Antioxidant activity of the spent coffee ground infused virgin coconut oil under different extraction conditions and the efficiency of emulsifier types

NADTHANAN JUNPRATUG
5810459

A special project submitted to the school of Biotechnology, Assumption University in part of fulfilment of the requirement for the degree of Bachelor of Science in Biotechnology

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By	**: Nadthanan Junpratug**
Advisor	**: Sireerat Laodheerasiri**
Department	**: Agro-Industry**
Faculty	**: Biotechnology**
Academic Year	**: 2018**

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Sireerat Laodheerasiri
(Advisor)

ABSTRACT

The spent coffee ground (SCG) contains large amount of total phenolic compounds as a source of antioxidant compound. To examine the antioxidant activity of SCG, oil infusion has been used in extraction of oil soluble compounds from the SCG via virgin coconut oil (VCO) at 30% (w/w) in varied conditions which were heated (42°C) and unheated (room temperature approximately 30°C) for 24, 120, and 240 hours. SCG-VCO infused oil was then used to perform an emulsion as the primary study for produced lyophilized VCO enriched antioxidant compound by freezing dry. DPPH radical scavenging method reported that antioxidant compounds can be extracted out from SCG via VCO at heated condition (0.484±0.004, 0.509±0.018, 0.557±0.002 mgTE/g sample) significantly higher than unheated condition (IC_{50} values were 0.441±0.007, 0.458±0.001, and 0.484±0.005 mgTE/g sample) at 24, 120, and 240 hours respectively. Nevertheless, non-infused VCO (300 mg/ml) showed the lowest antioxidant activity. Duration time of infusion also enhanced the amount of antioxidant compounds isolated from SCG, the longer infusion period the higher antioxidant activity. Additionally, incubation at 42°C, 240 hours indicated the highest amount of total phenolic compounds (TPC) as 237.38±0.1 mgGAE/ml sample and the Trolox equivalence IC_{50} value was 0.557±0.002 mgTE/ml sample. Visible spectrophotometer was used to determine color intensity of SCG-VCO infused oil, results showed that heated condition influenced the brown color development more than unheated condition. Absorbance value of heated condition increased from 0.9235, 0.9935 and 1.0605 respectively while unheated condition the absorbances were not significantly difference at 0.849±0.02 (24, 120, 240 hours). An efficient emulsifier to perform SCG-VCO emulsion was Gum Arabic (GA) since it exhibited the percentage creaming index (%CI) of 86.47±0.4% in secondary SCG-VCO emulsion and spoilage developed slowly while comparing with whey protein (WP) and 1:1 ratio (w/w) mixed emulsifier GA:WP. The 4% (w/w) GA can stabilize an emulsion and produced the smallest average diameter of oil disperse phase in secondary emulsion as 0.117±0.030 mm.

Keywords: spent coffee ground (SCG), virgin coconut oil (VCO), oil infusion, antioxidant activity, total phenolic compounds (TPC), VCO emulsion

ACKNOWLEDGEMENTS

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Nadthanan Junpratug

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CHAPTER I

INTRODUCTION

Coffee becomes the most widely consumed beverages aside from water in all region around the globe. There are two species which are *Coffea arabica* (Arabica coffee) and *C. canephora* (Robusta coffee) are commercially cultivated. 70% of Arabica are produced in total world coffee according to high quality and sold 2-3 times higher than Robusta. (Tornincasa, Furlan, Pallavicini, & Graziosi, 2010)

The coffee powder will be gotten after the process of roasting and milling. Cups of coffee are made from the brewing process, and the coffees' residue was called as "spend coffee ground or SCG". SCG mostly disposed of in the form of fertilizer because of non-commercial value. Some cases, SCG was used as the ingredient of further processes of other industries. (Allesina, Pedrazzi, Allegretti, & Tartarini, 2017)

Chlorogenic acid is the main component of green bean coffee that formed by esterification consists of trans-cinnamic acids (caffeic, ferulic and *p*-coumaric acids) with hydroxyl groups on quinic acid. Chlorogenic acid is degraded by heat and hydrolysis such as caffeine and soluble compounds during the coffee brewing process. The process of roasting which applied high temperature, chlorogenic acid will be transformed into melanoidins which presented in brown colored compounds that come from Maillard reaction between reducing sugar and amino acid. Oil infusion process has been used for extracting water non-soluble substances from herbs, seeds and etc. which various oil carrier types (canola, olive, sunflower, etc.) to extract phenolic compounds which contain actively anti-oxidant. Virgin coconut oil (VCO) is widely used particularly in food products, cosmetics, personal healthcare and pharmaceutical products since it highly contains medium chain fatty acids. Furthermore, many researches have been reported that VCO showed the antimicrobial activity. Previously research, oil infusion method with different oil carries (canola oil, corn oil, coconut oil, sunflower oil, and mineral oil) was studied to extract the compounds from SCG. The results showed an increase of antioxidant activity present in oil (Narita & Inouye, 2015).

In this study, VCO infusion method has been used in the extraction of water non-soluble active compounds from SCG by varying extraction temperature, extraction time to determine the condition showing the highest amount of total phenolic contents and antioxidant activity. Then SCG-infused VCO was encapsulated by different emulsifiers, The efficiency of emulsifier types included whey protein, gum Arabic, and mixed of whey protein with gum Arabic (mixed ratio of 1:1) were investigated to stabilize the emulsion of SCG-infused VCO after the emulsion was subjected to coating with wall material maltodextrin. The stability of emulsion was measured as percent creaming index (%CI). This SCG-infused VCO emulsion was prepared for the freeze-drying method in the further study. This project aims to study the amount of active compounds which contained in SCG by VCO infusion, the concentration of total phenolic compounds obtained from different extraction temperature conditions during VCO infusion both unheated ($25^{O}C$) and heated ($42^{O}C$) and study the emulsion stability prepared by different emulsifiers which are whey protein (WP), Gum Arabic (GA) and mixed emulsifier of WP + GA at ratio 1:1.

OBJECTIVES

1. To investigate the extraction condition of bioactive compounds in the spent coffee ground (SCG) extracted by virgin coconut oil (VCO) infusion.
2. To determine the stability of SCG-infused VCO emulsion obtained from different emulsifiers.
3. To study the formation of SCG infused VCO oil emulsion for further lyophilization by freeze-dry technology.

CHAPTER II

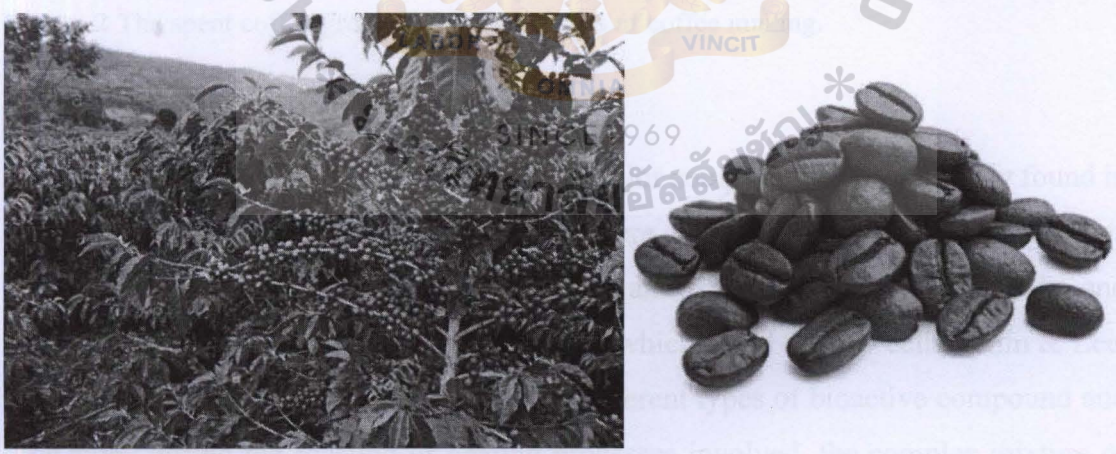
LITERATURE REVIEW

Coffee

coffees' species

Coffee is the most important in agricultural field (Spent coffee ground as a source of phenolic compounds and bioenergy) and economic plant for exporting product worldwide around 60 tropical and sub-tropical countries. The ranking of coffee consumption is in second order next from the petroleum consumption (International Coffee Organication, 2019). Total Coffee Production of Exporting Countries, London (UK), coffee was consumed as the beverage mainly produced by *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) species. (Esquivel & Jiménez, 2012).

Processing of two coffee species it would provide differently in its taste and aroma. Robusta contains higher bitter taste and less in the aroma. While Arabica gives stronger in the aroma but provide 50% less than Robusta. By these reasons, 100% Arabica coffee was considered as the best quality coffee in the world (Kositarat, 2016).



(a) (b)

Figure 1 (a) Coffee tree and the fresh coffee fruits (https://www.youtube.com/watch?v=8VIQoOT3i5o) (b)The roasted coffee (https://www.indiamart.com/proddetail/roasted-coffee-beans-14657195273.html).

Spent coffee ground

Spent coffee ground "SCG" is the residue after processed the coffee powder into the liquid. Most of SCG were sent to disposal factory according to non-economic value. (Zuorro & Lavecchia, 2011) while it contains high amount of sugar mainly polysaccharides especially celluloses and hemi-celluloses up to 50% of dried mass, oils, antioxidants (McNutt & He, 2019) . SCG could use as exhibitor for the Fe and Zn metals affinity (Claudio & Saigusa, 2011). Somehow, SCG was left as waste approximately 2.5 million tons in the year of 2016 and it is higher heating value up to 25 kJ/kg similar to coal that makes SCG become attractive for renewable energy (L & Ferreira, 2019).

Figure 2 The spent coffee ground after the process of coffee making.

Phenolic compounds in coffee

Phenolic compounds are found as the major component of plant mostly found in seeds and green leaves. Phenolic compounds were contained in coffee in high amounts as shown in Figure 1. Moreover, it is high in antioxidant activity in preventing and slowing down the oxidation reaction in natural which could destroy cells. (Kim & Lee, 2015) Source and age of coffee are provide different types of bioactive compound and it changed during the roasting or heating processes involved, the complex mixture of aroma compounds is changed by different chemical reactions such as Maillard reactions, Strecker degradation, caramelization, and oxidation (Wongsa, Nuttida, & Sineenat, 2019). The main group of bioactive compounds content in coffee are caffeine, chlorogenic acids and these bioactive compounds will change related with heated time and temperature used (Rostagno, Celeghini, Debien, Nogueira, & Meireles, 2015).

