil droplet diameters than that of prepared by homogenized samples. However, when the sterilization process was applied, the diameter has been increased and the differences between these two methods then cannot be seen (Table 4.3 and Fig. 4.4).

Table 4.3 Coarse oil droplet diameter (μm) of coconut milk emulsions containing gums by different mixing methods of gums

Mixing methods	Agitation (stir)		Homogenization	
	Non-sterilization	Sterilization	Non-sterilization	Sterilization
0.05%Xanthan gum	7.50 ± 2.39	8.52 ± 2.86	8.47 ± 3.81	8.89 ± 4.10
0.10% Xanthan gum	6.52 ± 2.65	7.24 ± 3.21	7.53 ± 2.71	9.63 ± 4.89
0.15% Xanthan gum	8.57 ± 3.15	7.43 ± 2.58	8.46 ± 2.88	8.39 ± 2.74
0.05% Guar gum	7.98 ± 2.27	8.99 ± 2.88	11.04 ± 4.47	8.60 ± 3.41
0.10% Guar gum	8.52 ± 2.19	9.39 ± 3.32	8.94 ± 2.76	7.39 ± 2.59
0.15% Guar gum	7.06 ± 2.17	8.24 ± 1.96	8.68 ± 3.13	6.64 ± 2.56

From the experiment, coarse oil droplet diameter was compared between agitation (stir) and homogenization methods at non-sterilization and sterilization. The results in Table 4.3 showed that most of coarse oil droplet diameter had a similar diameter around 7 or 8 μm. Coarse oil droplet diameter by agitation (stir) gave smaller coarse oil droplet diameter than those made of homogenization process.

0.15% guar gum   0.15% guar gum

0.15% xanthan gum   0.15% xanthan gum

(a) agitation   (b) homogenization
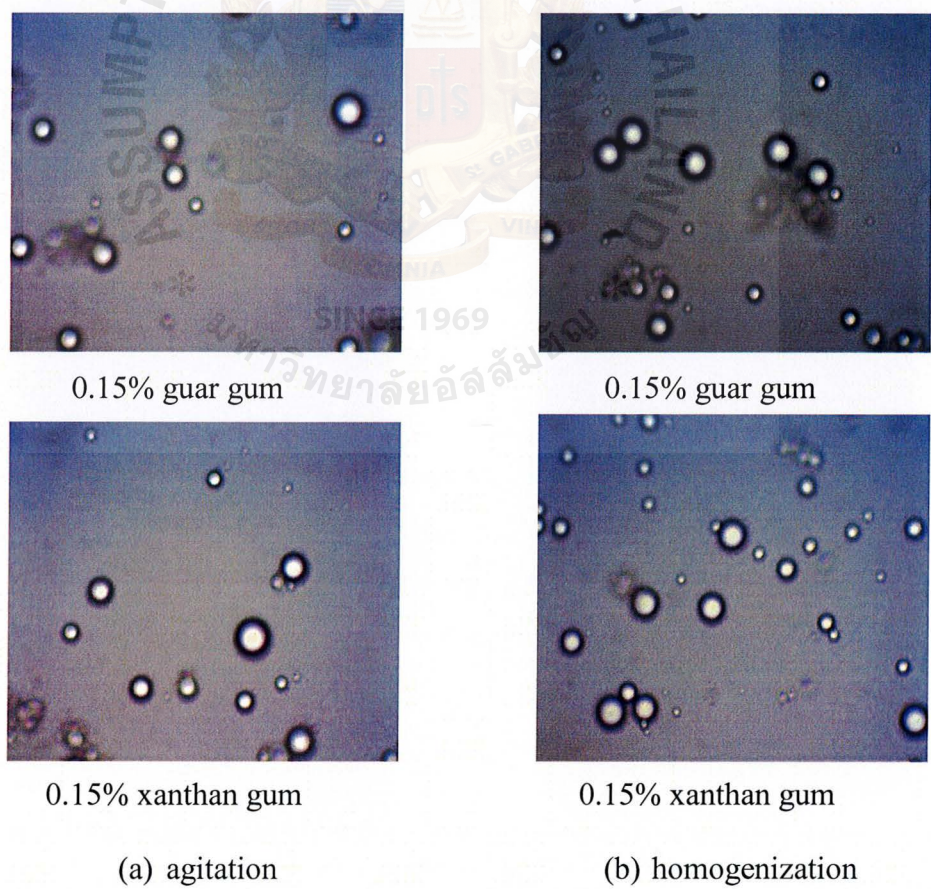
Figure 4.4 Micrographs of sterilized coconut milk emulsions containing 0.15% gums prepared by different mixing methods of gums

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For example, at 0.10% xanthan gum agitation(stir) both non-sterilization and sterilization give smaller coarse oil droplet diameter in order $6.52 \pm 2.65\mu m$ and $7.24 \pm 3.21\mu m$, while homogenization give $7.53 \pm 2.71\mu m$ and $9.63 \pm 4.89\mu m$ respectively. As expected, by increasing the concentrations of gum in emulsions can reduce the coarse oil droplet size however, the effect was rather small (Fig. 4.4). Small coarse oil droplet diameter is better than big because it gives better stability.

Considering creaming index, higher in creaming index value refers to larger phase separation of cream layer in an emulsion. As shown in Table 4.4 and Fig 4.5 – 4.8, the emulsions prepared by agitation and homogenized methods showed relatively the same creaming profile during storage. The differences can be seen on the sample prepared by agitation method which gave lower creaming index especially at storage day 7. Compare between non-sterilization and sterilization, found that sterilization gave lower creaming index than non-sterilization

In this experiment, all samples were kept in various temperatures at 6ºC, 20ºC and 30ºC. From the Figure 4.8 – 4.15, found that, at low temperature give lower separate phase of creaming index as show in figure of the test tube above. Moreover, at higher percentage of each sample also give lower creaming index. Therefore, at lower temperature give better lower separate phase of creaming index than higher temperature.

Table 4.4 Creaming index compare between homogenization and agitation

| Sample		Creaming index (%)			
		Non-sterilization		Sterilization	
		Day 1	Day 7	Day 1	Day 7
0.05%					
Homo	0:100	52.00±6.62	61.59±3.79	21.89±7.47	34.36±7.90
	100:0	10.06±9.28	25.30±17.29	6.00±2.18	20.85±7.13
Agitation	0:100	49.06±13.36	58.05±7.62	25.10±7.06	33.70±10.94
	100:0	5.67±4.94	18.83±6.29	3.84±5.38	24.08±10.32
0.10%					
Homo	0:100	47.76±8.08	59.59±2.91	24.02±1.34	36.04±7.79
	100:0	0.00±0.00	5.12±1.65	0.00±0.00	6.80±5.29
Agitation	0:100	50.22±11.38	59.09±4.27	25.59±9.29	47.55±8.40
	100:0	0.00±0.00	1.58±1.70	0.85±1.34	11.43±5.62
0.15%					
Homo	0:100	38.37±16.39	55.92±6.10	21.53±3.84	39.66±3.43
	100:0	0.00±0.00	0.00±0	0.00±0.00	4.37±6.77
Agitation	0:100	43.07±20.27	56.25±8.76	10.78±2.79	39.16±6.26
	100:0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

(a) non-sterilization (b) sterilization
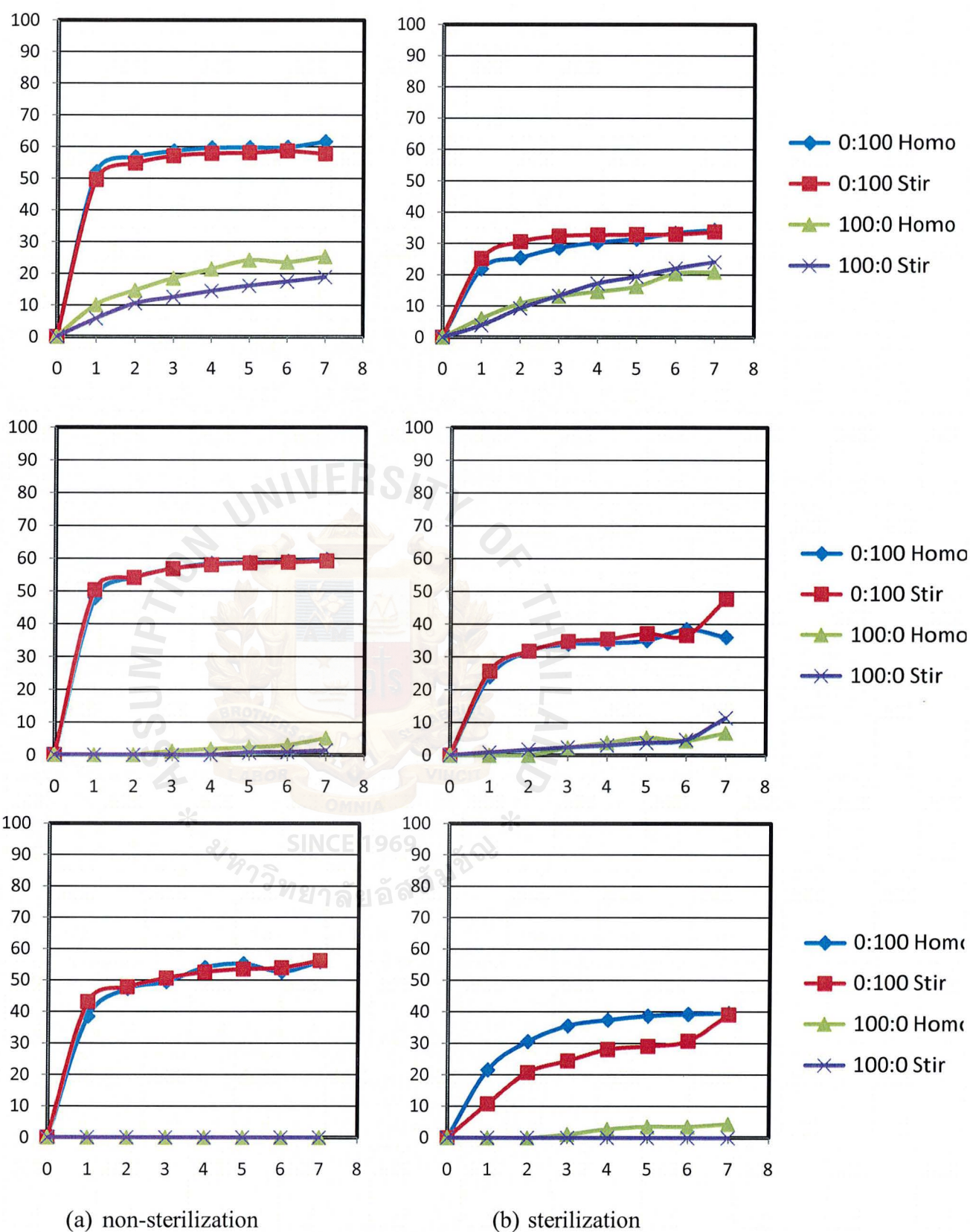
Figure 4.5 Creaming index of coconut milk emulsions containing gums (xanthan:guar) stored at 30°C prepared by different mixing methods of gums.

