APPLICATION OF KONJAC GLUCOMANNAN TO ENCAPSULATE AND STABILIZE THE CURCUMIN IN MILK SYSTEM

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การประยุกต์บุกกลูโคแมนแนนเพื่อกักเก็บและเสริมความคงตัวของขมิ้นชันในระบบนม

น.ส.อาฟวา ฮายุนิงทียาส

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อาฟวา ฮายุนิงทียาส : การประยุกต์บุกกลูโคแมนแนนเพื่อกักเก็บและเสริมความคงตัวของขมิ้นชันในระบบนม. (APPLICATION OF KONJAC GLUCOMANNAN TO ENCAPSULATE AND STABILIZE THE CURCUMIN IN MILK SYSTEM) อ.ที่ปรึกษาหลัก : ชาลีดา บรมพิชัยชาติกุล, อ.ที่ปรึกษาร่วม : สาริศา สุริยรักษ์

งานวิจัยนี้ศึกษาอิทธิพลของความเข้มข้นของบุกกลูโคแมนแนนที่ระดับความเข้มข้น 0, 0.1, 0.2, 0.3% w/v ต่อเสถียรภาพของอิมัลชันเคอร์คูมินในระบบน้ำนม โดยใส่น้ำมันที่มีเคอร์คูมินผสมอยู่ ในระดับ 10%, 20% และ 30% v/v ตรวจวัดดัชนีการเกิดครีม (creaming index) ความเข้มข้นของเคอร์คูมิน ความหนืดและสี เพื่อตรวจสอบความเสถียรของระบบอิมัลชันดังกล่าว พบว่าระบบอิมัลชันมีความเสถียรทางกายภาพ (physical stability) จนถึงวันที่ 14 ของการเก็บรักษาที่อุณหภูมิ 4 °C จากนั้นวิเคราะห์ฤทธิ์การต้านอนุมูลอิสระ ขนาดอนุภาค ค่าศักย์ซีต้า และความสามารถในการปลดปล่อยในระบบทางเดินอาหารจำลอง (GIT) ผลการวิจัยชี้ให้เห็นว่า การเพิ่มความเข้มข้นของบุกกลูโคแมนแนนส่งผลต่อความหนืดในวัฏภาคน้ำอย่างมีนัยสำคัญ นอกจากนั้นยังส่งผลต่อความหนืดโดยรวมของระบบอิมัลชัน ซึ่งผลของความหนืดมีความสัมพันธ์กับเสถียรภาพของครีม (creaming stability) กล่าวคือ เมื่อเพิ่มความเข้มข้นของกลูโคแมนแนนขึ้นจาก 0.1% เป็น 0.2% w/v ทำให้ปริมาณน้ำมันที่มีเคอร์คูมินผสมอยู่เพิ่มมากขึ้น แต่อย่างไรก็ตามระบบอิมัลชันที่มีบุกกลูโคแมนแนน 0.3% w/v กลับมีเสถียรภาพของครีมลดลง เพราะความเข้มข้นของบุกกลูโคแมนแนนเพิ่มมากขึ้น สามารถขัดขวางการรวมตัวของน้ำมันเป็นชั้นครีม (depletion flocculation) และพบว่าความเข้มข้นของบุกกลูโคแมนแนนที่มากขึ้น ไม่ส่งผลต่อความเข้มข้นของเคอร์คูมินและค่าสีเหลือง (b^* value) ในระบบอิมัลชัน แต่การเพิ่มขึ้นของวัฏภาคน้ำมันและค่าสีเหลืองของระบบอิมัลชันขึ้นอยู่กับการเพิ่มความเข้มข้นของเคอร์คูมิน เพราะเนื่องด้วยปริมาณเคอร์คูมินที่มากขึ้นในวัฏภาคน้ำมัน โดยระบบอิมัลชันที่มีน้ำมัน 20% v/v มีความสามารถในการกักเก็บสารเคอร์คูมินได้ดีกว่าเมื่อเปรียบเทียบกับที่ 10% v/v และ 30% v/v อาจเป็นเพราะระบบมีความเข้มข้นของอิมัลซิไฟเออร์ที่พอเหมาะ อันได้แก่ โปรตีนในน้ำนม และบุกกลูโคแมนแนน ซึ่งมากพอที่จะห่อหุ้มเม็ดไขมันที่มีสารเคอร์คูมิน ในขณะที่ฤทธิ์การต้านอนุมูลอิสระ จากการทดสอบด้วยวิธี DPPH และ FRAP แสดงให้เห็นว่าฤทธิ์ต้านอนุมูลอิสระของอิมัลชันยังคงมีเสถียรภาพตลอด 14 วันของการเก็บรักษาที่ 4 °C ซึ่งแสดงถึงความคงตัวของสารเคอร์คูมินในระบบ แต่ความเข้มข้นของบุกกลูโคแมนแนนไม่ได้ส่งผลต่อฤทธิ์การต้านอนุมูลอิสระ แต่บุกกลูโคแมนแนนในวัฏภาคน้ำกลับมีผลต่อการปลดปล่อยสารเคอร์คูมินในระบบทางเดินอาหารส่วนต้น ดังนั้นการผสมบุกกลูโคแมนแนนปริมาณต่ำในวัฏภาคน้ำจึงสามารถประยุกต์ใช้ในอิมัลชันเคอร์คูมินในระบบน้ำนม เพื่อเพิ่มความคงตัวของระบบอิมัลชันในระบบน้ำนมที่มีวัฏภาคน้ำมันมาก และสามารถควบคุมการปลดปล่อยของสารกักเก็บได้