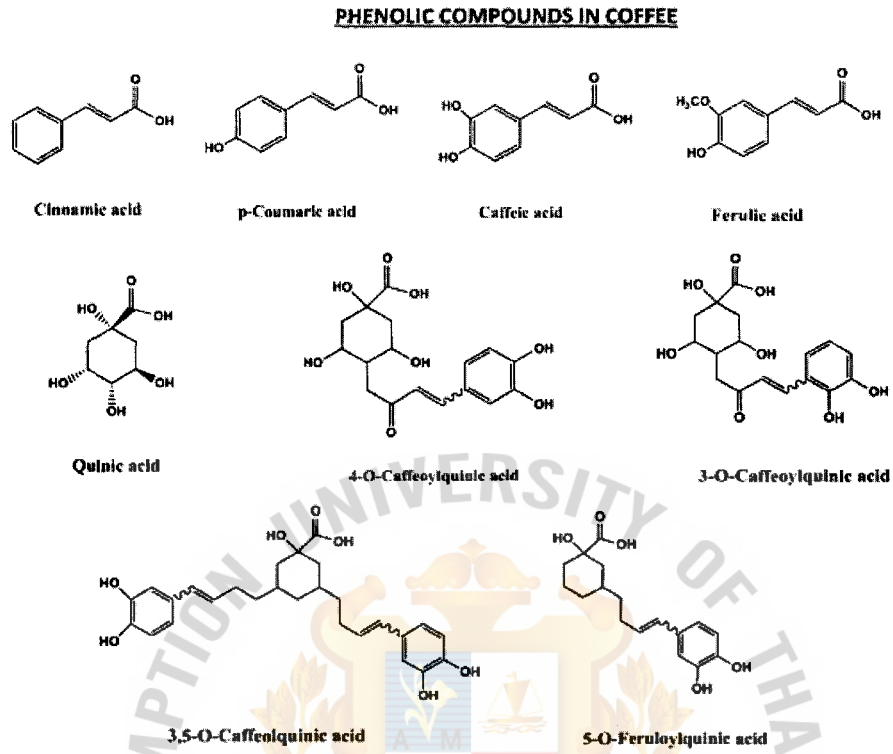
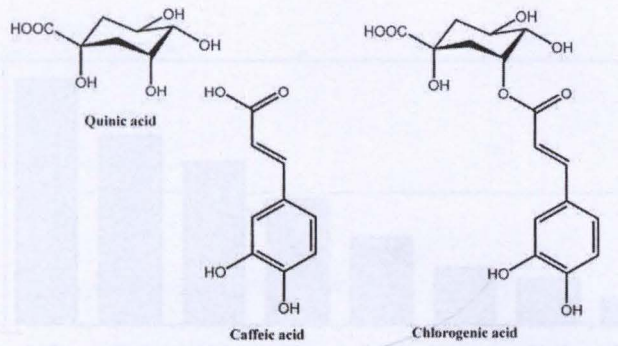


Figure 3 The chemical compound structures which containing in coffee plant. (https://noughtyscience.wordpress.com/2015/04/22)

Chlorogenic acid (CGA)

Chlorogenic acid (CGA) consisted of ester's family that formed between quinic acid with trans-cinnamic acids. The most common foods and beverages majorly contain 5-O-caffeoylquinic acid (5CQA). The 5-CQA can classify as subgroups are: monoesters included caffeoylquinic acid (CQAs), *p*-coumaroylquinic acid, and feruloylquinic acid; diesters and triesters included diCQA and triCQA; and mixing diesters between ferruric acid and caffeic acid. (Stefanello, Spanevello, Passamonti, & Porciúncula, 2018)



(Image modified from: dos Santos MD, et al. Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. Biol Pharm Bull. (2006))

Figure 4 Transforming of melanoidins in coffee beans during the roasting process.

Melanoidins

Melanoidins are high molecular weight of brown-colored and nitrogen content such as foods with sugar, amino acid, and protein which generated the Maillard reaction (MR), the non-enzymatic browning reaction (E., 2008), which originated from Maillard reaction products (MRPs) from heat applied during the processes. The products are affected especially on the color and aroma formations. Coffee and some foods have the complex characterization of melanoidins from the model of melanoidins because of the diversity of reactants. Covalent or non-covalent interactions are holding the melanoidins' core which are phenolics (mainly chlorogenic acid). Thus, spent coffee ground and coffee silver skin can use as the sources of melanoidins that many researchers interested in comparing to the other melanoidins sources (figure 3.), due to the water-soluble melanoidin content. (Mesias & Delgado-Andrade, 2017). Coffee infused in the main source in obtaining the melanoidins among human foods. Moreover, coffee melanoidins are used as the antimicrobial agents, modulators of the gut microbiota, or even prebiotic (Rufian-Henares & Pastoriza, 2015).

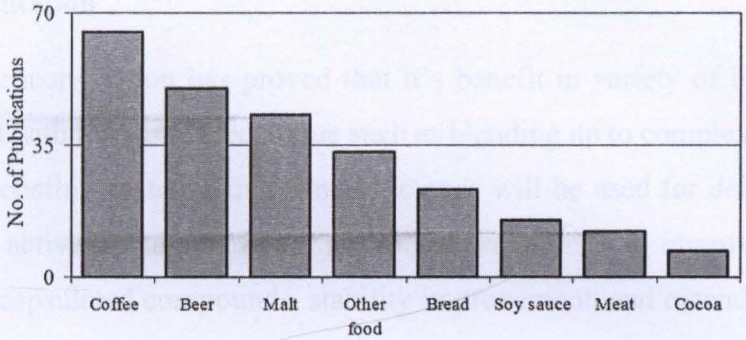


Figure 5 Food stuff distribution which related on publications of melanoidins in several food categories for the period 1900-2007.

Virgin coconut oil

Virgin coconut oil (VCO) is highly functional nutrient comparing to another type of eatable oil due to its benefits for health such as immune system boosting, a cholesterol level of maintaining, unique odor, rancidity resistance, low melting point, and clear crystal forming. Furthermore, VCO can directly be converted to energy from fatty acid chains without storing in adipose tissue because of shorter branches and smaller structure molecules present. But VCO has the unique characteristics which are VCO will become solid at 24^{O}C temperature because it contains a highly amount of saturated fatty acid (Amin, Koh, Hamid, Tan, & Long, 2017). VCO extraction processes should not exposed to the light and heat and cold compression method was considered as the better extraction method comparing to fermentation process that highly moisture content and could lead to rancid faster (Nagdeve, 2019). Moreover, the VCO also used to improve the physical appearances especially on face such as face lifting and improve winkles by massaging with specific movement. VCO consuming benefits, improve the skin to become stronger, gastrointestinal health from consuming of good fat (Astra, 2019).

Microencapsulation

Microencapsulation has proved that it's benefit in variety of industry that has been involved with the simple technique such as blending up to complex technique such as polymeric coating systems, in cosmetic science will be used for delivery system in personal care actives. The benefits of microencapsulation is to improve the aesthetic, protect the encapsulated compounds, stability improvement, and extended the shelf life of active compounds (Scott , Nava , Wolf, Guyard, & Greenburg, 2005). The sensitive ingredient will be entrapped by using encapsulation technique. The coating material or wall will inhibit the reactions between internal reactant from the environment. The simplest method of encapsulation is emulsifying which normally use with oil (Desobry, Netto, & Labuza, 1997).

Gum Arabic

Gum Arabic (GA) or Acacia fiber is the substrate that commonly use in food and pharmaceutical fields. It is a branches-complex structure of polysaccharide which slightly acidic and found in Acacia Senegal tree a native in Africa, Pakistan, and India. The highly soluble of GA is helping to lower the cholesterol level, protect diabetes. Soluble fiber, one of dietary fiber form gel-like character when dissolved in water (Wong, 2019). GA was used as the emulsifier to stabilize emulations (Hu, et al., 2018). It is the biopolymers with complex mixtures, the surface-active composted of branched arabinogalactan blocks attached with polypeptide chain. The function of gum Arabic in aqueous phase can stabilize the droplet in encapsulation process (McClements, 2009).

Figure 6 The characteristic of Acacia Senegal or Gum Arabic tree (https://www.dairyfoods.com/blogs/14-dairy-foods-blog/post/92225-on-the-trail-of-acacia-gum-part-2-a-visit-to-the-orchard).

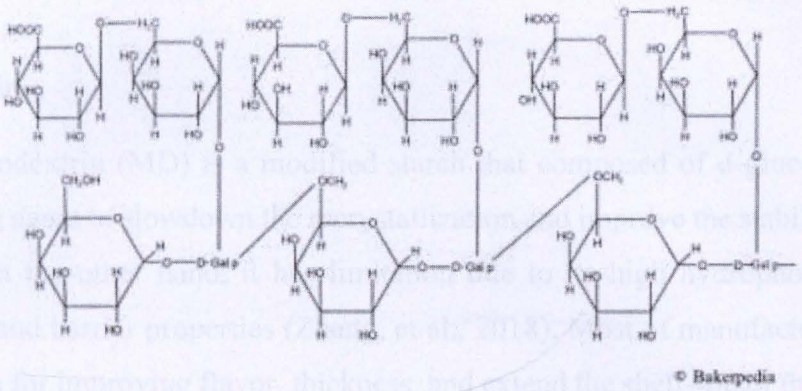


Figure 7 structure of Gum Arabic or Acacia Gum. (https://bakerpedia.com/ingredients/acacia-gum/acacia-gum-composition/)

Whey protein

Whey protein (WP) is one of the protein which found in milk with casein that will occur as by-product of cheese production and it was considered as the complete protein as it contains 9 types of essential amino acids and low lactose content (Nordqvist, 2017). There are four main classes content are β-lactoglobulin (50% of total protein content), α-lactalbumin, serum albumin (5% of protein content) and several immunoglobins. Most of these have a globular conformation and sensible for denaturation and aggregation by heat and high pressure (Arriaga, 2011). As protein has property about stabilizing of droplets due to the protein structures are protruded (attaching to the surface of oil droplets) once it absorbed it will reacted to form the strong interfacial interaction (Singh & Dalgleish, 1998).

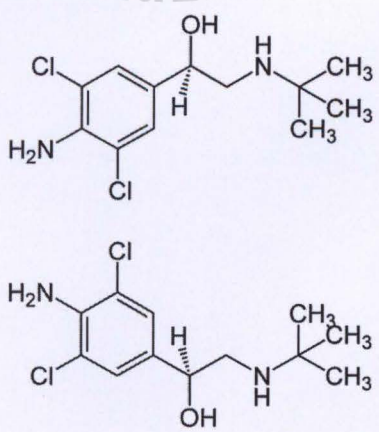


Figure 8 The whey protein chemical structure (http://opening.download/spring-opening.html).

Maltodextrin

Maltodextrin (MD) is a modified starch that composed of d-glucose units for film forming agent to slowdown the recrystallization and improve the stability shelf-life of foods. On the other hand, it has limitation due to its high hydrophobicity, weak mechanical and barrier properties (Zhang, et al., 2018). Most of manufacturing use the maltodextrin for improving flavor, thickness, and extend the shelf-life of their products. As the maltodextrin has the characteristics similar to starch products, in order to use it commercially in production it requires hydrolysis process to breakdown starch structure molecules into sugars molecules. Moreover, it's gluten free and have no nutritional value. About 20% or less of sugar content in maltodextrin that the reason of using MD instead of sugar in dietary food such as sweetener or nutrition bars but it would cause the serious obstruction in vein due to glycemic Index (GI) is high (Lisa, n.d.).

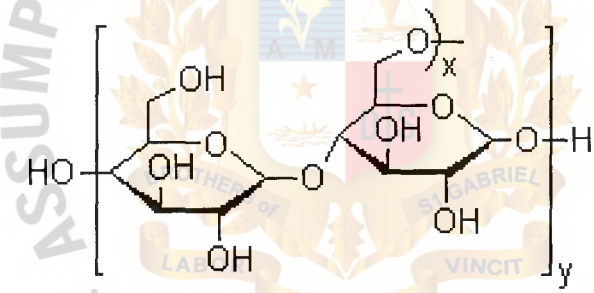


Figure 9 The chemical structure of maltodextrin.

CHAPTER III

METHODOLOGY

MATERIALS

1. Samples

Samples	sources
Coffee	
Arabica SCG	Fresh coffee shop
Oil	
VCO	King Island, Thailand

2. Chemical substances

Chemical substances	Company
Potassium iodine (KI MW =166)	Ajax Finechem, New Zealand
Gallic acid monohydrate ($C_7H_6O_5$ MW= 170.12)	Sigma-aldrich, China
2,2-Dipheny-1-Picrylhydrazyl(DPPH) ($C_{18}H_{12}N_5O_6$ MW = 394.23)	Srichem, India
Sodium Thiosulphate ($Na_2S_2O_3$ MW = 158.11)	Ajax Finechem, Australia
Sodium carbonate (Na_2CO_3 MW = 105.98)	Ajax Finechem, New Zealand
Trolox (C14H18O4 MW =250.294g/mol)	Acros Oganics, US

3. Equipments

Equipments	Company
Balance	A&D Company Limited, Japan
Micropipette	Biohit company, Germany
Vortex mixer	VELP, Italy
Spectrophotometer	Milton Roy, USA
Stirrer	VELP, Italy
Shaker	Ika Labortechnik, Malaysia
Thermostat oven	Jebsen & Jessen (Thailand) Co. Ltd VELP, Italy

Magnetic stirrer	Milton Roy, US
Spectrophotometer	ANMO Electronics Co.,Ltd Thailand
Microscope eye-piece camera	

4. miscellaneous

Plasticware	Company
Pipette tip (100µl, 1,000µl)	QSP, USA

Glass-wares	Company
Beaker	Pyrex, USA
Funnel	Pyrex, USA
Erlenmeyer Flask	Pyrex, USA
Stirring rod	Pyrex, USA
Magnetic bar	Pyrex, USA

METHODS

Spent coffee ground preparation

The SCG contain high moister content after passed brewing process were collected and further for drying process by hot air oven at 45^{O}C until SCG weight was constant. In order to maintain the chemical composition after drying, dried SCG were collected triple, pool mixed and kept in plastic bags with aluminum foil at 4^{O}C refrigerator.