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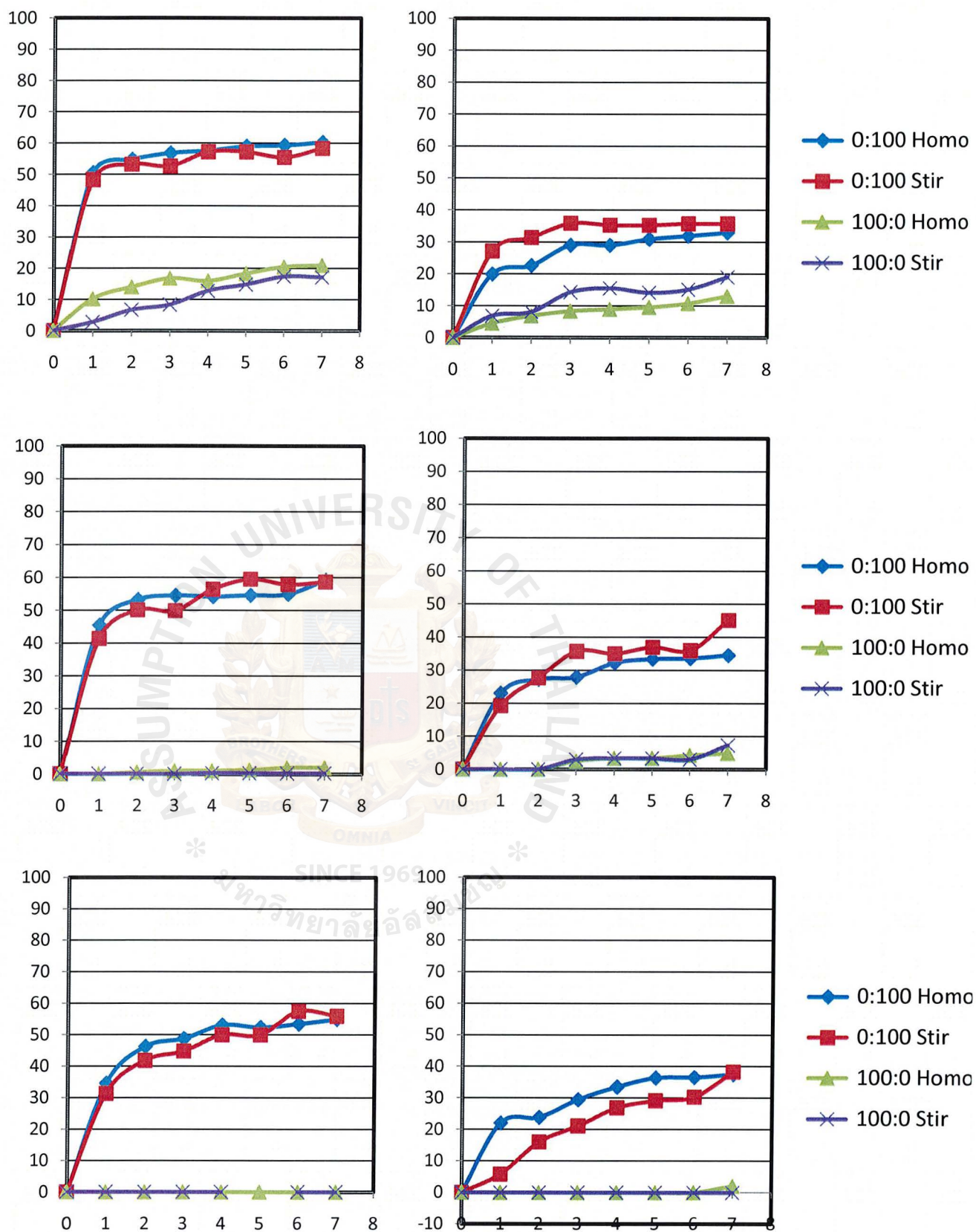
Figure 4.6 Creaming index of coconut milk emulsions containing gums (xanthan:guar) stored at 20°C prepared by different mixing methods of gums.
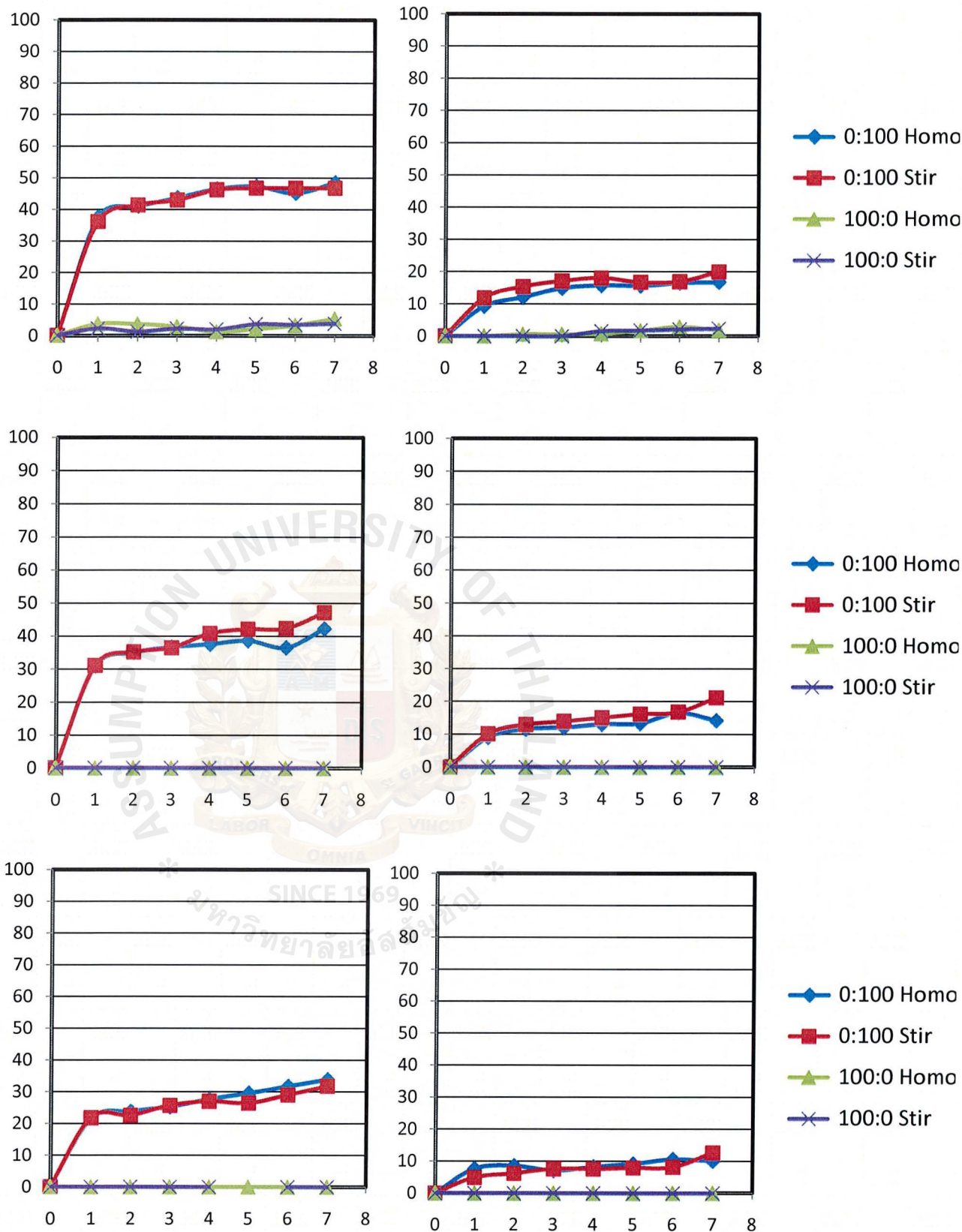
Figure 4.7 Creaming index of coconut milk emulsions containing gums (xanthan:guar) stored at 6°C prepared by different mixing methods of gums

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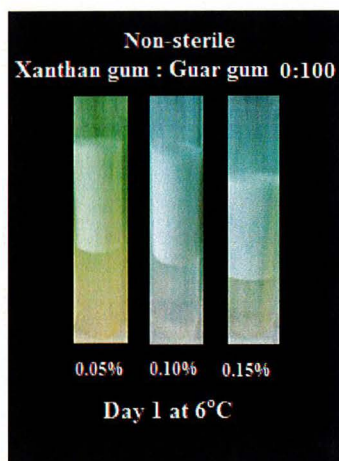
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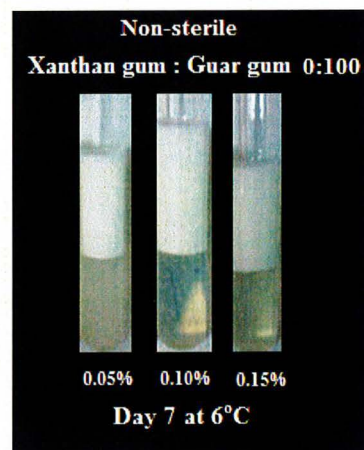
(b)

(c)
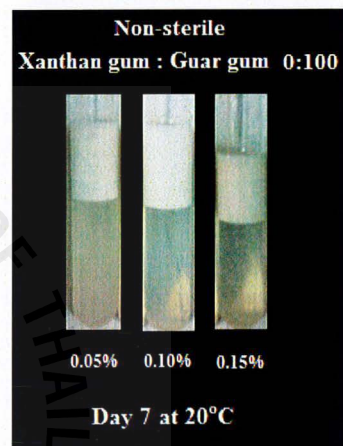
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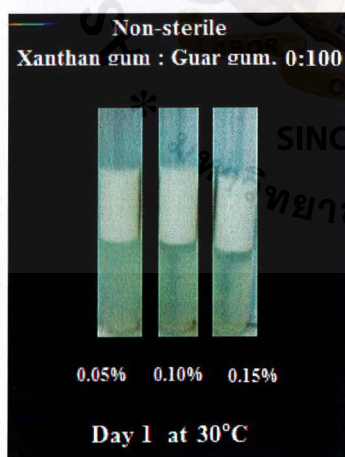
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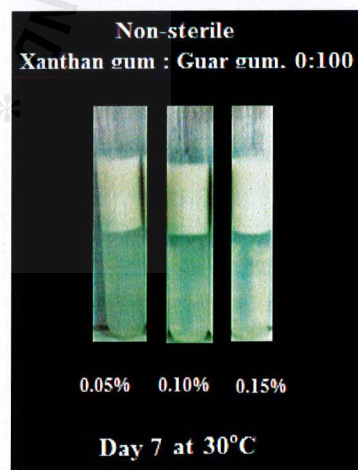
(f)

Figure 4.8 Non-sterilization of (0:100) Xanthan gum: Guar gum in stabilized coconut milk emulsion (20% fat) stored of day 1 at 6°C (a), 20°C (c), 30°C (e), and day 7 at 6°C (b), 20°C (d), and 30°C (f) by homogenization method
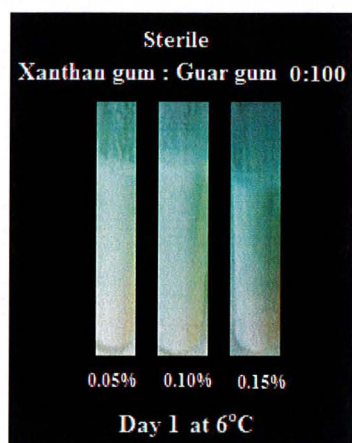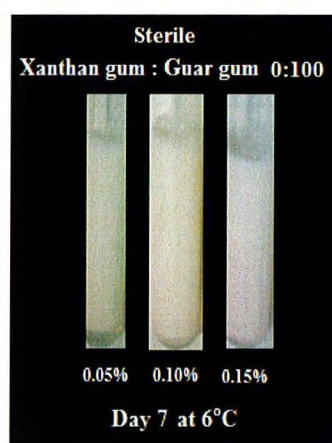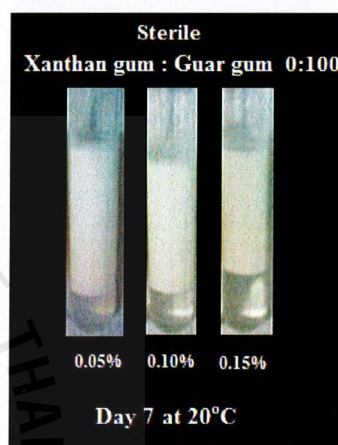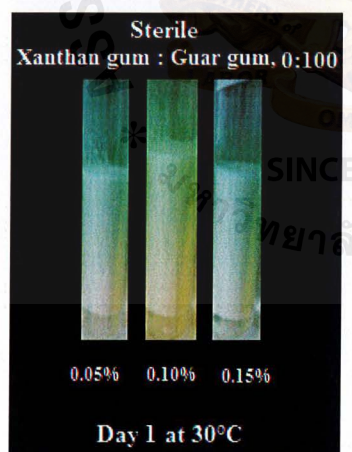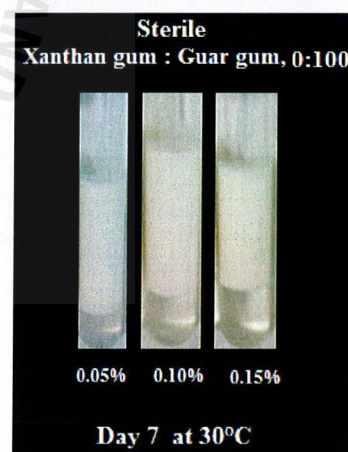
Figure 4.9 Sterilization of (0:100) Xanthan gum: Guar gum in stabilized coconut milk emulsion (20% fat) stored of day 1 at 6°C (a), 20°C (c), 30°C (e), and day 7 at 6°C (b), 20°C (d), and 30°C (f) by homogenization method

Figure 4.10 Non-sterilization of (100:0) Xanthan gum: Guar gum in stabilized coconut milk emulsion (20% fat) stored of day 1 at 6°C (a), 20°C (c), 30°C (e), and day 7 at 6°C (b), 20°C (d), and 30°C (f) by homogenization method