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Afwa Hayuningtyas : APPLICATION OF KONJAC GLUCOMANNAN TO ENCAPSULATE AND STABILIZE THE CURCUMIN IN MILK SYSTEM. Advisor: Assoc. Prof. Dr. CHALEEDA BOROMPICHAICHARTKUL Co-advisor: Dr. Sarisa Suriyarak

This study investigated the influence of different concentration of konjac glucomannan (KGM) (0, 0.1, 0.2, 0.3 % (w/v)) on the stability of curcumin emulsion in milk system containing 10%, 20%, and 30% (v/v) oil volume fraction. The data of creaming index, curcumin concentration, apparent viscosity, and color were obtained to investigate the stability of the system. Emulsions that reached the physical stability until 14 days of storage at 4°C were subjected to further analysis including antioxidant, particle size, zeta potential, and bioaccessibility by the simulated gastrointestinal tract (GIT). The result suggested that increasing KGM concentration significantly increased the viscosity of the water phase, following by increasing the viscosity of the emulsion system. The viscosity result is related to the increase of creaming stability when KGM concentration is increased from 0.1% to 0.2% w/v KGM containing higher oil volume fraction. However, emulsions containing 0.3% KGM showed poor creaming stability due to depletion flocculation. The result showed that increasing KGM concentration had no significant impact on the concentration of curcumin and had no significant impact on the yellowness (*b** value) in the final emulsion. However, the increased oil phase volume fraction significantly increased the curcumin concentration and *b** value of emulsions due to the increasing of curcumin content that added together with oil into the system. The emulsions containing 20% v/v oil significantly showed a better loading capacity compared to 10% v/v and 30% v/v oil, suggesting that there were a sufficient concentration of native emulsifier (milk protein) and KGM to cover and stabilize oil droplet containing curcumin in 20% v/v oil. DPPH and FRAP assay showed the antioxidant activity of emulsion remained stable over 14 days of storage at 4°C and was related to the concentration of curcumin in the system. However, there is no significant effect of KGM concentration on the antioxidant activity. Addition of KGM can potentially slower the release of curcumin from the emulsion droplet during the upper part of GIT. The result indicated that introducing KGM to the water phase of emulsions is feasible to achieve a controlled release of curcumin from emulsions. Finding in this study denoted that structuring water phase with the low concentration of KGM could be possible to design curcumin in milk system containing high oil-phase fraction with potential emulsion stability and control release properties.

Field of Study:	Food Science and Technology	Student's Signature .....
Academic Year:	2018	Advisor's Signature .....
		Co-advisor's Signature .....

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Afwa Hayuningtyas

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INTRODUCTION

1.1. Research Background

Curcumin, one of the active components within the turmeric plant (*Curcuma longa)*, has been globally investigated and demonstrated by researchers on their potential health benefits and disease risk reduction. Despite curcumin's potential health benefit, curcumin needs a great strategy to be delivered effectively in human body as it is a highly lipophilic compound, poor stability, and low oral bioavailability highly limit its application in food and beverage system. Encapsulation is established as a great strategy to protect functional compounds from adverse conditions by incorporating them within the encapsulation droplet core. Emulsion system is one such encapsulated template which has been largely developed to overcome the limitations of lipophilic bioactives. However, limited study is reported about the influence of modifying aqueous phase on the physicochemical stability of the curcumin in milk system and the digestion of emulsion droplets during the gastrointestinal tract.

Natural polysaccharide, due to their high availability, edibility, excellent physicochemical stability, and low cost, has been widely used to stabilize an emulsion by several mechanisms. Previous studies have reported that adding polysaccharide into the aqueous phase of the whey protein-stabilized emulsion as a second stabilizer can significantly improve emulsion stability by modifying the droplet interface, gelation, forming network structure in the water phase, or forming a double-layer interface (Dickinson, 2011, Bouyer et al., 2012b). Konjac glucomannan (KGM), a polysaccharide from tubers of *Amorphophallus muelleri,* is reported to possess many beneficial health effects (Chua et al., 2010, Lu et al., 2018). The previous study also reported that simply structuring oil in water protein-stabilized emulsions with KGM, resulting in better emulsion stability to encapsulate carotenoid (Lu et al., 2018, Hu et al., 2016). The free KGM enhances the viscosity of emulsion by structuring the water phase and thus limiting the mobility of oil droplets (Bouyer et al., 2012b).