Spent coffee ground oil infusion

The essential compounds in SCG were extracted by oil infusion technique. Virgin coconut oil (VCO) has been used in the process. The infusion ratio of SCG: VCO was used at 30%w/w, extracting in (25^{O}C) and (42^{O}C) temperatures for 24hrs (1 day), 120hrs (5 days), and 240hrs (10 days), each container was agitated 1 time per day. The SCG infused oil were collected and filed with a double layer of white cloth and kept in a non-transparent container at room temperature. The pure VCO were also incubated as a control oil.

Chemical analysis of sample preparations

DPPH scavenging assay sample preparation

Dissolved 4g of SCG infused oil sample in 10 ml of ethyl acetate solvent, the concentration of sample prepared was 400 mg/ml, then dilute sample solution into 300, 200, and 100 mg/ml in ethyl acetate. These four concentrations will be used to determine IC_{50} value of the antioxidant activity by DPPH scavenging method. VCO without SCG has been used as a control, ethyl acetate has been used as a blank solution. The scavenging activity was compared as mg Trolox which used as standard.

Total phenolic compounds sample preparation

The solution was prepared by dissolve 5g of SCG infused with 25 ml hexane in focal tube mixed well and add 10ml of 60% methanol. Focal tube solutions were vertex for 1 minute, stand for 10 minutes until solution separate into two layers. The lower part was token for rotary extraction at 40^{O}C temperature for 5 minutes.

Chemical characteristics studies

Anti-oxidant activity by DPPH scavenging method

The modified DPPH in Scavenging technique that measures the antioxidant activity in the percentage reduction of DPPH. The 0.1mM of in ethyl acetate was prepared in 100 ml Erlenmeyer flask, 0.0030 g of DPPH was dissolved in ethyl acetate (0.1 mM)

Preparing SCG infused oil 0.5ml in 2ml of ethyl acetate then mixed with the 0.1 mM of DPPH solution in a test tube, mix vigorously by vertex for 10 seconds then leave the mixed solution for 30 minutes in the dark place. The reaction was measured by spectrophotometer at 517 nm. (0.5ml of ethyl acetate + 2ml of 0.1 mM DPPH was used as control solution and ethyl acetate used as a blank solution).

Percentage of antioxidant activity in each time condition will be plotted in the graph versus with concentration in order to examine IC_{50} value from the linear equation, comparing to Trolox standard curve. Percentage of the antioxidant activity increased from carrier oil will be calculated from these formulas.

$$\%\boldsymbol{inhibition\ increased\ from\ carrier\ oil} = \left(\frac{\boldsymbol{A_i - A_c}}{\boldsymbol{A_c}}\right) \times \mathbf{100}$$

A_c = absorbance control (carrier oil)

A_i = absorbance infused oil

Color intensity

The SCG infused oil in different temperature and time conditions will be measured the color intensity by a spectrophotometric technique for determining the percentage of color intensity of SCG infused oil with different time infusion and different temperature conditions comparing with VCO without SCG. all were measured in absorbance at 420 nm.

Total phenolic compounds by Folin-Ciocalteu's method

Prepared fresh Folin-Ciocalteu's reagent by diluting with distilled water (1:10 v/v, Folin-Ciocalteu's reagent / distilled water). The 500µl of sample and 1ml of Folin-Ciocalteu's reagent were mixed in the test tube, 1 ml of 7.5%(w/v) of sodium carbonate (Na_2CO_3) was added and kept in room temperature and dark condition for 30 minutes. Measured by spectrophotometer at 765 nm. The total phenolic compounds were expressed as milligram Gallic acid equipment (mgGAE/g of sample). The concentration of total phenolic compounds was calculated from the standard curve of gallic acid standards.

Encapsulation process

Coating materials preparation

Maltodextrin (MD) solution was prepared at 30%(W/W) in distilled water stir until the maltodextrin completely dissolved approximately 120 minutes then keep the solution at room temperature.

Gum Arabic (GA) solution was prepared at 4.45%(W/W) in distilled water stir with magnetic stirrer for 6-8 hours and keep the solution in room temperature until the Gum Arabic powder completely dissolved overnight.

Whey protein (WP) solution was prepared at 4.45% (W/W) in distilled water for 120 minutes with magnetic stirrer then kept the completely dissolved WP in refrigerator ($4^{O}C$) overnight. Further use these solutions in the next experiment.

Primary emulsion (1st emulsion) preparation

There were three types of emulsifier used in the research study, whey protein, Gum Arabic, and a mixed solution of whey protein and Gum Arabic at ratio of 1:1 by these three emulsifiers were prepared for two set are i) SCG infused in VCO sample ii) pure VCO. SCG infused in VCO 10% (w/w) are mixed with 90% (w/w) of emulsifiers with wisely drop SCG infused into emulsifiers while stirring with a magnetic stirrer and further mixed with a homogenizer at speed 12,000 rpm for 1 minute. The 1st emulsions were measured pH by 10%(w/w) samples were dissolved with ethyl acetate, and 10 g of the 1st emulsion has been taken for calculating of emulsion stability (%CI Equation 1) by standing sample for 24 hours for the formula below.

$$\%CI = [\frac{H_S}{H_T}] \times 100$$

Equation 1

Where CI is creaming index, H_S is high of serum part, and H_T is total high.

Secondary emulsion (2nd emulsion) preparation

First emulsions were mixed with 30% MD solution at a ratio of 1:1 by using magnetic stirrer and further mixed by homogenizer at speed 12,000 rpm for 1 minute in for obtaining the second emulsion. The second emulsions were examined the %IC **Equation 1**, total solubility (^{O}Brix value), microscopic observation, and pH (10%(wt) samples were dissolved with ethyl acetate).

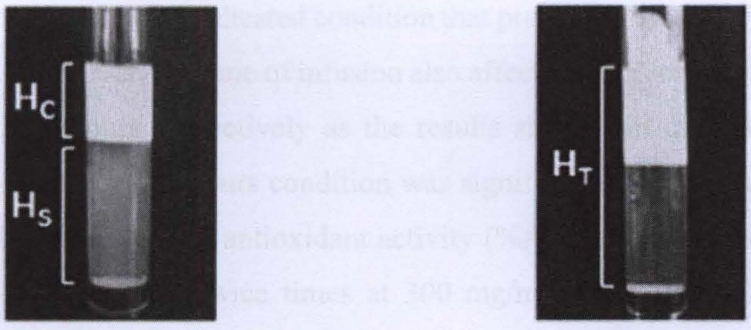


Figure 10 The description of emulsion layer, where H_C is creaming thickness, H_S is serum thickness, and H_T is the total high.

CHAPTER IV

RESULTS AND DISCUSSIONS

Antioxidant activity by DPPH Scavenging method

The spent coffee ground (SCG) obtained from a fresh coffee shop in each batch was dried to evaporate out the liquid content as the conducted process with oil (virgin coconut oil, VCO) in order to extract the compounds out from dried SCG. Three batches of dried SCG were pool mixed before the process of infusion. Virgin coconut oil (VCO) has been used as the compounds extracting solvent at 30%(w/w). In the extracting process of oil infusion, temperature and time condition have been varied. Temperature condition was varied as a heated condition at 42^{O}C and unheated condition at room temperature of Thailand approximately 30^{O}C and in each temperature condition was also varied the infusion times at 1 day (24 hours), 5 days (120 hours), and 10 days (240 hours). After infusion, oil liquid was filtrated by a double layer of sheet cloth and collected at room temperature.

After the infusion process, infused SCG oil in differences time infusion were varied the concentration at 100, 200, 300, and 400 mg/ml to measure percent antioxidant activity (%AA) by radical Scavenging DPPH method 517 nm for further calculation of IC_{50} values (half concentration inhibitory). From this varying concentration found out that, heated condition gave %AA higher than unheated condition and both conditions also have %AA higher than pure VCO or non-infused VCO. The IC_{50} indicates the infusion in heated condition provided a higher efficiency ranging between 158.08 to 181.99 mg/ml compared to the unheated condition that provided values between 180.57 to 199.59 mg/ml. Moreover, the time of infusion also affect the increasing of IC_{50} ability as 240, 120, and 24 hours respectively as the results showed in table 1. While the statistical test at heated at 240 hours condition was significantly difference from other treatments (α=0.05). The percent antioxidant activity (%AA) of SCG infused oil were higher than non-infused VCO twice times at 300 mg/ml concentration of unheated condition, each concentration and infusion time also gave different values of %AA as the results showed in table 6.

Figure 11 to 13 represented the %AA value of non-infused VCO compared with SCG infused VCO under difference infusion times, the results clearly indicated that bioactive compounds in SCG could be extracted by VCO at 42°C.

As the sample of bioactive compounds extraction especially tannin from tea (*Camelia sinesis)* in commercial brands by varying the times (2, 4, 6, 8, and 10 min) and temperatures (90, 95, and 100 ^{O}C) found out that increase in boiling time and temperature can increase tannin concentration but longer time is decrease the sensory properties such as essential oil which responsible for aroma (Rehman, Almas, Shahadi, Nighat, & Saleem, 2002). Also the higher temperature and longer time of roasting are significantly develop the color and non-significantly increase chemical properties of rice germ oil (In-Hwan , et al., 2002).

The virgin coconut oil (VCO) has found out that it has relationship between the total phenolic content, scavenging activity and the reducing power which could contributed to the antioxidant activity (%) in VCO (Marina, Che man, & Nazimah, 2009).

Table 1 The inhibition concentration activity (IC_{50}) of SCG infused oil in unheated condition and heated (42^{O}C) conditions at 24, 120, and 240 hours of infusion times.

Unheated condition			
Infusion time (hrs)	IC_{50} (mg/ml)	Trolox equivalence (mgTE/g sample)	TPC (mg GAE/ml sample)
24	199.59^{a}	0.441	193.45±0.1^{e}
120	192.42^{a}	0.458	210.03±0.3^{d}
240	180.57^{a}	0.488	218.12±0.2^{c}
Heated (42^{O}C) condition			
Infusion time (hrs)	IC_{50} (mg/ml)	Trolox equivalence (mgTE/g sample)	TPC (mg GAE/ml sample)
24	181.99^{a}	0.484	208.25±0.1^{c}
120	172.95^{a}	0.509	214.89±0.4^{a}
240	158.08^{b}	0.577	237.38±0.1^{b}

Remark *- the different letters of super scrip indicate the significantly difference of each treatment (p>0.95).*

Total phenolic compounds

From table 1, the amount of total phenolic compounds (TPC) in SCG infused with VCO were significantly different among both infused conditions. The most efficient method of oil infusion was under the heated condition at 42^{O}C in which the amount of TPC from SCG was higher than unheated condition. Furthermore, the time of infusion affected the amount of TPC extracted from the SCG; the infused SCG oil for 10 days (240 hours) showed the highest efficiency at 237.38±0.1 mg GAE/mL sample followed by 5 days (120 hours) at 214.89±0.4 mg GAE/mL sample then 1 day (24 hours) at 208.25±0.1 mg GAE/mL sample respectively. As for the unheated condition, the results obtained were 218.12±0.2, 210.03±0.3, and 193.45±0.1 mg GAE/mL sample for 240, 120, and 24 hours respectively. The infusion process to obtain the total phenolic compounds by applied heat can extract more TPC out from SCG in the same way with longer time of infusion long infusion time also extract more TPC. The TPC content value of 30%(w/w) of SCG in VCO was increased according to the infusion time varied (24, 48, 72, 96, and 120 hr) (Kositarat, V, 2016).