34
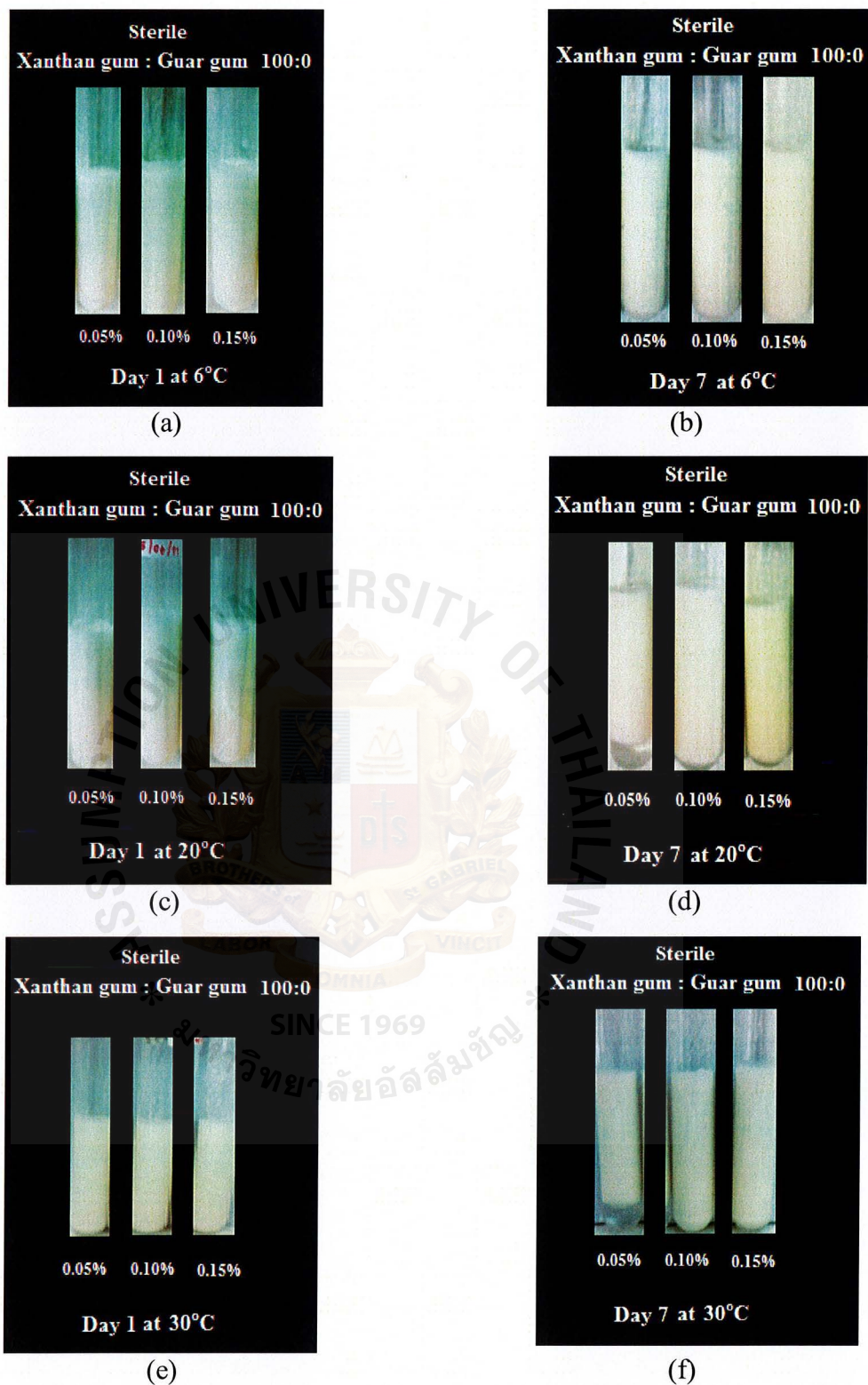
Figure 4.11 Sterilization of (100:0) Xanthan gum: Guar gum in stabilized coconut milk emulsion (20% fat) stored of day 1 at 6°C (a), 20°C (c), 30°C (e), and day 7 at 6°C (b), 20°C (d), and 30°C (f) by homogenization method
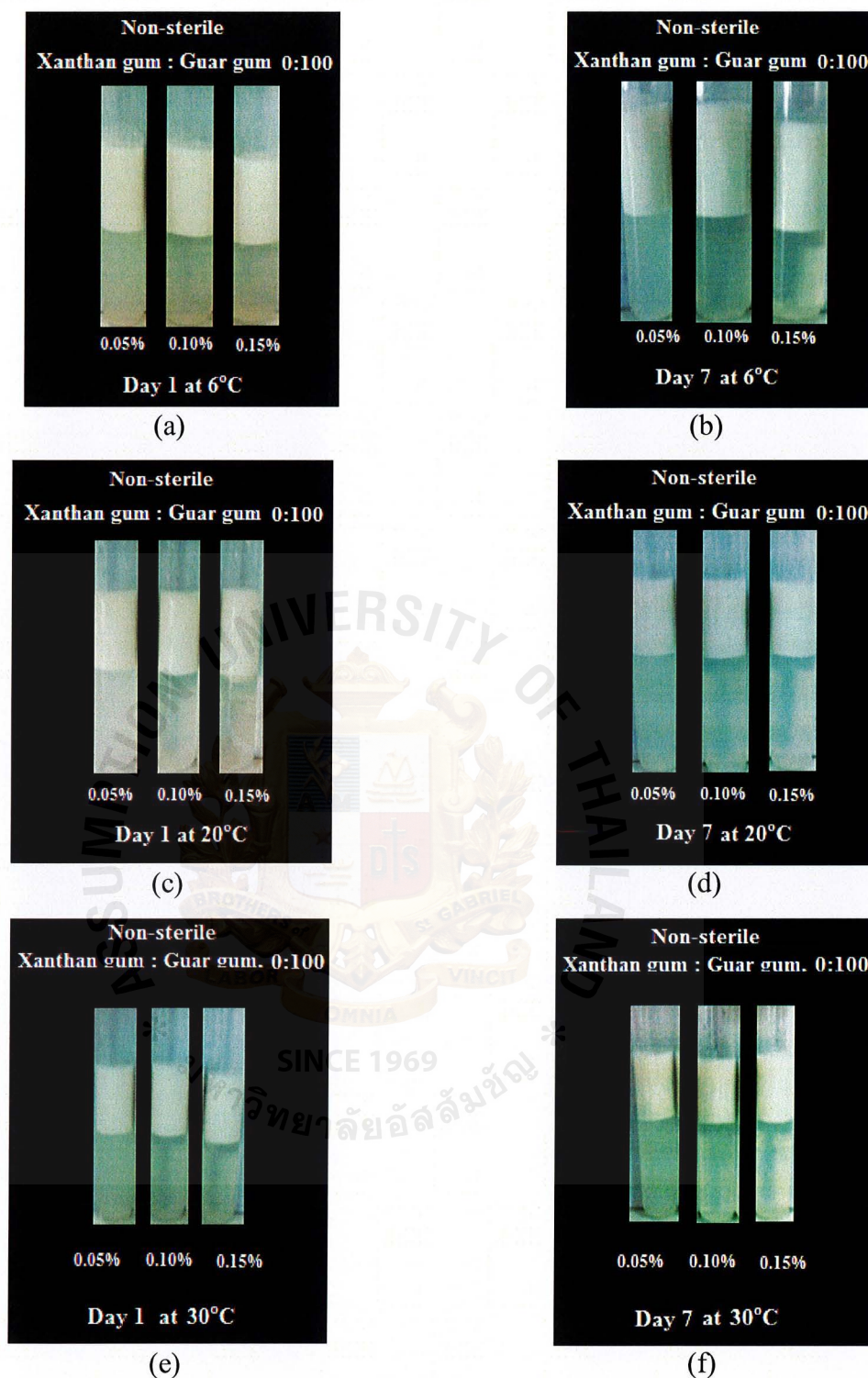
Figure 4.12 Non-sterilization of (0:100) Xanthan gum: Guar gum in stabilized coconut milk emulsion (20% fat) stored of day 1 at 6°C (a), 20°C (c), 30°C (e), and day 7 at 6°C (b), 20°C (d), and 30°C (f) by agitation method
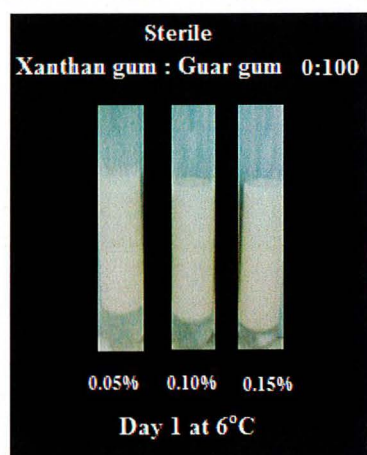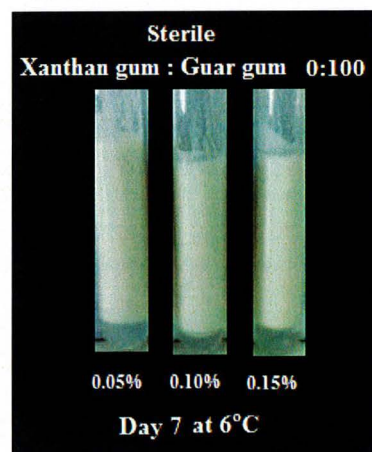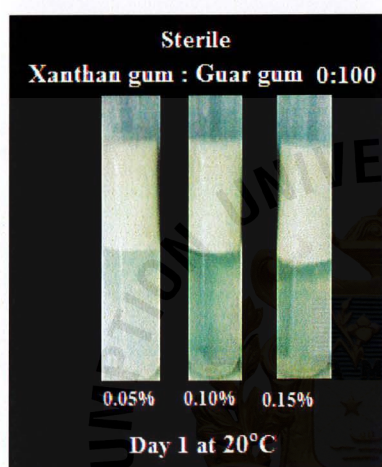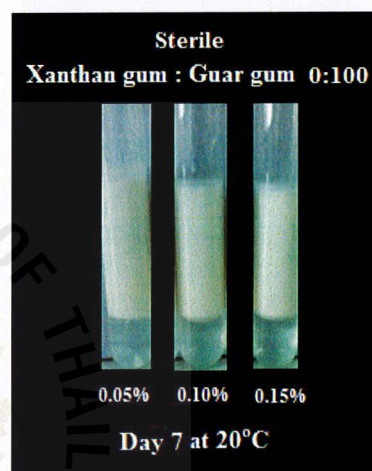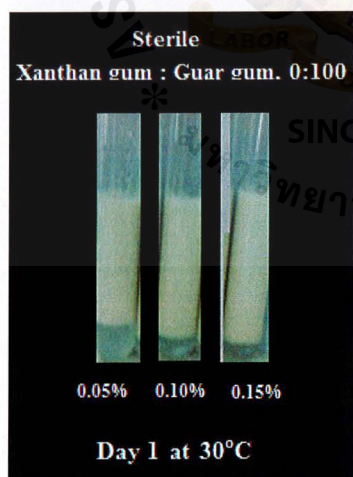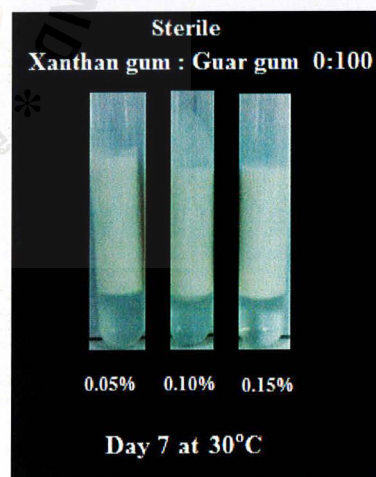
Figure 4.13 Sterilization of (0:100) Xanthan gum: Guar gum in stabilized coconut milk emulsion (20% fat) stored of day 1 at 6°C (a), 20°C (c), 30°C (e), and day 7 at 6°C (b), 20°C (d), and 30°C (f) by agitation method

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Figure 4.14 Non-sterilization of (100:0) Xanthan gum: Guar gum in stabilized coconut milk emulsion (20% fat) stored of day 1 at 6°C (a), 20°C (c), 30°C (e), and day 7 at 6°C (b), 20°C (d), and 30°C (f) by agitation method

Figure 4.15 Sterilization of (100:0) Xanthan gum: Guar gum in stabilized coconut milk emulsion (20% fat) stored of day 1 at 6°C (a), 20°C (c), 30°C (e), and day 7 at 6°C (b), 20°C (d), and 30°C (f) by agitation method

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4.3 Effect of gum concentrations on physicochemical properties of coconut milk emulsions

To study effect of gum concentrations on physicochemical properties of coconut milk emulsions. The results was shown in Table 4.5 found that, at higher concentrations gave lower n value and increasing in viscosity especially in 0.15% of all samples of mixing gum.

Table 4.5 Rheological parameters of coconut milk emulsions containing different gum solutions (xanthan:guar)

| Sample (xanthan: guar)	Ostwald-de Waele				Herschel-Bulkley					A_{TH}
	K	n	r	η_{eff}	τ_0	K	n	r	η_{eff}	Pa/s
	Pas^n	-	-	(100/s)	Pa	Pas^n	-	-	(100/s)	
				mPas					mPas	
0.05%										
0:100	0.198	0.597	0.991	26.30	0.058	0.129	0.699	1.000	27.985	10.03
15:85	0.215	0.593	0.995	33.04	0.044	0.159	0.665	1.000	9.613	3.85
50:50	0.369	0.569	0.997	45.96	0.065	0.289	0.620	1.000	10.740	13.09
85:15	0.538	0.489	0.997	50.87	0.097	0.425	0.541	1.000	12.630	10.45
100:0	0.537	0.504	0.998	50.80	0.082	0.442	0.542	1.000	52.013	17.91
0.10%										
0:100	0.346	0.606	0.992	46.16	0.096	0.232	0.689	0.999	48.755	46.59
15:85	0.354	0.585	0.995	50.78	0.070	0.265	0.655	0.999	14.545	16.15
50:50	0.629	0.517	0.998	67.11	0.090	0.520	0.560	1.000	12.554	23.19
85:15	1.035	0.475	0.999	90.81	0.099	0.920	0.485	1.000	10.278	29.66
100:0	1.000	0.462	1.000	78.93	0.046	0.992	0.458	1.000	79.233	14.90
0.15%										
0:100	0.315	0.649	0.995	57.44	0.064	0.233	0.710	0.999	59.827	26.16
15:85	0.471	0.582	0.996	67.21	0.090	0.357	0.648	1.000	18.343	29.30
50:50	0.941	0.502	0.999	92.74	0.101	0.819	0.531	1.000	12.419	32.85
85:15	1.566	0.438	1.000	114.37	0.065	1.505	0.443	1.000	5.398	37.07
100:0	2.062	0.383	1.000	119.02	0.00	2.115	0.681	1.000	928.289	32.13

Gums are used in foods primarily as thickeners and gelling agents as a result of their ability to alter the rheological properties of the solvent in which they are dissolved. The

change in viscosity occurs as a result of the high molecular weight polymeric nature of the gums and the interactions between polymer chains when gums are dissolved or dispersed (Yaseen, Herald, Aramouni, Alavi, 2004). As mentioned above, pure guar solution (0:100) gave high viscous non-Newtonian fluid behaviour, where xanthan solution showed semi-solid behavior When these two polysaccharides were mixed, the strong synergistic has been occurred more than expected (Tipvarakarnkoon, 2008). Guar gum has the general structure of galactomannans, some research found that the intermolecular binding mechanism between xanthan and galactomannans is still controversial, and different models have been proposed (KHOURYIEH, HERALD, ARAMOUNI, BEAN, ALAVI, 2007). The first model was proposed by Morris and others (1977) and Dea and others (1977), who suggested the intermolecular binding concept rather than mutual exclusion to explain the gelation mechanism between xanthan and galactomannans. The authors proposed that the synergistic interaction between xanthan and galactomannan is based on a cooperative interaction, depending on the fine structure of the galactomannan. The intermolecular binding involves binding of unsubstituted regions of the galactomannan to the ordered xanthan helical structure. McCleary (1979) modified the above model in order to explain the strong interaction between xanthan and certain high-galactose galactomannans. They suggested that the interaction involve the ordered xanthan and sequences along the mannan backbone where the galactosyl units arelocated on one side only. The 2nd model was proposed by Cairns and others (1986, 1987)in which intermolecular binding occurred between the disordered xanthan and galactomannans, and disordering of xanthan helical structure is necessary for gelation. They proposed that xanthan has a disordered, extended,2-fold, cellulose-like conformation, rather than a 5-fold helix, when interacting with galactomannan. Cheetham and others (1986) and Cheetham and Mashimba (1988, 1991) proposed that the interaction occurs between the disordered segments of the xanthan chains and galactomannan. The 3rd model was proposed by Tako and others (1984), Tako and Nakamura(1985) and Tako (1991) in which the intermolecular binding occurs between the side chains of xanthan in the helical form and backbone of the galactomannans. They suggested that the side chains of the xanthan are inserted into adjacent unsubstituted regions of the galactomannan backbone, which adopted an extended, 2-fold, ribbon-like conformation. From the result, show that the maximum yield point was observed at a ratio of 85:15. These results show the influence of structure would dominant by xanthan. When solutions contain xanthan more than 50 %, X/G mixtures showed yield point and