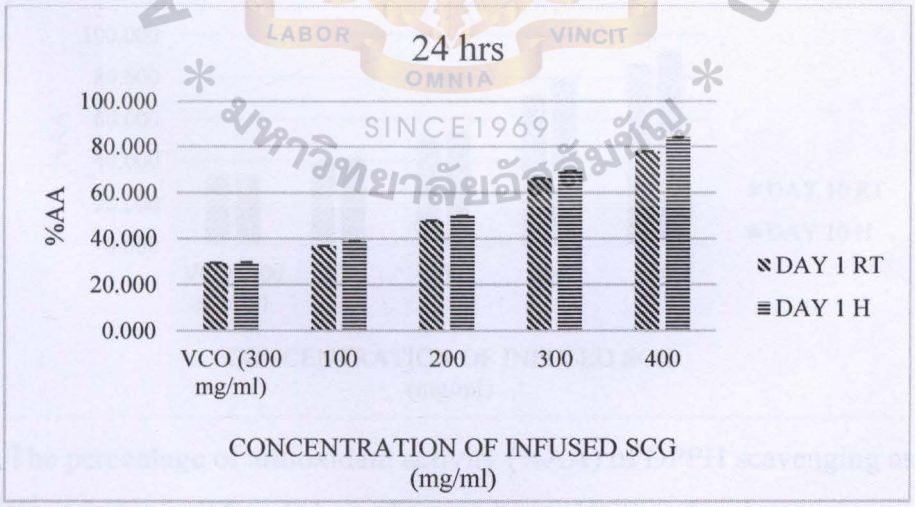


Figure 11 The percentage of antioxidant activity (%AA) of DPPH scavenging assay of 24 hrs SCG infusion in RT and H conditions of non-infused VCO and various concentrations (100, 200, 300, and 400 mg/ml).

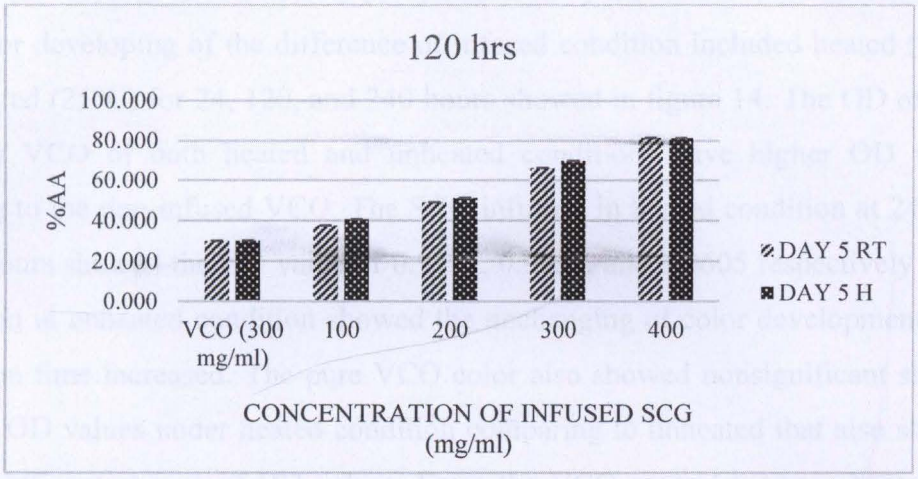


Figure 12 The percentage of antioxidant activity (%AA) of DPPH scavenging assay of 120 hrs SCG infusion in unheated (25^{O}C, RT) and Heated (42^{O}C) conditions of non-infused VCO and various concentrations (100, 200, 300, and 400 mg/ml).

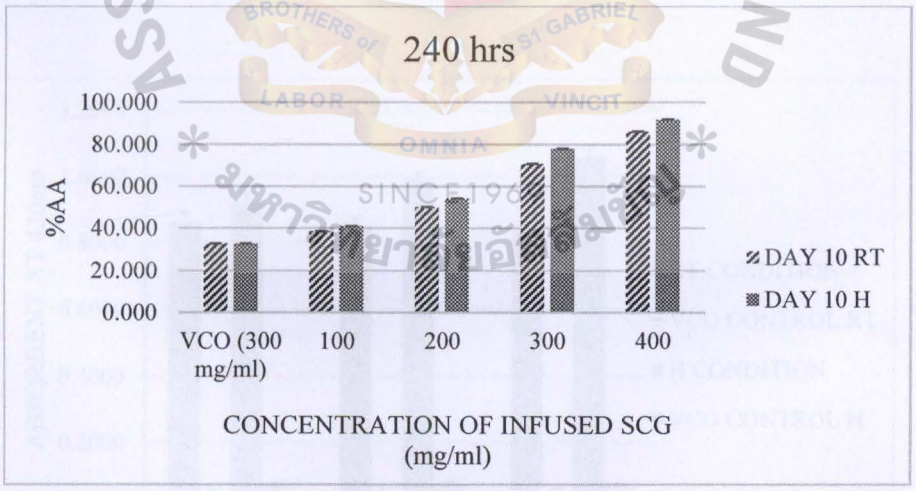


Figure 13 The percentage of antioxidant activity (%AA) of DPPH scavenging assay of 240 hrs SCG infusion in RT and H conditions of pure VCO and various concentrations (100, 200, 300, and 400 mg/ml)

As the result of optical density (OD) measured by the visible spectrometer at 420 nm of color developing of the difference of infused condition included heated (42°C) and unheated (25°C) for 24, 120, and 240 hours showed in figure 14. The OD of SCG infused in VCO of both heated and unheated conditions gave higher OD values comparing to the non-infused VCO. The SCG infusion in heated condition at 24, 120, and 240 hours showed the OD value of 0.9235, 0.9935, and 1.0605 respectively while the infusion in unheated condition showed the unchanging of color development even the infusion time increased. The pure VCO color also showed nonsignificant slightly change of OD values under heated condition comparing to unheated that also showed the nonsignificant change of OD values. From the VCO control between heated and unheated conditions, heated condition was slightly increasing the color intensity significantly. The heating process affect the extraction of the bioactive compounds from SCG include changing the property of VCO, darker in color development of SCG infused. The SCG infused in unheated condition for 24, 120, and 240 hours indicated the OD values approximately the same which are 0.8620, 0.8480, and 0.8385 respectively. On the other hand, the heated condition OD values significantly increased in 24, 120, and 240 hours (0.9235, 0.9935, and 1.0605 respectively).

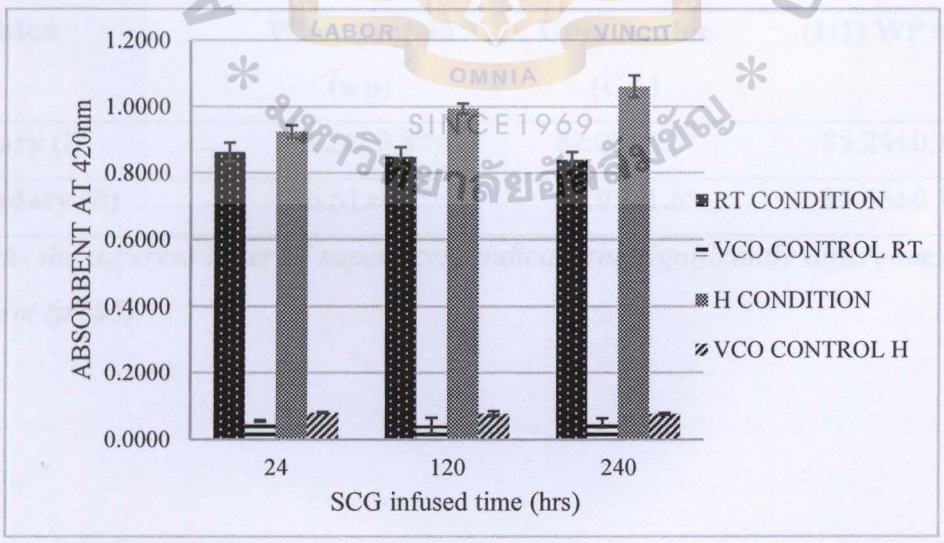


Figure 14 The development of color intensity compares to the time of SCG infusion (24, 120, and 240 hrs) in unheated condition (RT) and heated condition (H) of the infusion process.

These results revealed that as the infusion temperature increased, the more bioactive compounds such as the TPC could be extracted from the SCG more than the infusion under room temperature condition. The radical scavenging DPPH assay indicated the antioxidant activity in SCG infused oil increased which was related with TPC compared with the non-infused VCO, furthermore the higher in color intensity could refer to higher amount of bioactive compounds isolated from SCG in VCO.

Table 2 The percent creaming index (%CI) in primary and secondary emulsions of whey protein (wp), Gum Arabic (GA), and mixed WP+GA at ratio (1:1) for 24 hrs.

SCG			
	Emulsifiers		
Emulsion	**Whey protein (wp)**	**Gum Arabic (GA)**	**(1:1) WP+GA**
Primary (i)	85.34 ± 1.2^{bc}	84.29 ± 1.4^{c}	82.38 ± 0.8^{d}
Secondary (ii)	86.41 ± 0.6^{b}	89.74 ± 0.4^{a}	$85.24\pm0.8b^{c}$
VCO			
	Emulsifiers		
Emulsion	**Whey protein (wp)**	**Gum Arabic (GA)**	**(1:1) WP+GA**
Primary (i)	85.24 ± 0.8^{bc}	82.08 ± 0.7^{d}	85.24 ± 0.1^{bc}
Secondary (ii)	86.51 ± 0.7^{b}	89.05 ± 1.6^{a}	85.65 ± 0.1^{bc}

***Remark**- the different letter of super scrip indicate the significantly difference of each treatment (p>95).*

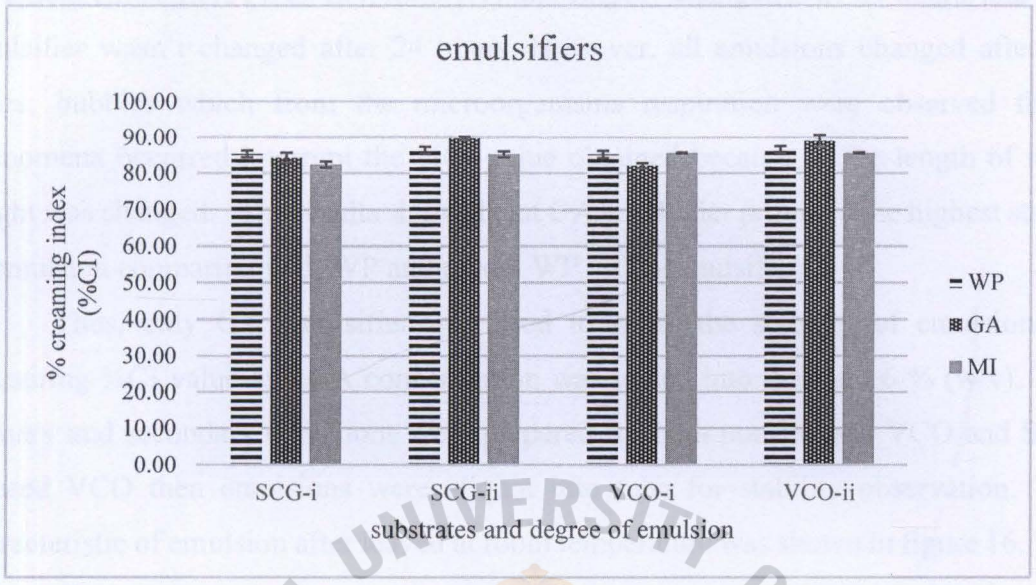


Figure 15 The %CI comparison results between emulsifiers; whey protein (WP), Gum Arabic (GA) , and Mixed WP + GA at ratio 1:1.