higher value was found compared to pure xanthan solution. A complex network formed which influenced by the xanthan conformation. Xanthan which highly extend in solution had entanglement with network interaction and would bind with guar. Hydrogen bonding between two or more molecules of xanthan by the side chain of guar may arise. At high concentration of xanthan, a formation of strong network interaction between xanthan/guar mixtures. The semi-solid with high degree of structural stability well represents for this system. However, at low concentration of xanthan, the hydrophilic interaction between xanthan/guar is less. Two or more molecules of guar gave only high viscous behavior which exhibited less n value however much higher viscosity. It can be assumed that only small amount of xanthan presented in mixtures have been binding with guar. Guar can form only weakly network between itself, highest internal friction would occur, results in lower n value. Xanthan/guar interaction molecules would immobilize water, results in higher in viscosity.
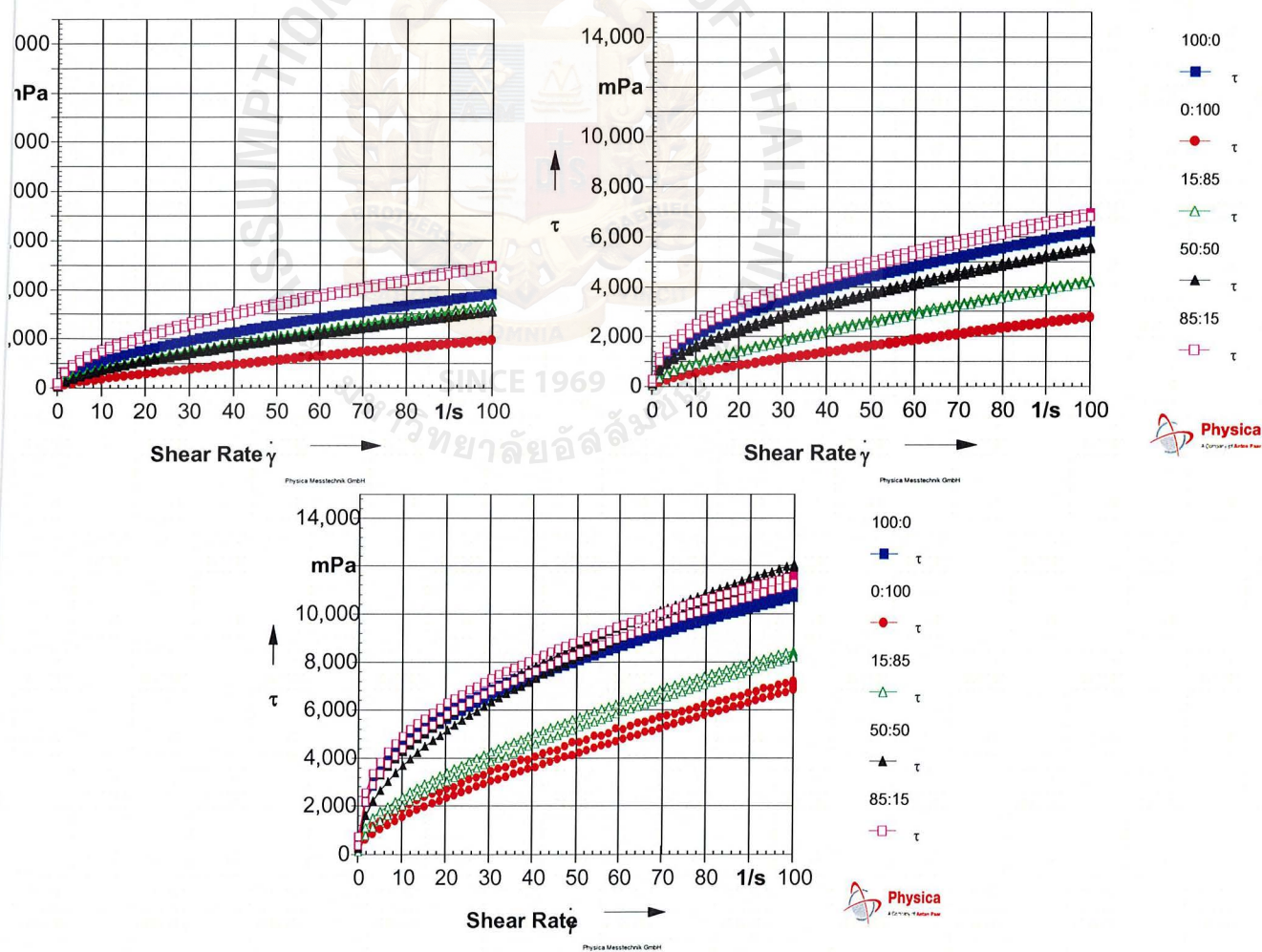
Figure 4.16 Flow curve of coconut milk emulsion containing mixed gum (xanthan:guar) at (a) 0.05% w/w, (b) 0.10% w/w, and (c) 0.15% w/w.

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Figure 4.17 Frequency sweep curve of coconut milk emulsion containing gum mixtures (xanthan:guar) at (a) 0.05% w/w, (b) 0.10% w/w, and (c) 0.15% w/w

The viscosity of gum solution was increasing gradually with increasing the concentrations of gum in water. In this part, mixing xanthan gum and guar gum to promote high solution viscosity and stability of coconut milk emulsion. The mixing gum were various to 0:100, 15:85, 50:50, 85:15 and 100:0 by xanthan:guar, it found that at higher percentage of xanthan gum are better than lower percentage of xanthan gum because it gave higher viscosity. From Table 4.6 showed that at high concentration of all samples of gum mixing gave higher viscosity especially in 85:15 and 100:0. From Fig 4.18 showed that at 85:15 give highest viscosity follow by 100:0, 50:50, 15:85 and 0:100 respectively. From Figure 4.19 showed the frequency sweep curve of coconut milk emulsion, clearly showed that, at higher concentration of all samples of mixing gum gave higher G'. It gave solid-like behavior and gave higher viscosity in coconut milk emulsion therefore that higher concentration is better than lower concentrations.

It is noted that if G' is much greater than G'' the materials will behave as solid-like or elastic. However, if the G'' is much greater than G' the material will behave as liquid-like. From the results, it showed that G' was higher than G'', therefore, coconut milk emulsion gave higher stability.

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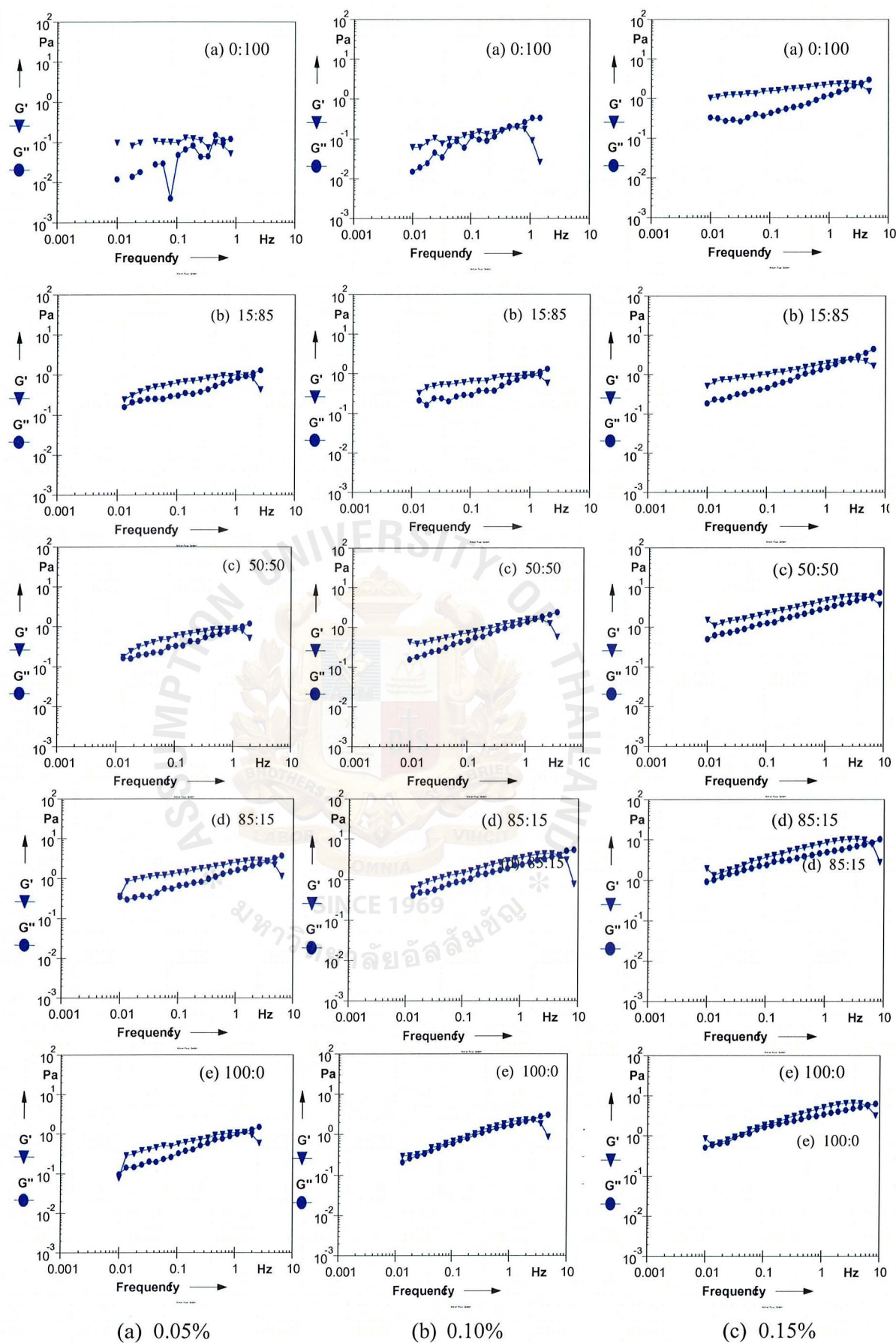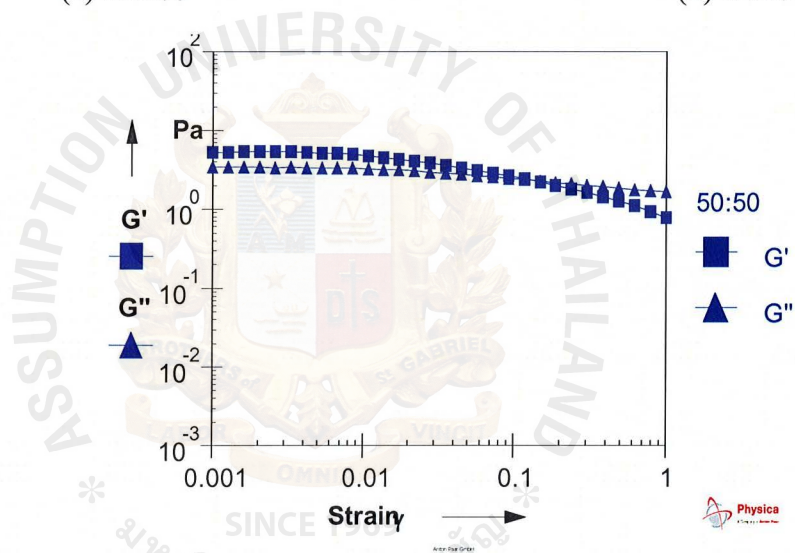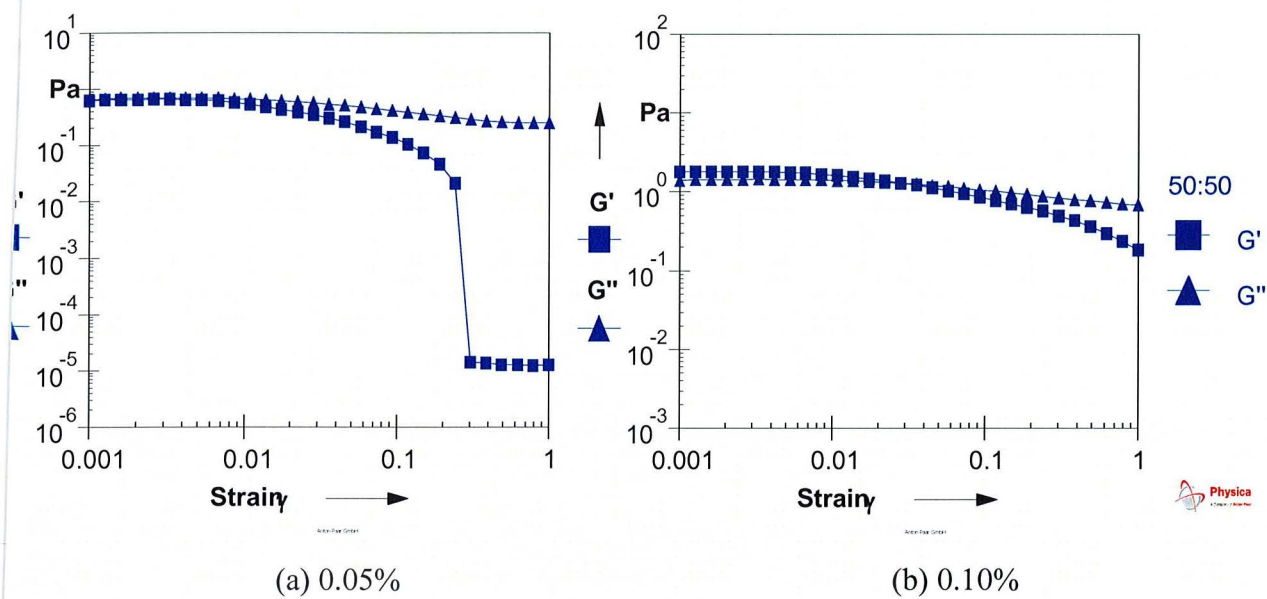(a) 0.05%

(b) 0.10%

Figure 4.18 Amplitude sweep curve of coconut milk emulsion containing gum mixtures
at a ratio of 50:50 (xanthan:guar) at (a) 0.05% w/w, (b) 0.10% w/w, and (c) 0.15% w/w

From Table 4.6, the results indicated that at higher of concentration give higher G' and G'' and also at higher percentage of xanthan gum gave higher G', and G' is higher than G''. It indicated that solution give semi-solid structure.

Table 4.6 G', G'' as a function of frequency at 1 Hz of coconut milk emulsion containing gum mixtures (xanthan:guar)

Xanthan: guar	0.05%		0.10%		0.15%	
	G'	G''	G'	G''	G'	G''
0:100	1.207	0.530	1.579	0.744	1.775	1.165
15:85	1.178	0.922	1.719	1.222	1.613	1.402
50:50	1.763	1.238	2.189	1.625	3.413	2.473
85:15	2.588	1.568	4.640	2.864	6.478	4.016
100:0	2.673	1.547	2.128	0.986	5.430	3.523

Viscoelastic properties were measured by determining the storage (G') and loss (G'') moduli as functions of amplitude or frequency. Viscoelastic "liquid" structure ($G' < G''$) and viscoelastic "solid" structure ($G' > G''$) It is noted that if G' is much greater than G'' the material will behave as solid-like or elastic. If the G'' is much greater than G' the material will behave as liquid-like. The G' and G'' increased as well when the concentrations increased.

From the results, it showed that where percentage of xanthan gum increased G' also increased, it's indicated the stronger hydrophilic interaction found in the solutions and give more stability of coconut milk emulsion. The semi-solid structure of xanthan was confirmed by frequency sweep measurements (Tipvarakarnkoon, 2009) where G' always much larger than G'' mean that at higher percentage of xanthan give higher G', and by parallel rather than flat graphs throughout the frequency range. At higher concentrations of mixing gum solutions showed stronger structures when G' has been markedly increased. Moreover, where increasing concentration of mix gum solution G' also increase it gave more viscosity and stability of coconut milk emulsion.

Table 4.7 Coarse oil droplet diameter of coconut milk emulsion containing gum mixtures (xanthan:guar)

Sample	Coarse oil droplet diameter (μm)	
	Non-sterilization	sterilization
0.05%		
0:100	7.98 ± 2.27	8.99 ± 2.88
15:85	9.16 ± 4.04	9.27 ± 4.13
50:50	8.76 ± 3.58	9.91 ± 3.46
85:15	6.07 ± 3.29	9.28 ± 3.66
100:0	7.50 ± 2.39	8.32 ± 2.86
0.10%		
0:100	8.52 ± 2.19	9.39 ± 2.32
15:85	8.64 ± 4.23	8.24 ± 3.35
50:50	5.97 ± 2.07	7.55 ± 2.74
85:15	6.56 ± 2.83	8.38 ± 4.07
100:0	6.52 ± 2.65	7.24 ± 3.21
0.15%		
0:100	7.06 ± 2.17	8.24 ± 1.96
15:85	6.87 ± 2.60	8.72 ± 3.55
50:50	7.40 ± 3.67	8.29 ± 3.68
85:15	6.82 ± 2.64	7.17 ± 3.11
100:0	8.57 ± 3.15	7.43 ± 2.58

For highly concentrated emulsions, a dramatic increase in the viscosity occurs when the droplet size is reduced (Pal, 1998). The shear-thinning behavior of concentrated emulsions is also enhanced upon the reduction in droplet size. From Table 4.7 and in Figure 4.19 and 4.20 clearly showed that at xathan:guar 50:50 and 85:15 gave smaller coarse oil droplet diameter. For smaller coarse oil droplet diameter gave more viscosity and more stability in coconut milk emulsion.
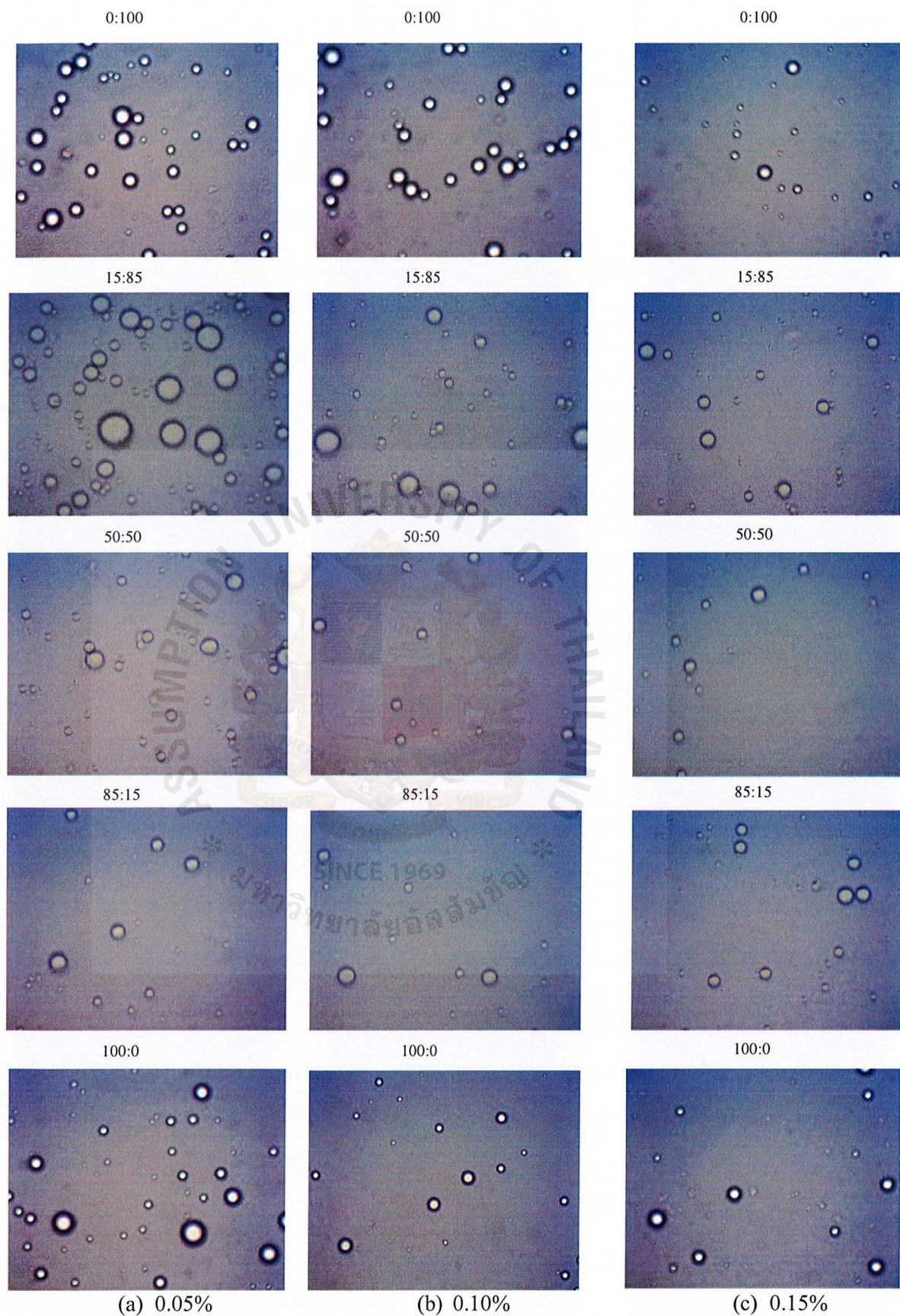
Figure 4.19 Micrograph of non-sterilized coconut milk emulsion containing gum mixtures (xanthan:guar) at (a) 0.05% w/w, (b) 0.10% w/w, and (c) 0.15% w/w
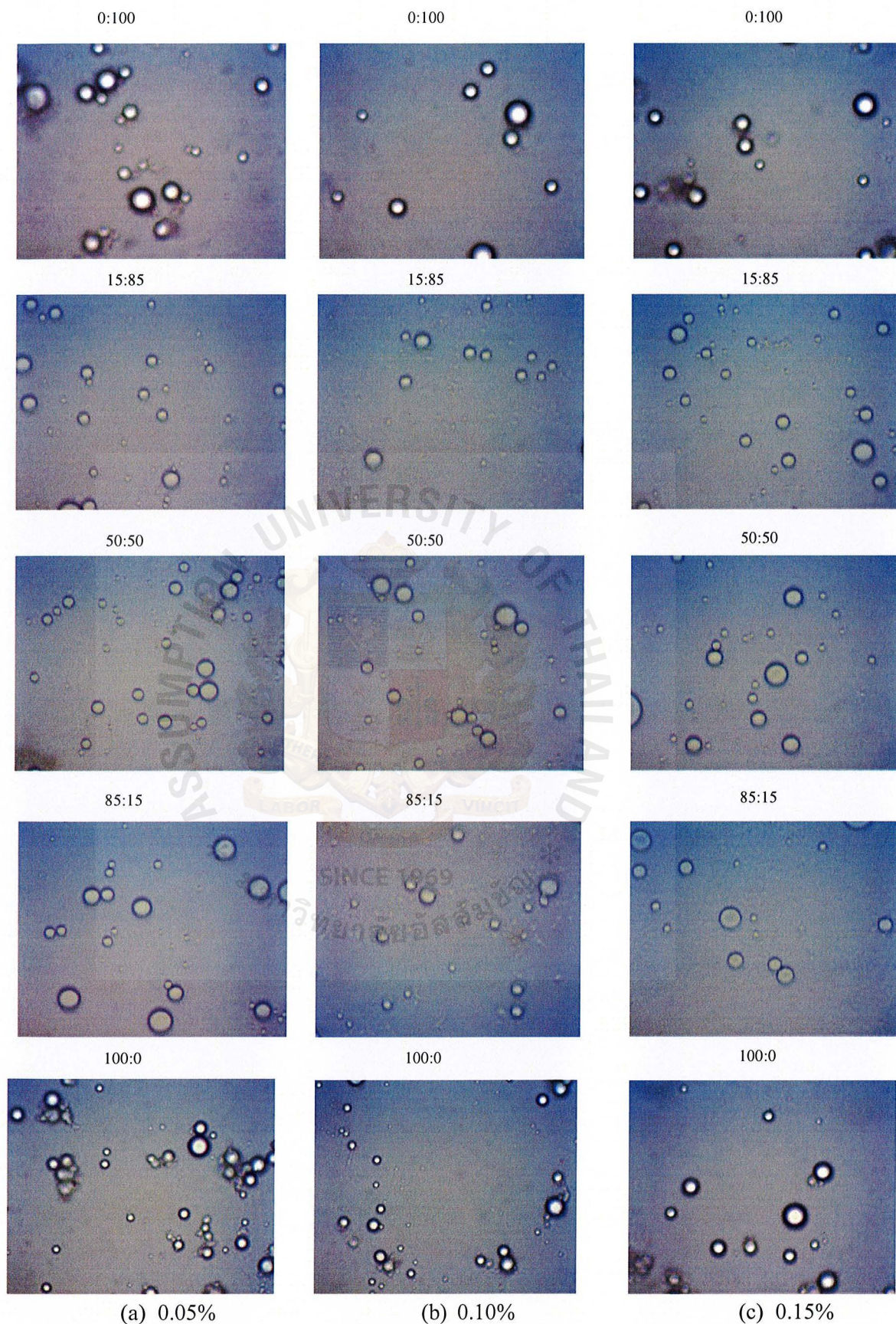
Figure 4.20 Micrograph of sterilized coconut milk emulsion containing gum mixtures (xanthan:guar) at (a) 0.05% w/w, (b) 0.10% w/w, and (c) 0.15% w/w

Particle size distribution obtained from a Malvern laser diffraction particle analyzer were investigated for the emulsion after sterilization and stored at 30 °C As shown in Fig. 4.21, Two broad ranges of particle size were observed from 0.1 to 5 μm (peak I) and from 5 μm to approx. 50 μm. In this experiment, only the sterilized emulsions were kept at 30°C over night prior measurement. By increasing percentage of xanthan gum in the solution induced smaller diameter of the oil droplets and give lower specific surface area of oil droplets. When compare oil droplet size between obtained from the Malvern laser particle analyzer and coarse oil droplet size obtained from microscopic method, found that, the coarse oil droplet size showed similar tendency of oil droplet at various types of food additives and concentrations. In particularly, coarse oil droplet size showed the size diameter close to that of $d_{3,2}$ obtained from a laser scattering method. A bit bigger size of oil droplets found in $d_{3,2}$ than in coarse oil droplet size may be because of longer time storage in sample (overnight) and of a flocculation of droplets detected as mono-droplets in a laser scattering method while detected as poly-droplets in microscopic method. From Figure 4.21 found that at diameter around 20 μm gave the highest quality and the smaller diameter of partical size were xanthan:guar at 50:50 and 85:15.
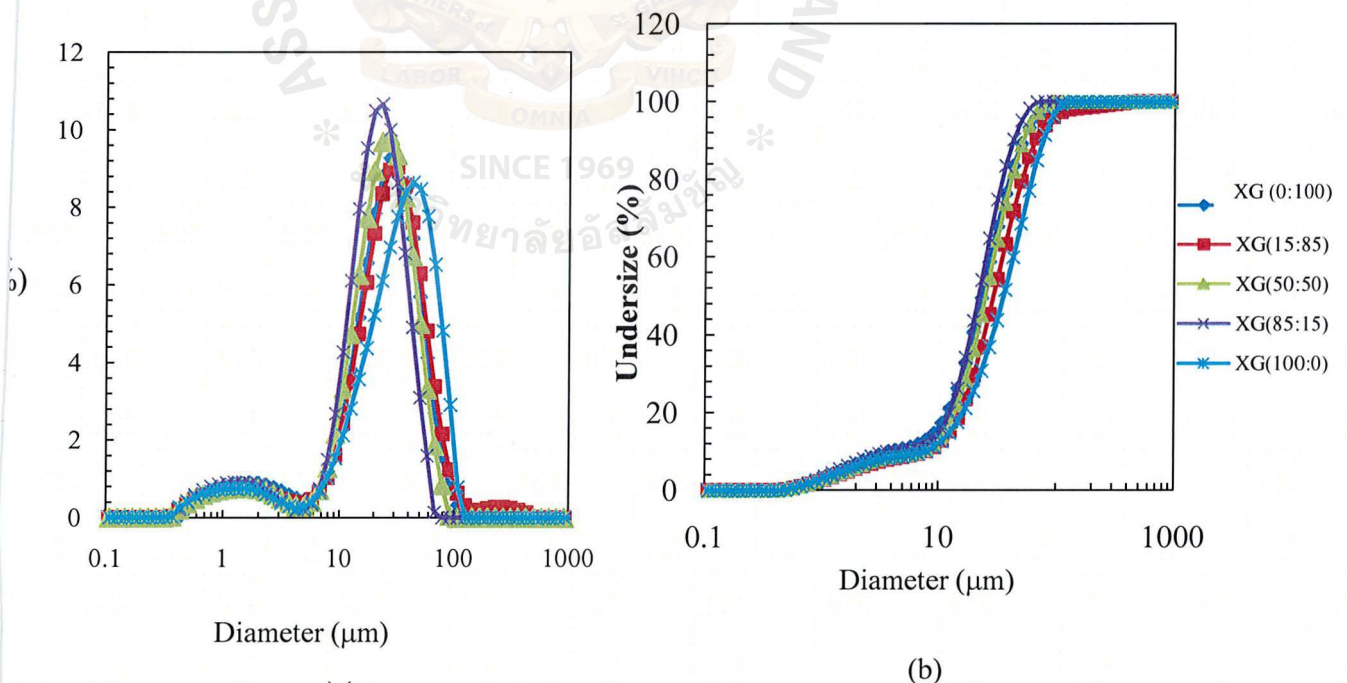
Figure 4.21 Particle size distribution (a) and accumulated particle size distribution (b) in μm of sterilized coconut milk emulsions measured by Mastersizer 2000.