Emulsification of SCG infused VCO

Emulsification of SCG infused in VCO was studied for prepare the primary emulsion for freeze drying to produce the lyophilized SCG infused VCO. To produce emulsion, emulsifiers are required to mix immiscible compounds as the oil phase and water phase together. The droplet of oil will be dispersed in the continuous phase of water by emulsify with the emulsifier. Furthermore, wall material is a substance which shell the oil droplet and help in the freeze drying method.

In this experiment, whey protein (WP), gum Arabic (GA) were chosen as emulsifiers. Three different emulsifier types were prepared which were 4.45% WP, 4.45% GA and 1:1 ratio of mixed WP:GA. Visual observation of emulsions showed that type of emulsifiers affect on the percent creaming index (%CI) in primary and secondary emulsion of non-infused VCO and SCG infused VCO. Table 3 indicated that %CI that obtained from GA emulsifier in both non-infused VCO and SCG infused VCO of secondary emulsion were 89.05±1.6 and 89.74±0.4 % respectively while comparing to secondary emulsion of WP and mixed WP+GA of SCG infused VCO were 86.41±0.6 and 85.24±0.8% and %CI of non-infused VCO were 86.51±0.7 and 85.65±0.1%. Moreover, after 24 hours of leaving emulsion in room temperature condition, some of emulsifiers used were spoiled since the prepared emulsions were not added preservative

because WP is rich in protein and important for microorganism metabolism followed by the mixed emulsifiers (WP+GA, ratio 1:1) that slightly changed after 24 hours, and GA emulsifier wasn't changed after 24 hours. However, all emulsions changed after 48 hours; bubbles which from the microorganisms respiration were observed those phenomena occurred interrupt the %CI value obtained because of the length of total height was changed. The results showed that GA emulsifier provided the highest stable of emulsion comparing with WP and mixed WP + GA emulsifiers.

Thus, only GA emulsifier was used to study the stability of emulsion by measuring %CI value and GA concentration was varied into 2, 4 and 6 % (w/v). The primary and secondary emulsions were prepared for both non-infused VCO and SCG infused VCO then emulsions were aliquot into tube for stability observation. The characteristic of emulsion after leaved at room temperature was shown in figure 16. The oiling-off of emulsion has occurred after leaving mixture for 24 hours; inside the tube consisted of 3 layers which be able to distinguish by naked eyes, the bottom layer (serum layer; H_S), middle layer (creaming layer; H_C), and the top later (oiling-off layer). The oiling-off layer has generated by assembling of oil droplets that closed together in the middle layer to form the bigger droplets at once stage assembled droplet will form pure oil layer.

The %CI values were analyzed and presented in table 4. For primary emulsion, the % CI of 2% (w/v) GA at 0 and 24 hours showed 83.8±1% presenting unchanged %CI value whereas the 4 and 6 % (w/v) GA had a slightly increased %CI after leave 24 hours, the value were 81.6±0 and 82.1±1% at 0 and 24 hours respectively of 4% (w/v) and 79.9±3 and 81.7±2% at 0 and 24 hours respectively of 6% (w/v). The value of %CI in secondary emulsion showed higher efficiency due to the emulsion solutions was mixed by 50% of maltodextrin (MD) so the amount of oil was decreased.

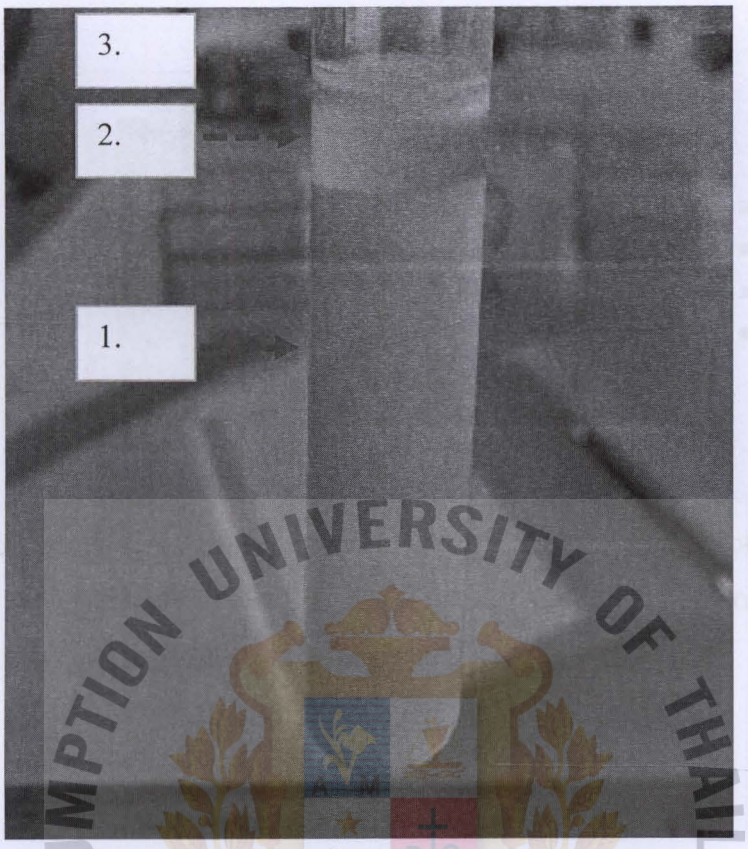


Figure 16 The oiling-off effect in emulsion test.

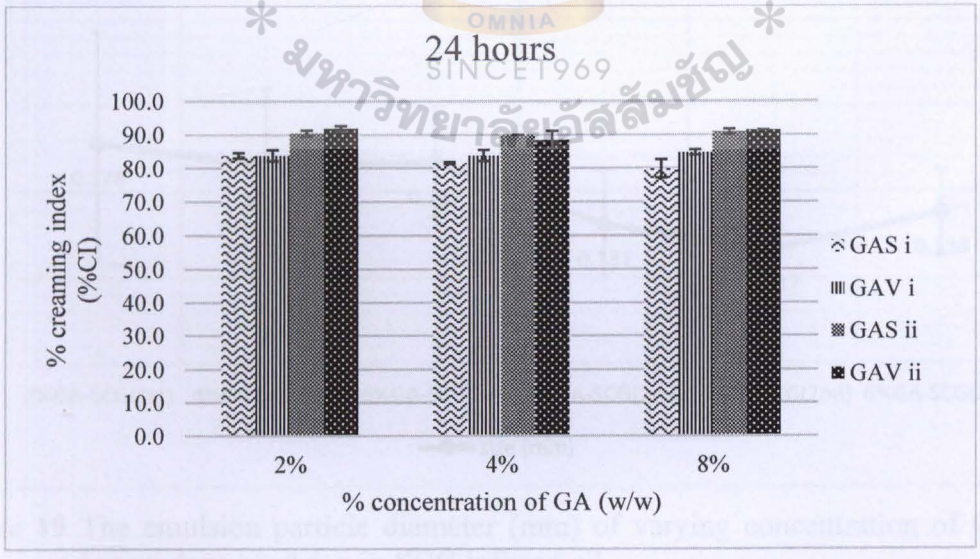


Figure 17 The results of percent creaming index (%CI) by varying of Gum Arabic concentrations at 2, 4, and 8% (w/w) in primary and secondary emulsion of GAS and GAV after finish preparation 24 hours.

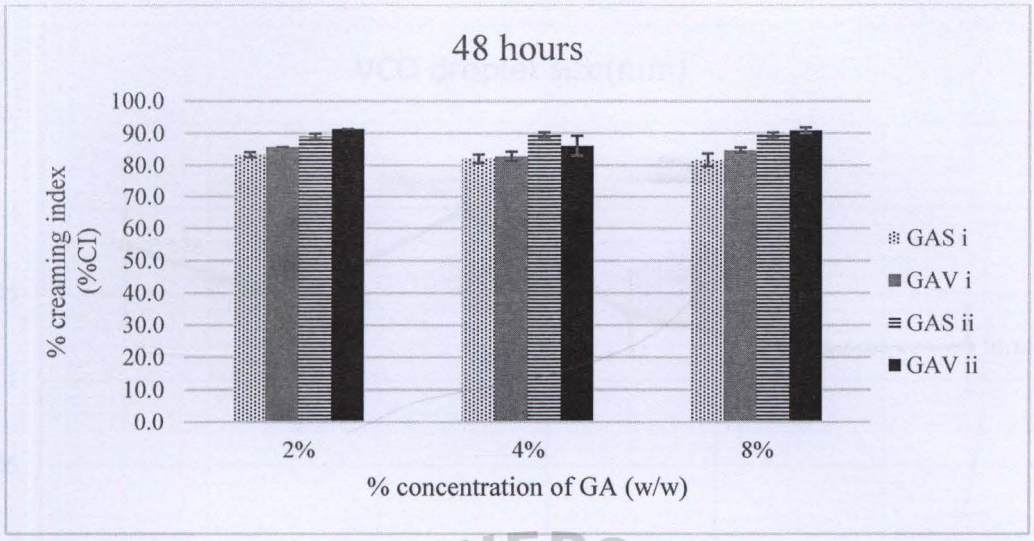


Figure 18 The results of percent creaming index (%CI) by varying of Gum Arabic concentrations at 2, 4, and 8% (w/w) in primary and secondary emulsion of GAS and GAV after 24 hours of preparation 48 hours.

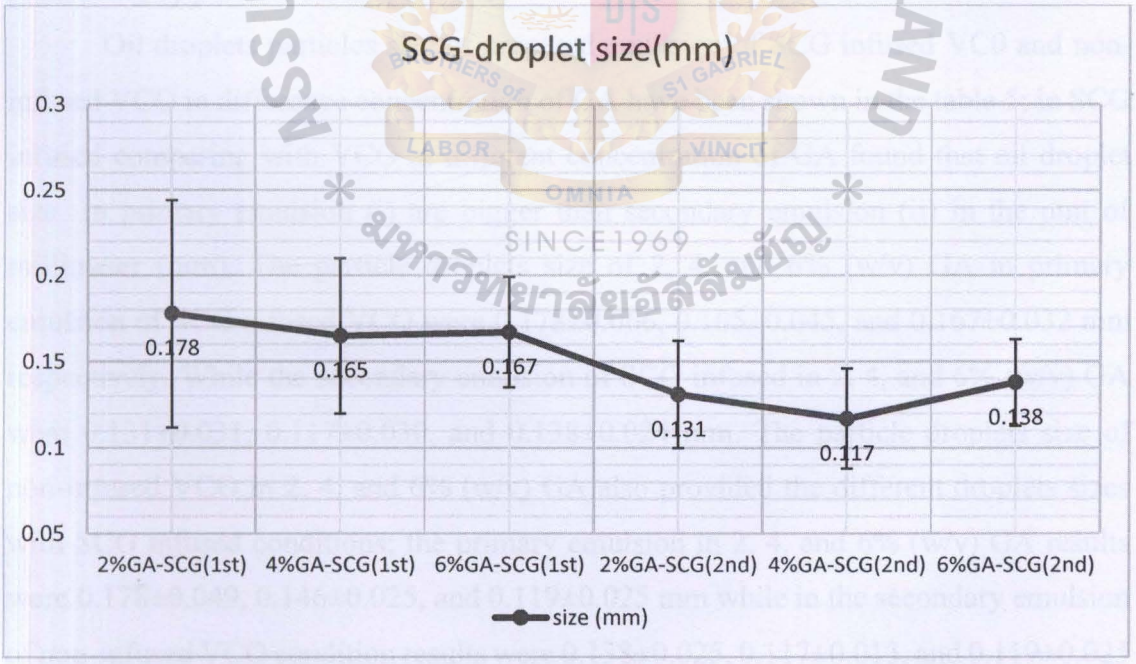


Figure 19 The emulsion particle diameter (mm) of varying concentration of GA in primary and secondary emulsion in SCG infused oil.