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Table 4.8 Droplet diameter and specific surface area (SSA) of sterilized coconut milk emulsions containing xanthan and guargum (X:G) kept at 30°C over night.

sample	D4,3 (µm)	D3,2 (µm)	d0.1 (µm)	d0.5 (µm)	d0.9 (µm)	Span (µm)	SSA (m²/g)
0:100	22.20	7.14	3.07	19.93	40.91	1.89	0.86
15:85	29.23	8.59	5.59	25.46	52.99	1.86	0.71
50:50	26.21	8.38	5.76	24.08	48.15	1.76	0.73
85:15	23.94	7.87	4.14	21.96	43.87	1.81	0.78
100:0	33.81	8.85	6.44	30.89	64.11	1.86	0.69

From Table 4.9 and Fig. 4.22 showed that higher concentration of gum gave lower creaming index and at higher ratio of xanthan gum also gave lower creaming index. Compare between non-sterilization and sterilization found that non-sterilization gave lower creaming index at day 7 in all samples. When increasing the percentage of xanthan gum in the emulsion the semi-solid like behavior were found. At day 7 of xanthan: guar 50:50 and 85:15 gave the lowest creaming index. By increasing concentration of mixed gum solution samples showed lower creaming index. Also it found that lower temperature gave lower creaming index as shown in Figure 4.22 – 4.24.

Table 4.9 Creaming index for stabilized coconut milks containing gum mixtures (xanthan:guar) at 30°C

Sample	Creaming index (%)			
	Non-sterilization		Sterilization	
	Day 1	Day 7	Day 1	Day 7
0.05%				
0:100	49.06±13.36	58.05±7.62	25.10±7.06	33.70±10.94
15:85	28.39±4.78	54.16±2.30	19.73±4.90	38.28±1.29
50:50	13.97±3.28	40.15±3.67	5.60±4.35	25.51±5.91
85:15	1.98±3.08	18.61±1.26	5.96±9.26	27.47±6.89
100:0	5.67±4.94	18.83±6.29	3.84±5.38	24.08±10.32
0.10%				
0:100	50.22±11.38	59.09±4.27	25.59±9.29	47.55±8.40
15:85	5.33±4.14	23.77±14.74	5.61±4.36	24.86±3.77
50:50	0.00±0	6.99±0.65	5.61±0	24.86±1.38
85:15	0.00±0	2.82±0.39	0.00±0	6.11±1.80
100:0	0.00±0	1.58±1.70	0.85±1.34	11.43±5.62
0.15%				
0:100	43.07±20.27	56.25±8.76	10.78±2.79	39.16±6.26
15:85	0.00±0	17.14±2.61	2.26±3.50	13.48±2.95
50:50	0.00±0	0.00±0	0.00±0	2.59±2.37
85:15	0.00±0	0.00±0	0.00±0	0.00±0
100:0	0.00±0	0.00±0	0.00±0	0.00±0
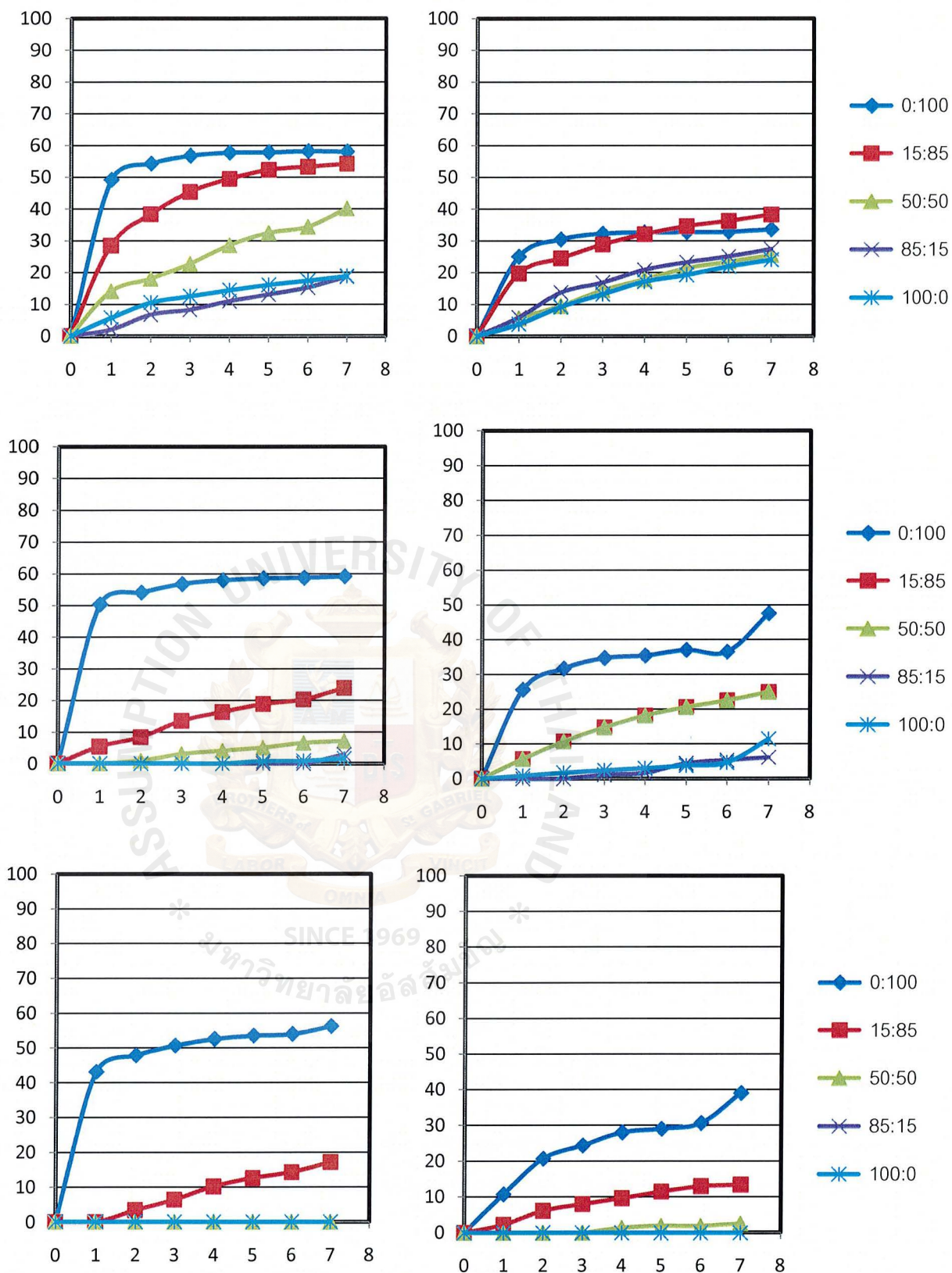
Figure 4.22 Creaming index of coconut milk emulsions containing gums (xanthan:guar) stored at 30°C prepared by different mixing methods of gums
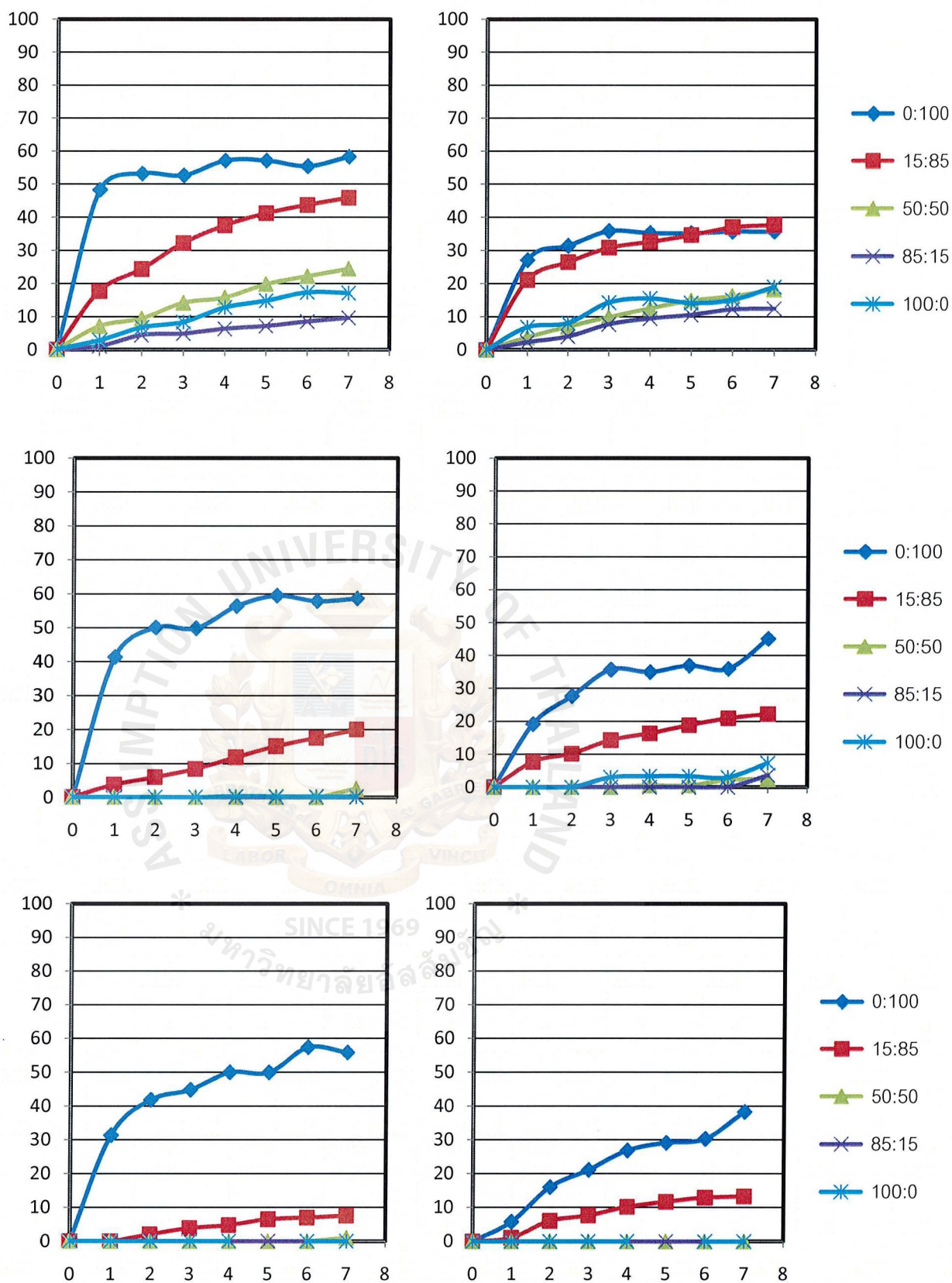
Figure 4.23 Creaming index of coconut milk emulsions containing gums (xanthan:guar) stored at 20°C prepared by different mixing methods of gums
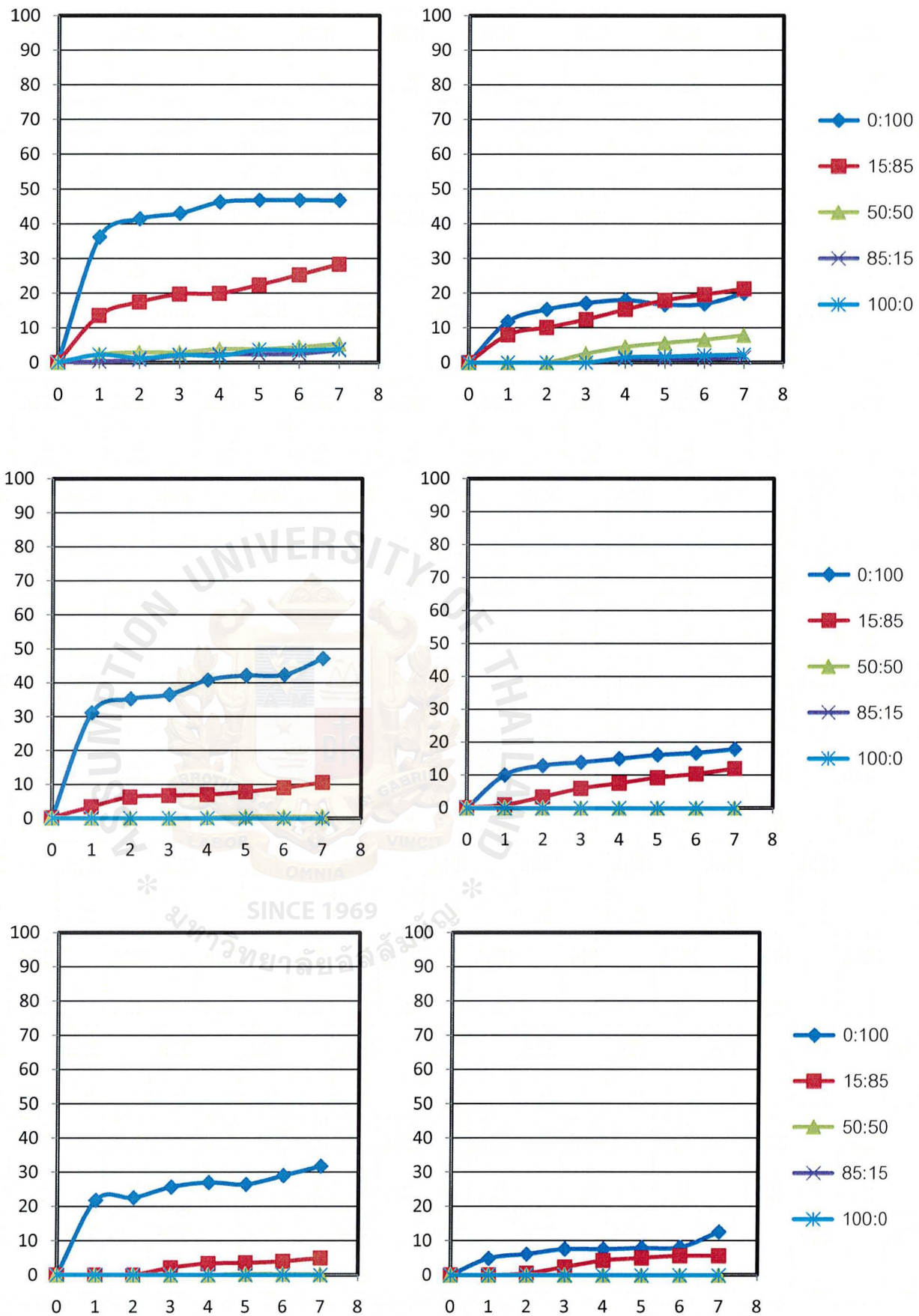
Figure 4.24 Creaming index of coconut milk emulsions containing gums (xanthan:guar) stored at 6°C prepared by different mixing methods of gums

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Photo had been taken during the storage of coconut milk emulsion in test tube. The creaming was observed where the storage had been done under three storage temperatures of 30°C, 20°C and 6 °C. The appearance of serum layer has been seen from these photos (Fig. 4.25 – 4.30).

(a)   (b)

(c)   (d)

(e)   (f)

Figure 4.25 Non-sterilization of (15:85) Xanthan gum: Guar gum in stabilized coconut milk emulsion (20% fat) stored of day 1 at 6°C (a), 20°C (c), 30°C (e), and day 7 at 6°C (b), 20°C (d), and 30°C (f) by agitation method

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Figure 4.26 Non-sterilization of (50:50) Xanthan gum: Guar gum-stabilized coconut milk emulsion (20% fat) stored of day 1 at 6°C (a), 20°C (c), 30°C (e), and day 7 at 6°C (b), 20°C (d), and 30°C (f) by agitation method
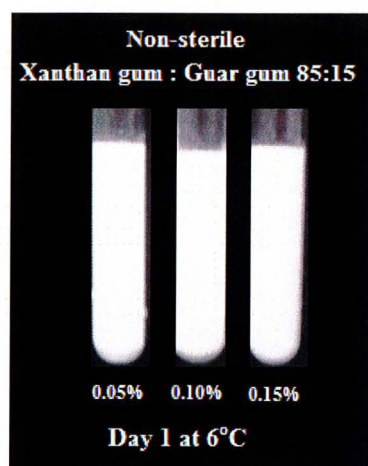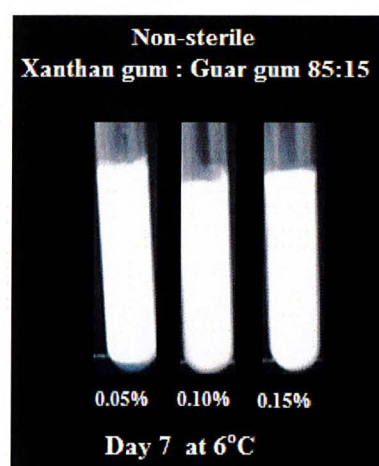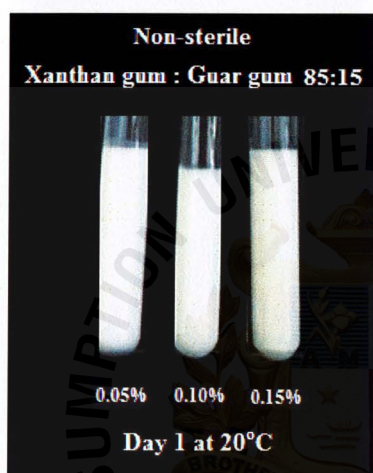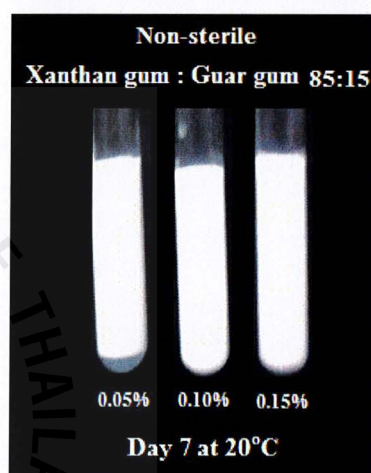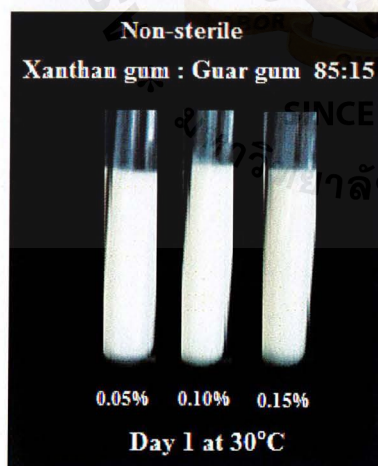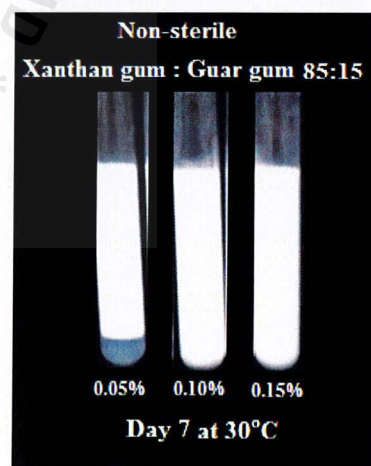
Figure 4.27 Non-sterilization of (85:15) Xanthan gum: Guar gum in stabilized coconut milk emulsion (20% fat) stored of day 1 at 6°C (a), 20°C (c), 30°C (e), and day 7 at 6°C (b), 20°C (d), and 30°C (f) by agitation method
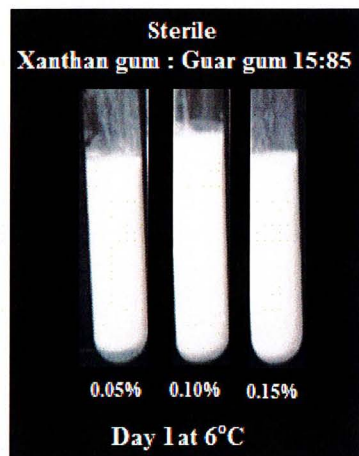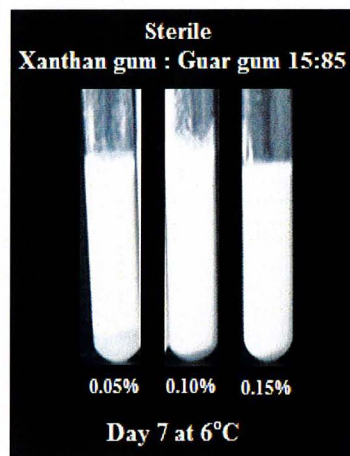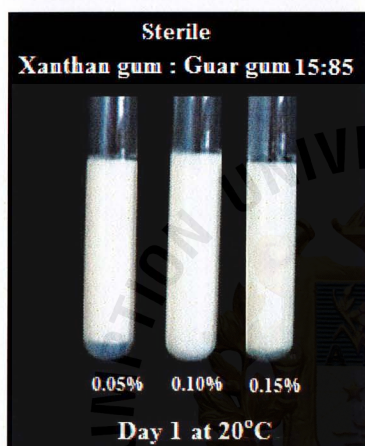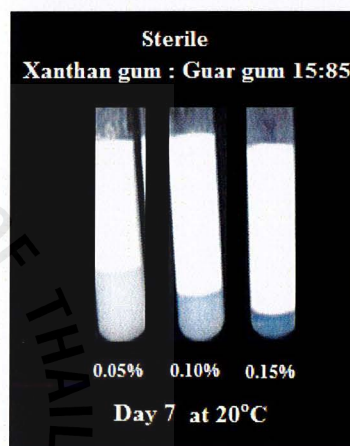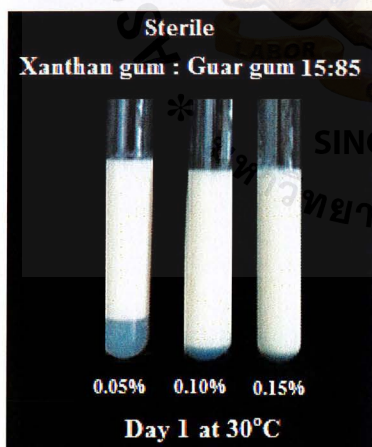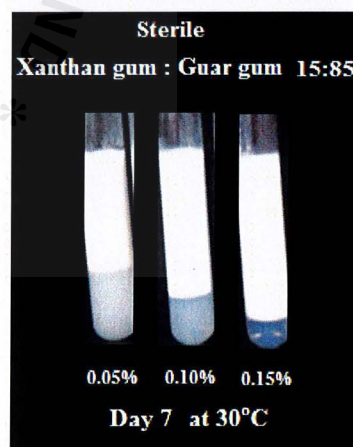
Figure 4.28 Sterilization of (15:85) Xanthan gum: Guar gum-stabilized coconut milk emulsion (20% fat) stored of day 1 at 6°C (a), 20°C (c), 30°C (e), and day 7 at 6°C (b), 20°C (d), and 30°C (f) by agitation method

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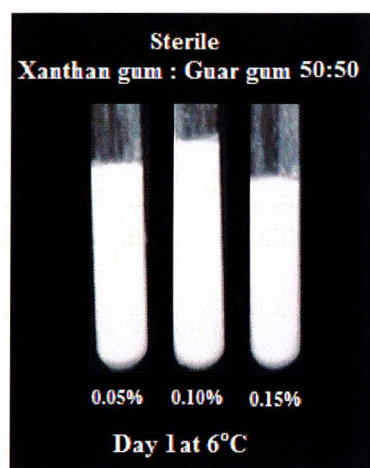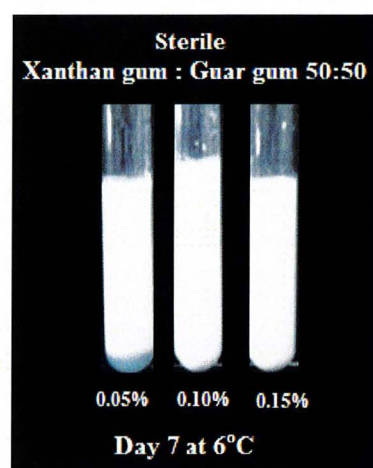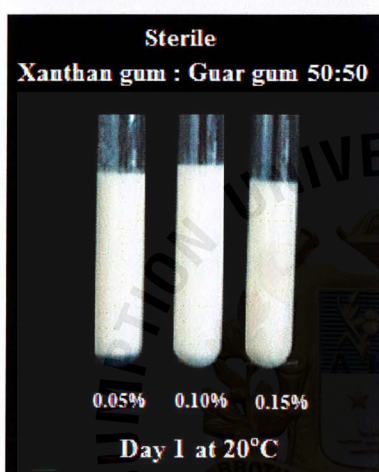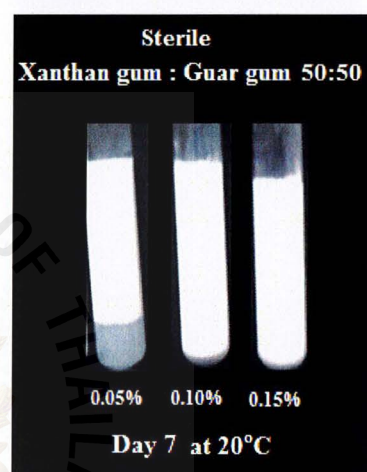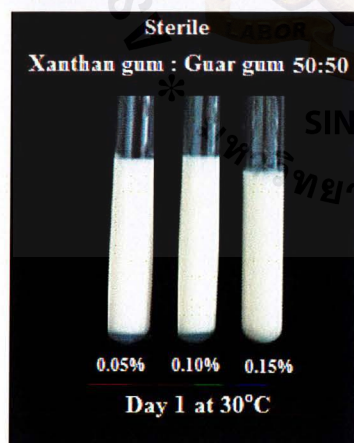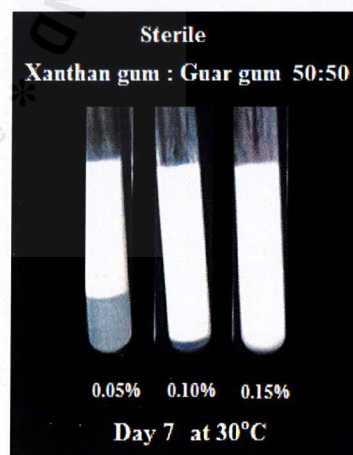
Figure 4.29 Sterilization of (50:50) Xanthan gum: Guar gum-stabilized coconut milk emulsion (20% fat) stored of day 1 at 6°C (a), 20°C (c), 30°C (e), and day 7 at 6°C (b), 20°C (d), and 30°C (f) by agitation method
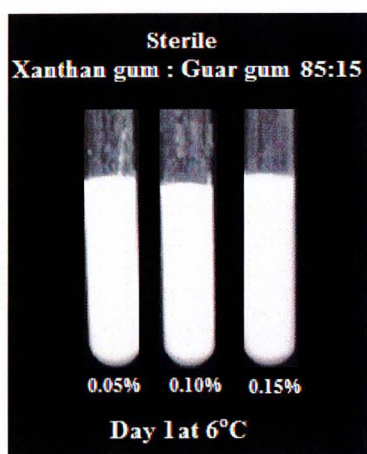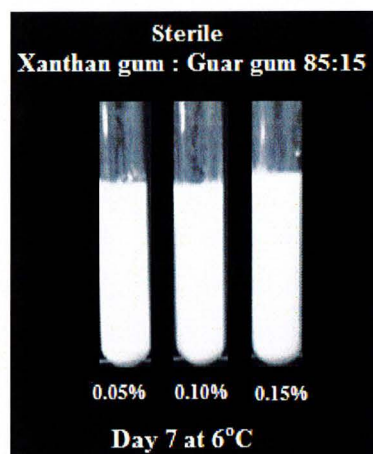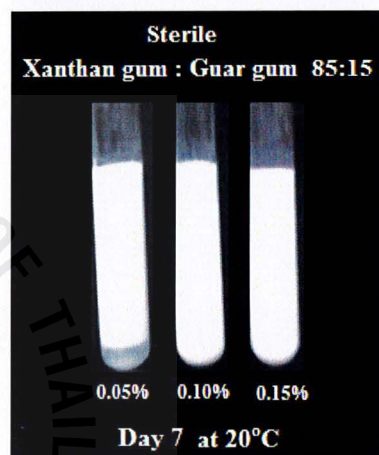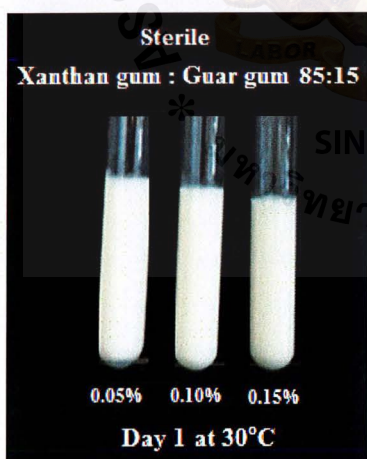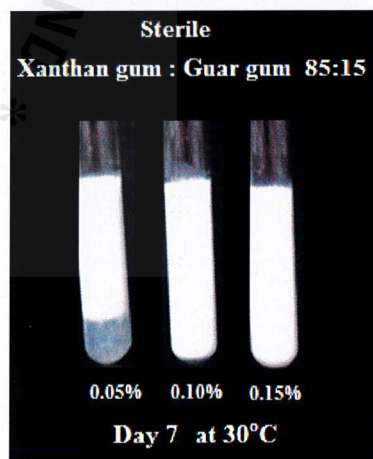
Figure 4.30 Sterilization of (85:15) Xanthan gum: Guar gum-stabilized coconut milk emulsion (20% fat) stored of day 1 at 6°C (a), 20°C (c), 30°C (e), and day 7 at 6°C (b), 20°C (d), and 30°C (f) by agitation method

5. Conclusion

1. For pure gum itself, xanthan gave higher effective viscosity than guar gum.

2. By increasing concentration of gum solutions, the semi-solid has been presented. This can delay creaming which gave lower creaming index.