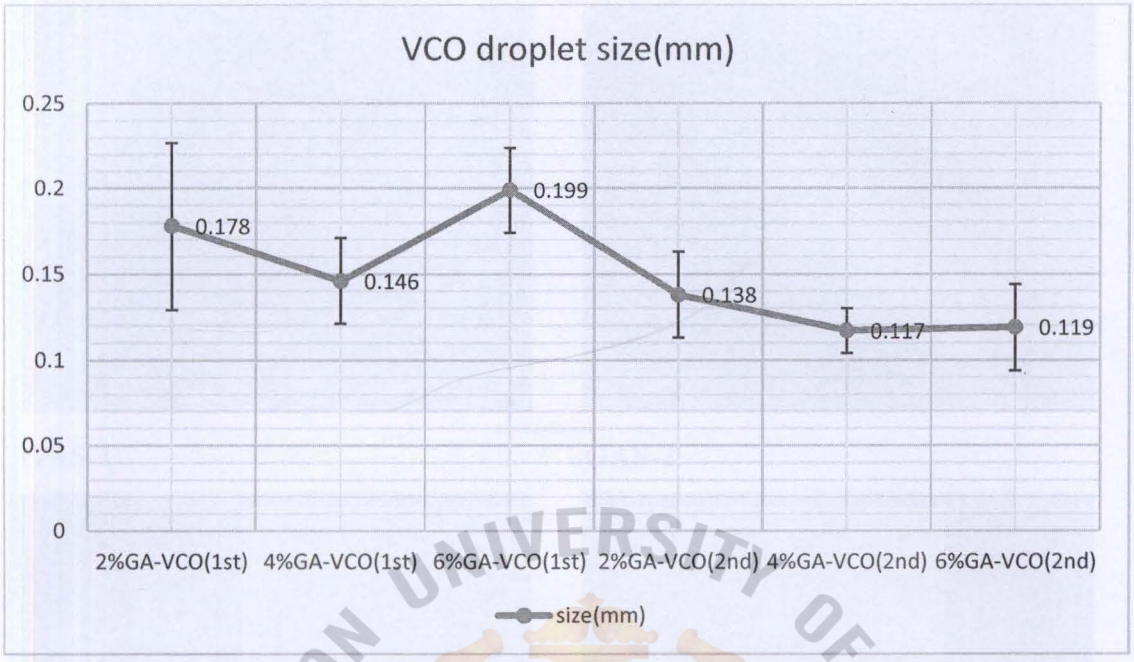
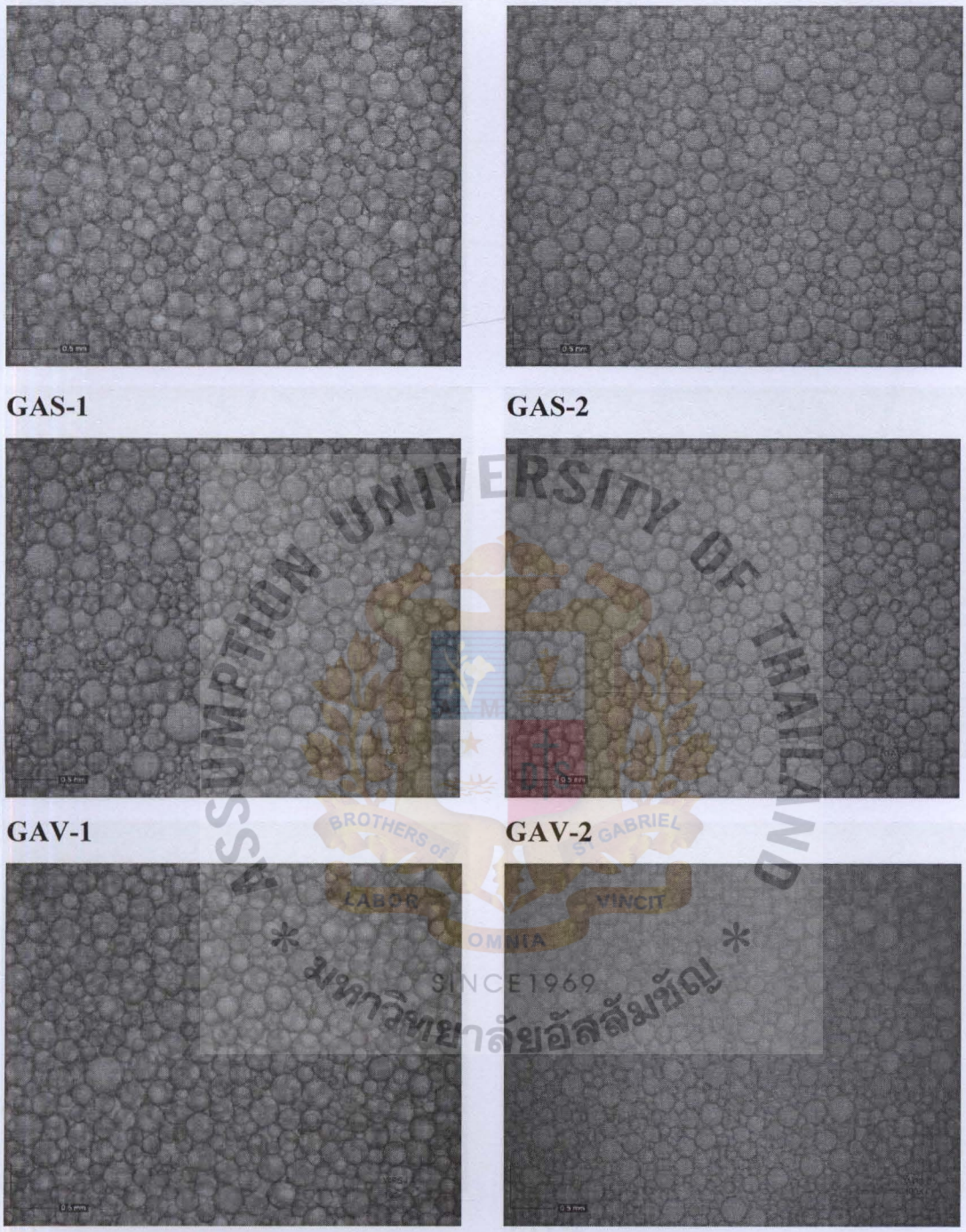


Figure 20 The emulsion particle diameter (mm) of varying concentration of GA in primary and secondary emulsion of pure VCO.

Measuring of particle oil droplets size

Oil droplets particles size of prepared emulsion of SCG infused VC0 and non-infused VCO in difference concentration of GA have been shown in the table 5; in SCG infused comparing with VCO at different concentration of GA found that oil droplet size in primary emulsion (i) are bigger than secondary emulsion (ii) in the unit of millimeter (mm). The particle droplets size of 2, 4, and 6% (w/v) GA in primary emulsion of SCG infused VCO were 0.178±0.066, 0.165±0.045, and 0.167±0.032 mm respectively. While the secondary emulsion of SCG infused in 2, 4, and 6% (w/v) GA were 0.131±0.031, 0.117±0.030, and 0.138±0.024 mm. The particle droplets size of non-infused VCO in 2, 4, and 6% (w/v) GA also provided the different droplets sizes with SCG infused conditions; the primary emulsion in 2, 4, and 6% (w/v) GA results were 0.178±0.049, 0.146±0.025, and 0.119±0.025 mm while in the secondary emulsion of non-infused VCO condition results were 0.138±0.025, 0.117±0.013, and 0.119±0.025 mm. From these results show that primary emulsion gave bigger size of droplet particles comparing to the secondary emulsion which both treatments are not significantly different ($p>0.95$). On the other hand, in VCO conditions gave similar size of particles size droplets in both primary and secondary emulsions as the comparing the particles size pictures show in figure 19 and figure 20.

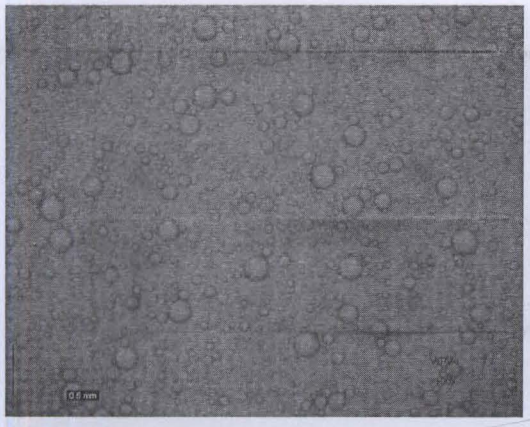


GAS-1 **GAS-2**

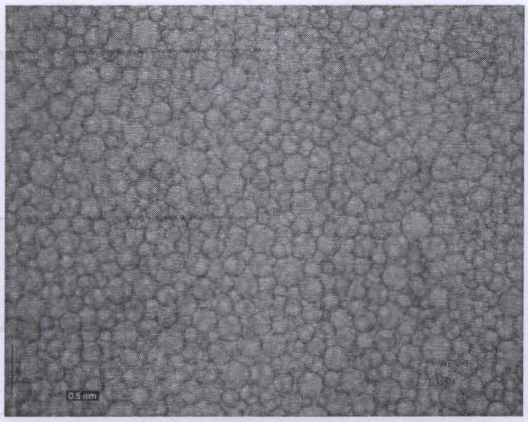
GAV-1 **GAV-2**

WPS-1 **WPS-2**

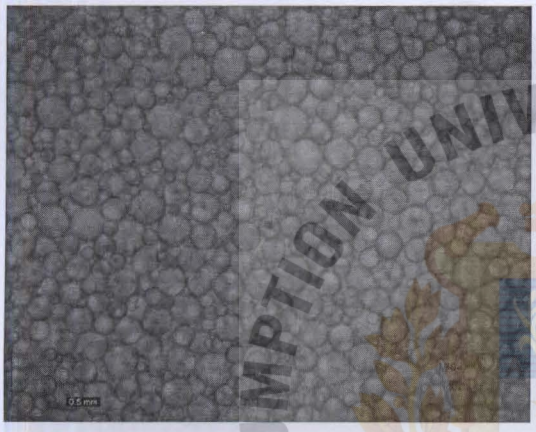
Figure 21 The microscopic observation of oil droplets size at magnification 100x where GA is Gum Arabic emulsifier, WP is whey protein emulsifier, MI is mixed GA+WP (1:1) emulsifier, S is Spent Coffee Ground infused, V is Virgin Coconut Oil, 1 is first emulsion.