3. At higher ratio of xanthan gave higher in viscosity and lower creaming index where at 50:50 and 85:15 (xanthan:guar) showed the highest emulsion stability.

4. Temperature during storage also had an effect to creaming index, at lower temperature (10 – 20 °C) it showed lower creaming index than those stored at higher temperature (30 °C).

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APPENDIX

Appendix A1

Table A.1: % creaming index of stabilized coconut milk emulsion at 0.05% of gum solution by different method at 30°C

	30°C			
	0:100 Homo	0:100 agitation	100:0 Homo	100:0 agitation
0	0	0	0	0
1	21.89	25.10	6.00	3.84
2	25.42	30.52	10.77	9.26
3	28.57	32.35	13.16	13.29
4	30.34	32.71	14.63	17.22
5	31.47	32.82	16.24	19.38
6	33.35	32.92	20.37	22.02
7	34.36	33.70	20.85	24.08

Table A.2: % creaming index of stabilized coconut milk emulsion at 0.05% of gum solution by different method at 20°C

	20°C			
	0:100 Homo	0:100 agitation	100:0 Homo	100:0 agitation
0	0	0	0	0
1	19.93	27.10	4.49	6.84
2	22.64	31.41	6.81	8.10
3	29.03	35.89	8.33	14.28
4	29.03	35.31	8.91	15.54
5	30.93	35.31	9.54	14.16
6	31.96	35.79	10.75	15.13
7	32.99	35.79	13.03	19.04

Table A.3: % creaming index of stabilized coconut milk emulsion at 0.05% of gum solution by different method at 6°C

	6°C			
	0:100 Homo	0:100 agitation	100:0 Homo	100:0 agitation
0	0	0	0	0
1	19.93	27.10	4.49	6.84
2	22.64	31.41	6.81	8.10
3	29.03	35.89	8.33	14.28
4	29.03	35.31	8.91	15.54
5	30.93	35.31	9.54	14.16
6	31.96	35.79	10.75	15.13
7	32.99	35.79	13.03	19.04

Table A.4: % creaming index of stabilized coconut milk emulsion at 0.10% of gum solution by different method at 30°C

	30°C			
	0:100 Homo	0:100 agitation	100:0 Homo	100:0 agitation
0	0	0	0	0
1	24.02	25.59	0.00	0.85
2	31.77	31.69	0.00	1.70
3	33.93	34.70	2.30	2.42
4	34.20	35.43	3.82	3.02
5	35.06	37.04	5.38	3.81
6	38.42	36.53	4.47	4.72
7	36.04	47.55	6.80	11.43

Table A.5: % creaming index of stabilized coconut milk emulsion at 0.10% of gum solution by different method at 20°C

	20°C			
	0:100 Homo	0:100 agitation	100:0 Homo	100:0 agitation
0	0	0	0	0
1	23.00	19.18	0.00	0.00
2	27.20	27.66	0.00	0.00
3	27.92	35.67	2.50	2.99
4	32.20	34.97	3.37	3.36
5	33.35	36.92	3.37	3.36
6	33.61	35.96	4.26	3.03
7	34.50	45.07	4.81	7.35

Table A.6: % creaming index of stabilized coconut milk emulsion at 0.10% of gum solution by different method at 6°C

	6°C			
	0:100 Homo	0:100 agitation	100:0 Homo	100:0 agitation
0	0	0	0	0
1	9.09	10.06	0	0
2	11.61	12.91	0	0
3	12.13	13.92	0	0
4	13.14	15.02	0	0
5	13.40	16.17	0	0
6	16.60	16.77	0	0
7	14.28	21.12	0	0

TableA.7: % creaming index of stabilized coconut milk emulsion at 0.15% of gum solution by different method at 30°C

	30°C			
	0:100 Homo	0:100 agitation	100:0 Homo	100:0 agitation
0	0	0	0	0
1	21.53	10.78	0.00	0.00
2	30.59	20.77	0.00	0.00
3	35.64	24.48	1.09	0.00
4	37.47	28.10	2.73	0.00
5	38.78	29.15	3.55	0.00
6	39.32	30.79	3.55	0.00
7	39.66	39.16	4.37	0.00

TableA.8: % creaming index of stabilized coconut milk emulsion at 0.15% of gum solution by different method at 20°C

	20°C			
	0:100 Homo	0:100 agitation	100:0 Homo	100:0 agitation
0	0.00	0.00	0.00	0.00
1	22.03	5.78	0.00	0.00
2	23.87	16.04	0.00	0.00
3	29.46	21.15	0.00	0.00
4	33.51	26.91	0.00	0.00
5	36.38	29.18	0.00	0.00
6	36.57	30.36	0.00	0.00
7	37.47	38.30	2.03	0.00

TableA.9: % creaming index of stabilized coconut milk emulsion at 0.15% of gum solution by different method at 6°C

	6°C			
	0:100 Homo	0:100 agitation	100:0 Homo	100:0 agitation
0	0.00	0.00	0.00	0.00
1	7.69	4.89	0.00	0.00
2	8.61	6.17	0.00	0.00
3	7.13	7.62	0.00	0.00
4	8.30	7.58	0.00	0.00
5	9.26	7.95	0.00	0.00
6	10.66	8.18	0.00	0.00
7	10.17	12.68	0.00	0.00

TableA.10: % creaming index of stabilized coconut milk emulsion at 0.05% of mix gum solution by agitation method at 30°C

	30°C				
	0:100	15:85	50:50	85:15	100:0
0	0	0	0	0	0
1	25.10	19.73	5.60	5.96	3.84
2	30.52	24.52	9.48	13.76	9.26
3	32.35	28.85	14.46	16.91	13.29
4	32.71	32.12	17.86	20.94	17.22
5	32.82	34.65	21.39	23.31	19.38
6	32.92	36.33	23.45	25.20	22.02
7	33.70	38.28	25.51	27.47	24.08

TableA.11: % creaming index of stabilized coconut milk emulsion at 0.05% of mix gum solution by agitation method at 20°C

	20°C				
	0:100	15:85	50:50	85:15	100:0
0	0	0	0	0	0
1	27.10	21.11	3.73	2.27	6.84
2	31.41	26.47	6.98	4.00	8.10
3	35.89	30.85	9.86	7.70	14.28
4	35.31	32.63	12.51	9.47	15.54
5	35.31	34.70	14.91	10.61	14.16
6	35.79	37.10	16.23	12.24	15.13
7	35.79	37.70	18.16	12.47	19.04

TableA.12: % creaming index of stabilized coconut milk emulsion at 0.05% of mix gum solution by agitation method at 6°C

	6°C				
	0:100	15:85	50:50	85:15	100:0
0	0	0	0	0	0
1	11.82	8.00	0.00	0.00	0.00
2	15.35	10.10	0.00	0.00	0.00
3	17.14	12.41	2.53	0.00	0.00
4	18.10	15.34	4.52	1.01	1.54
5	16.78	17.97	5.63	1.01	1.78
6	17.00	19.64	6.62	1.01	2.14
7	20.02	21.31	7.85	1.52	2.38

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TableA.13: % creaming index of stabilized coconut milk emulsion at 0.10% of mix gum solution by agitation method at 30°C

	30°C				
	0:100	15:85	50:50	85:15	100:0
0	0	0	0	0	0
1	25.59	5.61	5.61	0.00	0.85
2	31.69	10.64	10.64	0.00	1.70
3	34.70	14.67	14.67	1.11	2.42
4	35.43	18.08	18.08	1.67	3.02
5	37.04	20.50	20.50	4.42	3.81
6	36.53	22.47	22.47	5.33	4.72
7	47.55	24.86	24.86	6.11	11.43

Table A.14: % creaming index of stabilized coconut milk emulsion at 0.10% of mix gum solution by agitation method at 20°C

	20°C				
	0:100	15:85	50:50	85:15	100:0
0	0	0	0	0	0
1	19.18	7.66	0.00	0.00	0.00
2	27.66	10.14	0.00	0.00	0.00
3	35.67	14.23	0.00	0.00	2.99
4	34.97	16.31	0.52	0.00	3.36
5	36.92	18.80	0.52	0.00	3.36
6	35.96	20.94	2.24	0.00	3.03
7	45.07	22.20	2.50	3.62	7.35

TableA.15: % creaming index of stabilized coconut milk emulsion at 0.10% of mix gum solution by agitation method at 6°C

6°C					
	0:100	15:85	50:50	85:15	100:0
0	0	0	0	0	0
1	10.06	0.81	0.00	0.00	0.00
2	12.91	3.25	0.00	0.00	0.00
3	13.92	5.83	0.00	0.00	0.00
4	15.02	7.45	0.00	0.00	0.00
5	16.17	9.14	0.00	0.00	0.00
6	16.77	10.28	0.00	0.00	0.00
7	17.92	11.91	0.00	0.00	0.00

TableA.16: % creaming index of stabilized coconut milk emulsion at 0.15% of mix gum solution by agitation method at 30°C

	30°C				
	0:100	15:85	50:50	85:15	100:0
0	0	0	0	0	0
1	10.78	2.26	0.00	0.00	0.00
2	20.77	6.17	0.00	0.00	0.00
3	24.48	8.05	0.00	0.00	0.00
4	28.10	9.70	1.40	0.00	0.00
5	29.15	11.55	1.92	0.00	0.00
6	30.79	13.06	1.92	0.00	0.00
7	39.16	13.48	2.59	0.00	0.00

TableA.17: % creaming index of stabilized coconut milk emulsion at 0.15% of mix gum solution by agitation method at 20°C

	20°C				
	0:100	15:85	50:50	85:15	100:0
0	0.00	0.00	0.00	0.00	0.00
1	5.78	1.08	0.00	0.00	0.00
2	16.04	6.06	0.00	0.00	0.00
3	21.15	7.65	0.00	0.00	0.00
4	26.91	10.21	0.00	0.00	0.00
5	29.18	11.69	0.00	0.00	0.00
6	30.36	12.95	0.00	0.00	0.00
7	38.30	13.30	0.00	0.00	0.00

TableA.18: % creaming index of stabilized coconut milk emulsion at 0.15% of mix gum solution by agitation method at 6°C

	6°C				
	0:100	15:85	50:50	85:15	100:0
0	0	0	0	0	0
1	4.89	0.00	0.00	0.00	0.00
2	6.17	0.50	0.00	0.00	0.00
3	7.62	2.34	0.00	0.00	0.00
4	7.58	4.24	0.00	0.00	0.00
5	7.95	4.99	0.00	0.00	0.00
6	8.18	5.62	0.00	0.00	0.00
7	12.68	5.62	0.00	0.00	0.00

Appendix A.2

1. Fat content analysis for raw milk by Babcock method

(Ref: AOAC Official Method 989.04, 2006)

1. Area of application

This method is suitable for the raw, whole milk

2. Concept

Gerber method use for determine the fat content in sample in %.

3. Short description

Known weight of milk is placed into Babcock bottle and H_2SO_4 is added. Generated heat releases fat, which is then isolate by centrifuging and adding of H_2O. Fat is quantitated in graduated portion of Babcock bottle. Result is expressed as % fat by weight.

4. Reagents

4.1 Sulfuric acid

Specific gravity 1.82-1.83 at 20°C

4.2 Mercury

Purity $\geq 99.99\%$. Used for testing accuracy of Babcock bottles.

5. Equipment

5.1 Standard Babcock milk-test bottle. 18 g milk-test bottle, total height 160-170 mm. bottom of bottle is flat and axis of neck is vertical when bottle stands on level surface.

5.2 Pipet

DIN 10 283 –P for milk, Pipette DIN 12 837 – A for milk or corresponding to the equipment suitably.

5.3 PipetDIN 12 837 – B or the suitable one for sulfuric acid usage.

5.4 Centrifuge for milk fat determination with the suitable number of measurement probe.

The centrifuge must be heated, electric or otherwise to 46°-51°C during centrifuging.

5.5 Dividers or calipers – for measuring fat column

5.6 Water bath for tempering milk samples prior to weighing – provided with thermometer, device to maintain temperature of distill water at 50°-52°C, and device to deliver water into Babcock bottle.

5.7 Water bath for test bottles - provided with thermometer and device to maintain temperature of fat column at 48°±1°C

6. Determination

6.1 Test sample prepare and temperature adjustment – place test sample in water bath at 38°±1°C. Transfer 17.6±0.05 ml prepared test portion at 38°C to milk-test bottle. Adjust temperature of milk in bottle to 20°-22°C (a). Adjust temperature of H_2SO_4 to 20°-22°C (b).

6.2 add 17.5 ml of H_2SO_4 to bottle from (a)

6.3 place bottles in heated centrifuge, counter balance, and centrifuge 5 min after proper speed is reached. Add distilled water at 50°-52°C until bulb of bottle is filled. Centrifuge 2 min. Add distilled water at 50°-52°C until top of fat column approaches 7% mark on bottle neck. Centrifuge 1 min longer at ca 51°C.

6.4 use caliper to measure the golden-yellow.