WPV-1

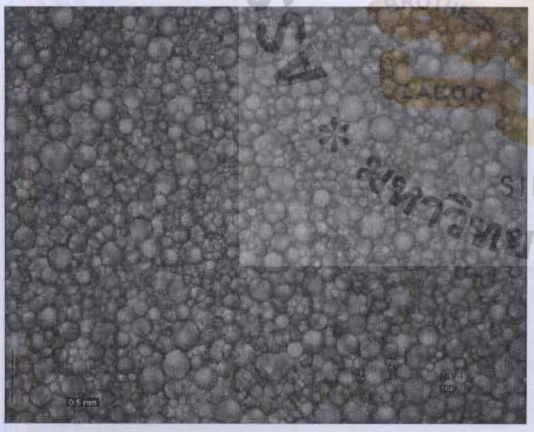


WPV-2

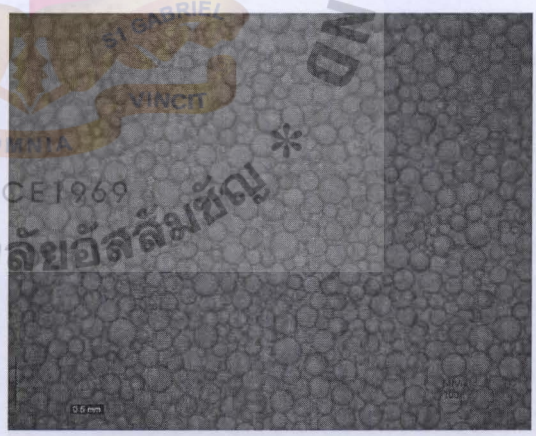


MIS-1

MIS-2

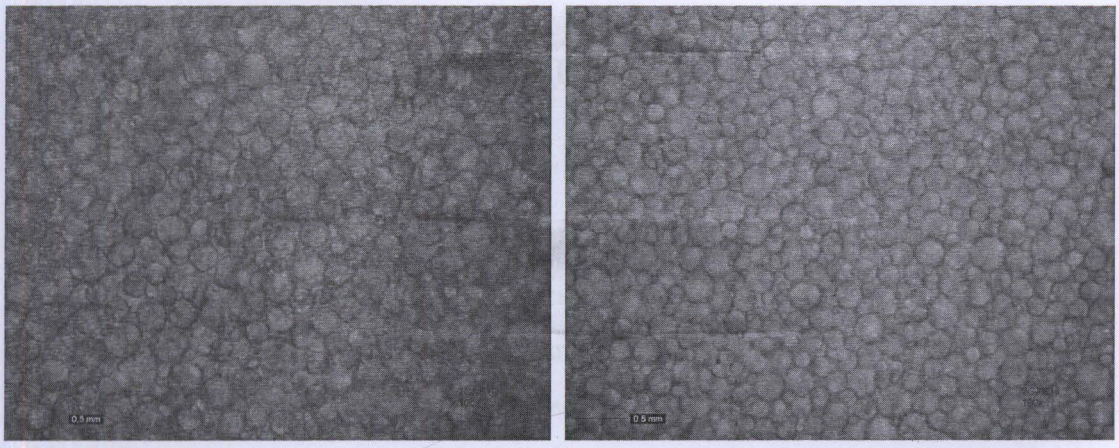


MIV-1

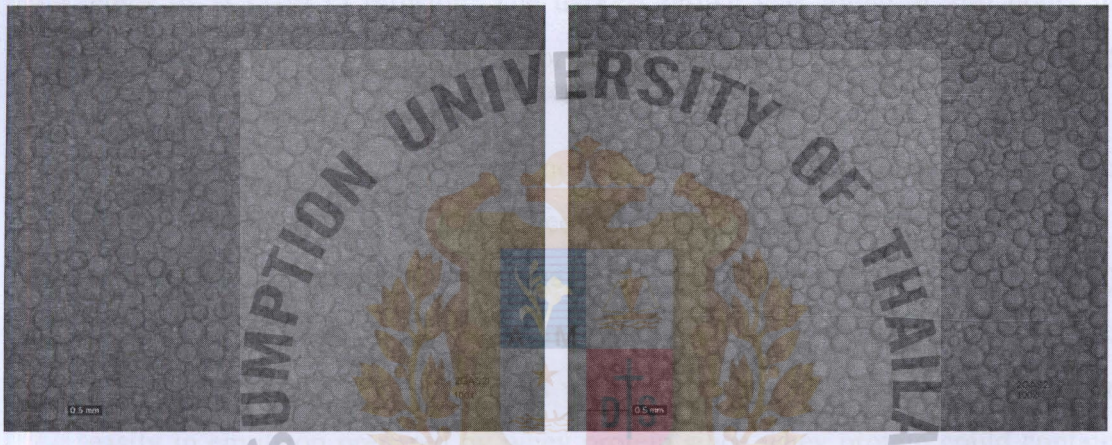


MIV-2

Figure 22 The microscopic observation of oil droplets size at magnification 100x where GA is Gum Arabic emulsifier, WP is whey protein emulsifier, MI is mixed GA+WP (1:1) emulsifier, S is Spent Coffee Ground infused, V is Virgin Coconut Oil, 1 is first emulsion, and 2 is second emulsion.



GAS1-i **GAS1-ii**



GAS2-i **GAS2-ii**

GAS3-i **GAS3-ii**

Figure 23 The varying Gum Arabic concentration in SCG infused microscopic observation results, where 1 is 2% GA concentration, 2 is 4% GA concentration, and 3 is 6% GA concentration. (i) is primary emulsion and (ii) second emulsion.

CHAPTER V

CONCLUSION

1. Heating condition at 42°C during the VCO infusion process can enhance the efficiency of extraction of total phenolic compounds from SCG. Additionally, longer duration time of infusion can promote highest TPC as 237.38 0.2 mg GAE/1 ml sample which found in heated condition for 240 hours.
2. Increasing of infusion time and infusion temperature can develop the brown color in SCG-VCO infusion. The darkest brown was found at 240 hours in heated condition, the intensity of brown color indicated the transformation of Chlorogenic acid to melanoidins.
3. The condition that can provide the most effective in half inhibition concentration (IC50) (158.08 mg/ml) was heated condition for 240 hours.
4. Mixed GA+WP at ratio 1:1 was the best emulsifier among Gum Arabic (GA) and Whey protein (WP) as creaming index (%CI) was lowest at 85.24%±0.8. However, emulsion was not added with preservative so spoilage could occur easily in emulsion prepared by whey protein since whey protein could contain nutrients providing for microorganism growth. In addition, emulsion prepared by gum Arabic showed the lower rate of spoilage.
5. The varying of Gum Arabic at 2, 4, and 6% (w/v), oil particles size of SCG infused VCO in secondary emulsion (0.129mm±0.01) was smaller than primary emulsion (0.17mm±0.01). While in VCO both primary and secondary emulsion particles size were occurred in the same size at 0.14mm±0.03 and 0.13mm±0.01 but secondary emulsion was more fine dispersion than primary emulsion.
6. The SCG infused VCO in heated condition for 240 hr with 4% GA(w/w) could perform the most effective stability emulsion where the %CI shows 89.5±0.3%.
7. The SCG infused in VCO in heated condition for 240 hr with 4% GA(w/w) could be suggested as the most suitable to perform an emulsion for further study of freeze-drying.

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APPENDIX

Antioxidant activity DPPH scavenging assay

- Control 0.1mM DPPH solution [0.1217] [1.194] [1.191]

Table 1 The raw data of percent inhibition of SCG-infused in VCO at 24, 120, and 240 hrs with various concentration of SCG infused sample (100, 200, 300, and 400 mg/ml) and pure VCO of the unheated condition.

24 hrs (RT)						
Concentration (mg/ml)	**Sample no. 1**			**Sample no. 2**		
	Absorbant at 517 nm.					
	[1]	**[2]**	**[3]**	**[1]**	**[2]**	**[3]**
VCO	0.849	0.853	-	-	-	-
100	0.774	0.755	0.753	0.751	0.769	0.757
200	0.623	0.646	0.676	0.614	0.609	0.593
300	0.448	0.455	0.449	0.359	0.441	0.360
400	0.314	0.307	0.303	0.237	0.208	0.128
120 hrs (RT)						
Concentration (mg/ml)	**Sample no. 1**			**Sample no. 2**		
	Absorbant at 517 nm.					
	[1]	**[2]**	**[3]**	**[1]**	**[2]**	**[3]**
VCO	0.840	0.842	-	-	-	-
100	0.747	0.753	0.750	0.756	0.740	0.750
200	0.604	0.611	0.606	0.630	0.609	0.613
300	0.407	0.410	0.375	0.398	0.418	0.396
400	0.234	0.224	0.203	0.230	0.217	0.225
240 hrs (RT)						
Concentration (mg/ml)	**Sample no.1**			**Sample no.2**		
	Absorbant at 517 nm.					
	[1]	**[2]**	**[3]**	**[1]**	**[2]**	**[3]**
VCO	0.808	0.810	-	-	-	-
100	0.785	0.780	0.768	0.741	0.736	0.718
200	0.633	0.634	0.647	0.611	0.565	0.559
300	0.411	0.399	0.368	0.297	0.295	0.307
400	0.251	0.222	0.203	0.072	0.098	0.112

Table 2 The raw data of percent inhibition of SCG infused in VCO at 24, 120, and 240 hrs with various concentration of SCG infused sample (100, 200, 300, and 400 mg/ml) and pure VCO at heated condition (42^{O}C).

24 hrs (H)						
Concentration (mg/ml)	**Sample no.1**			**Sample no.2**		
	Absorbant at 517 nm.					
	[1]	[2]	[3]	[1]	[2]	[3]
VCO	0.845	0.842	-	-	-	-
100	0.737	0.740	0.725	0.737	0.740	0.725
200	0.601	0.601	0.603	0.601	0.603	0.623
300	0.359	0.369	0.369	0.378	0.372	0.361
400	0.203	0.187	0.218	0.179	0.187	0.174
120 hrs (H)						
Concentration (mg/ml)	**Sample no.1**			**Sample no.2**		
	Absorbant at 517 nm.					
	[1]	**[2]**	**[3]**	**[1]**	**[2]**	**[3]**
VCO	0.839	0.830	-	-	-	-
100	0.748	0.717	0.721	0.710	0.687	0.697
200	0.637	0.627	0.607	0.526	0.536	0.540
300	0.402	0.390	0.409	0.322	0.315	0.308
400	0.200	0.263	0.133	0.198	0.158	0.234
240 hrs (H)						
Concentration (mg/ml)	**Sample no.1**			**Sample no.2**		
	Absorbant at 517 nm.					
	[1]	**[2]**	**[3]**	**[1]**	**[2]**	**[3]**
VCO	0.808	0.805	-	-	-	-
100	0.744	0.716	0.726	0.720	0.698	0.699
200	0.602	0.582	0.571	0.518	0.526	0.523
300	0.362	0.318	0.310	0.211	0.218	0.209
400	0.224	0.188	0.175	0.018	0.032	0.006

Table 3 Raw data standard Trolox equivalent at 517nm.

concentration (µl/ml)	ABS		
	No.1	No.2	No.3
control	0.961	0.961	0.954
90.00	0.505	0.429	0.468
45.00	0.715	0.686	0.653
22.50	0.803	0.806	0.784
11.25	0.864	0.883	0.875
5.625	0.919	0.929	0.914

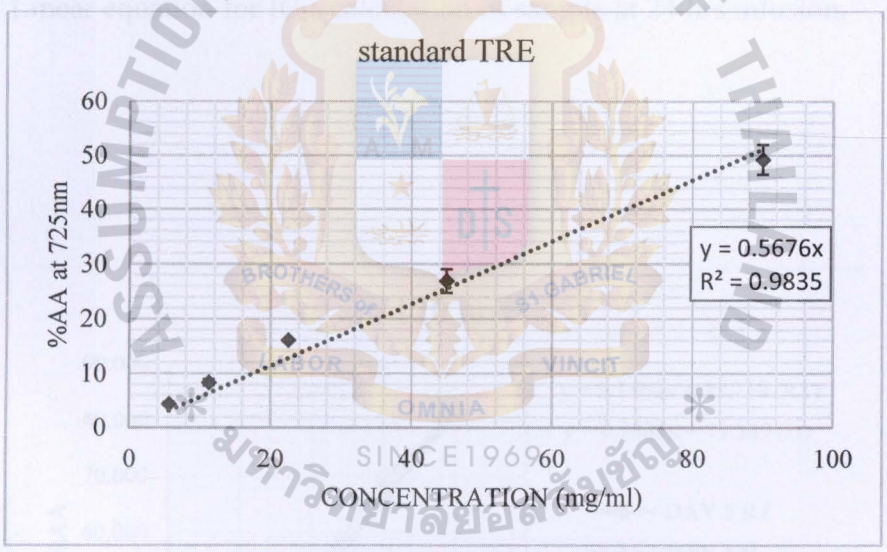

Figure 1 The standard Trolox equivalent.

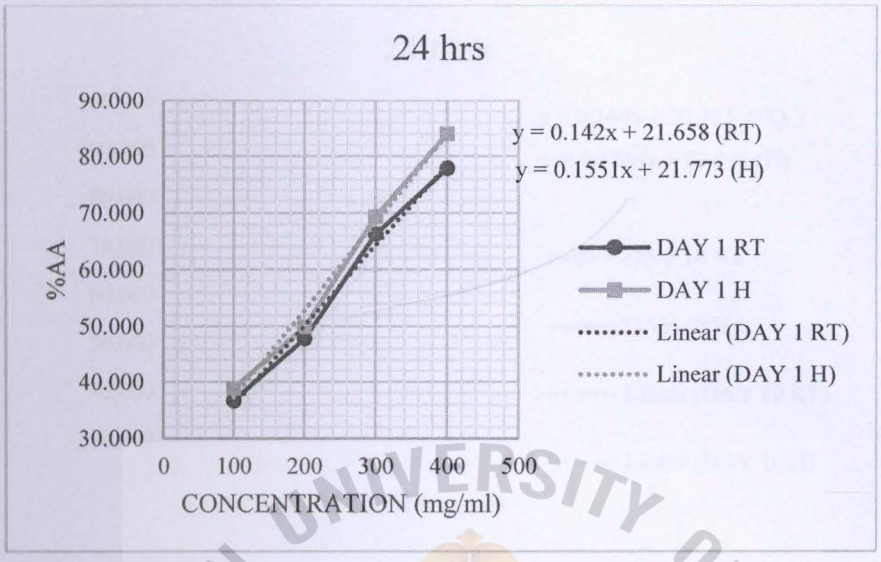

Figure 2 Linear equation for IC_{50} calculation of sample at 24 hrs infusion.

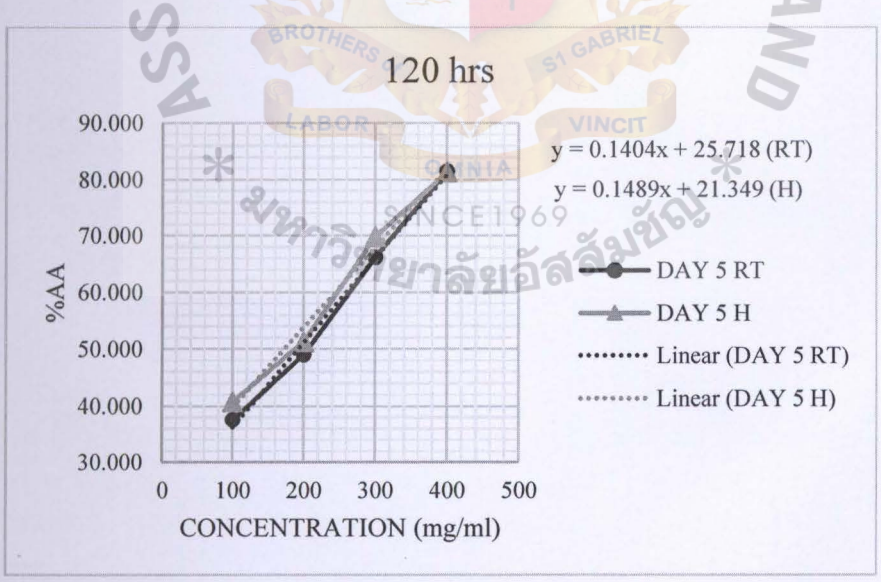

Figure 3 Linear equation for IC50 calculation of sample at 120 hrs infusion.

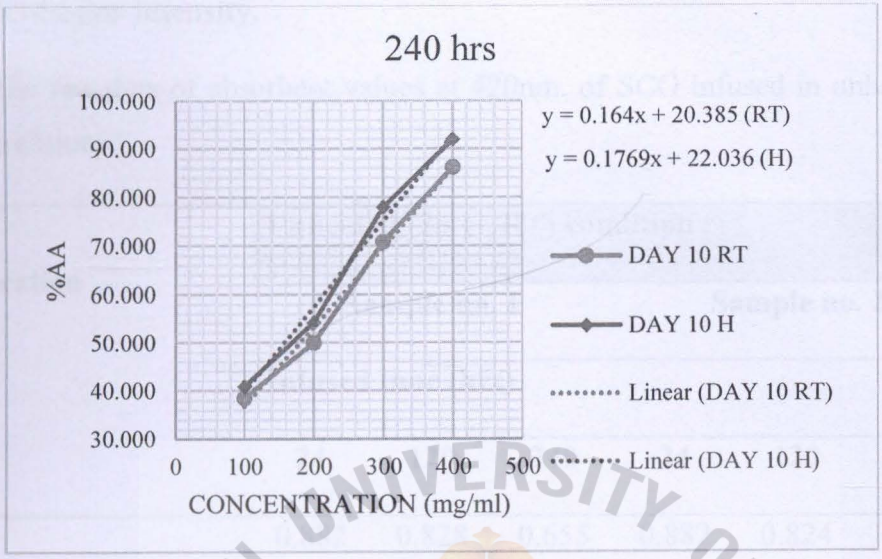

Figure 4 Linear equation for IC50 calculation of sample at 240 hrs infusion.

Raw data of color intensity.

Table 4 the raw data of absorbent values at 420nm. of SCG infused in unheated and heated conditions.

Duplication no.	Unheated (25°C, RT) condition					
	Sample no. 1			Sample no. 2		
	Infused time (hrs)					
	24	120	240	24	120	240
1	0.882	0.828	0.655	0.882	0.824	0.562
2	0.944	0.868	0.562	0.842	0.791	0.609
Duplication no.	**Heated (42°C) condition**					
	Sample no. 1			**Sample no. 2**		
	Infused time (hrs)					
	24	**120**	**240**	**24**	**120**	**240**
1	0.937	0.982	1.037	0.611	0.864	0.945
2	0.910	1.005	1.084	0.592	0.805	1.018

Table 5 The raw data of pure VCO color intensity of heated and unheated conditions.

Duplication No.	Unheated (25°C)			Heated (42°C)		
	Infused time (hrs)					
	24	120	240	24	120	240
1	0.054	0.058	0.059	0.081	0.082	0.078
2	0.058	0.052	0.040	0.078	0.072	0.074

Phenolic compounds

Table 6 The raw data of OD values phenolic compounds content of SCG infused measure at 765nm.

Time (hrs)	Replication No.	OD at 765nm					
		Unheated (RT)			Heated (42^{O}C)		
		[1]	[2]	[3]	[1]	[2]	[3]
24	**1**	0.571	0.542	0.550	0.522	0.465	0.531
	2	0.397	0.400	0.383	0.430	0.394	0.428
120	**1**	0.540	0.497	0.499	0.707	0.547	0.421
	2	0.425	0.439	0.434	0.434	0.447	0.447
240	**1**	0.428	0.443	0.388	0.521	0.491	0.487
	2	0.448	0.453	0.447	0.448	0.462	0.486

Table 7 Raw data of optical density (OD) of phenolic compounds in pure VCO in heated and unheated conditions measured by UV-spectrophotometer at 765 nm.

Time	Unheated (RT)			Heated (42^{O}C)		
(hrs)	[1]	[2]	[3]	[1]	[2]	[3]
24	0.315	0.323	0.275	0.646	0.613	0.666
120	0.463	0.448	0.448	0.81	0.83	0.951
240	0.63	0.586	0.637	0.828	0.73	0.771

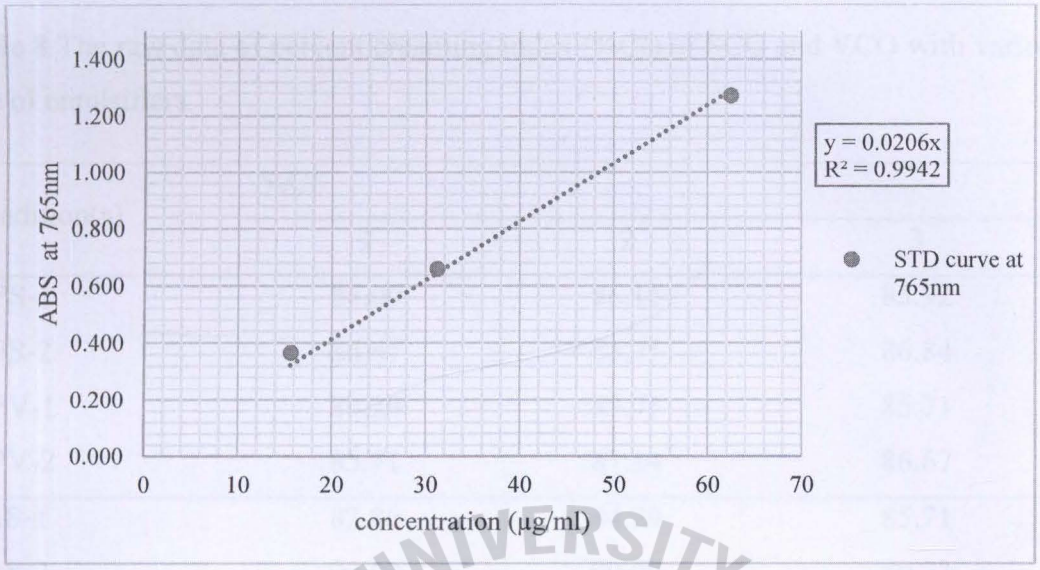

Figure 5 The standard curve of garlic acid equivalent (GAE/ml sample)

Table 8 The raw data of percent creaming index (%CI) of SCG and VCO with various type of emulsifiers.

Condition(s)	%CI		
	1	2	3
WPS-1	84.00	86.11	85.92
WPS-2	86.67	85.71	86.84
WPV-1	84.29	85.71	85.71
WPV-2	85.71	87.14	86.67
GAS-1	82.86	84.29	85.71
GAS-2	90.00	90.00	89.23
GAV-1	81.94	81.43	82.86
GAV-2	87.14	90.00	90.00
MIS-1	81.43	82.86	82.86
MIS-2	84.29	85.71	85.71
MIV-1	85.71	85.71	85.51
MIV-2	85.51	85.71	85.71

Where; wp is whey protein, GA is Gum Arabic, MI is mixed between wp and GA at ratio (1:1)

Table 9 The raw data of percent creaming index (%CI) of Gum Arabic concentration at 2,4, and 6% (w/w) between GAS and GAV at first emulsion and second emulsion.

	Hight (cm)				Hight (cm)		
		Time (hour)				Time (hour)	
condition		24	48	condition		24	48
GAS i	T0	T1	T2	GAS ii	T0	T1	T2
	7.0	5.9	5.8		7.0	6.4	6.3
2%	7.0	5.8	5.8	2%	7.0	6.3	6.2
	7.0	5.9	5.9		7.0	6.3	6.2
	7.0	5.7	5.8		7.0	6.3	6.3
4%	7.2	5.9	5.8	4%	7.0	6.3	6.2
	7.0	5.7	5.8		7.0	6.3	6.3
	7.0	5.7	5.8		7.0	6.3	6.3
6%	7.0	5.7	5.8	6%	7.0	6.4	6.3
	7.3	5.6	5.8		7.0	6.4	6.2
GAV i	T0	T1	T2	GAV ii	T0	T1	T2
	7.0	6.0	6		7.0	6.5	6.4
2%	7.0	5.8	6	2%	7.0	6.4	6.4
	7.0	5.8	6		7.0	6.4	6.4
	7.0	6.0	5.7		7.5	6.4	6.2
4%	7.0	5.8	5.8	4%	7.0	6.3	6.2
	7.0	5.8	5.9		7.0	6.3	6.1
	7.0	6.0	5.9		7.0	6.4	6.3
6%	7.0	5.9	5.9	6%	7.0	6.4	6.4
	7.0	5.9	6.0		7.0	6.4	6.4