In this study, milk system will be used as carrier to encapsulate curcumin, which will be previously dissolved in medium chain triglyceride (MCT) oil. Milk has been

studied on its potential application as carriers to deliver herbs and nutraceuticals for targeted health benefits (Sawale et al., 2012). Milk can provides several functional properties due to the diversity of milk proteins, several studies have been proved for milk utilization as carrier template of encapsulation and delivery of bioactive (Tavares et al., 2014). The nature milk proteins, mainly the group of casein, can be used as carriers of lipophilic molecules as they are excellent surface agents played an important role in the emulsion formation containing lipophilic bioactive (Tavares et al., 2014). Introducing KGM as stabilizer can form a chain structure in the water phase. The network structure can inhibit the creaming separation of emulsion by limiting the movement of oil droplets by the effect of steric hindrance (Lin et al., 2017). Therefore, the effect of structuring water phase of curcumin emulsion in milk system by KGM will be investigated in this study on the physicochemical properties and bioaccessibility of curcumin after passing through simulated gastrointestinal tract (GIT) digestion.

1.2. Objectives

i. To investigate the effect of different formulations of curcumin emulsion containing a various concentration of KGM on the emulsion stabilities
ii. To investigate the effect of adding KGM on physicochemical properties and bioaccessibility of curcumin emulsion in milk system during the storage period

1.3. Hypothesis

Konjac glucomannan can be used as a potential emulsifier and stabilizer to encapsulate and stabilize the curcumin in milk system.

CHAPTER 2
LITERATURE REVIEW

2.1. Curcumin

2.1.1. Structural Characteristics

Curcumin is a hydrophobic polyphenol, also known as diferuloylmethane, which has a symmetric molecule with the IUPAC name 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione (Wiggers et al., 2017a). Curcumin is the major constituent in the rhizome of *Curcuma longa,* which contains approximately 77% diferuloylmethane (curcumin), 17% demethoxycurcumin, and 6% bisdemethoxycurcumin (Priyadarsini, 2014). The chemical formula of curcumin is $C_{21}H_{20}O_6$ and it has the structure of a diferuloyl linked by methane with the molecular weight of 368.38. Figure 2.1 shows the chemical structure of curcumin. The structure contains the two aryl rings containing orthomethoxy phenolic group (OH-) are linked to a diketone moiety.

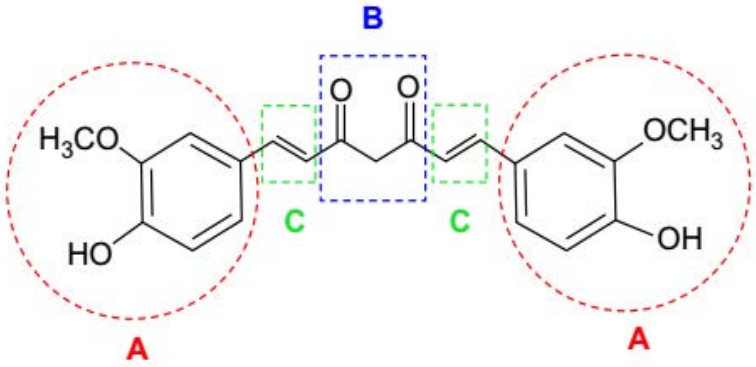

Figure 2. 1 Chemical structure of curcumin containing the functional regions: (A) aromatic rings, (B) b-diketone, and (C) olefinic linker. (Wiggers et al. (2017b)

2.1.2. Curcumin Benefits

Many previous studies have reported a broad variety of the beneficial effect of curcumin. A report by Mina et al. (2017) studied the therapeutic application of curcumin based on its potential antitumor properties in several tumors, including breast, pancreatic, colorectal, and prostate cancer. Curcumin has also been established for its biological effects, including antioxidant, antiangiogenic, anti-inflammatory, anticancer, and wound healing

(Maheshwari et al., 2006). The most primary mechanism that describes the majority of the biological effect of curcumin are antioxidant and anti-inflammatory properties (Hewlings and Kalman, 2017). One example of a condition that affected by inflammation is one such chronic joint disease, osteoarthritis. Daily et al. (2016) reported the potential application of the 8-12 weeks curcumin treatment, 1000 mg/day of curcumin, in decreasing arthritis symptoms. As systemic inflammation has been related to several Meta syndrome, curcumin has also been reported to improve insulin resistance (Na et al., 2013), weaken hypertension (Hlavačková et al., 2011), and lower triglyceride and cholesterol levels (DiSilvestro et al., 2012, Mohammadi et al., 2013). Curcumin is claimed as GRAS (generally regarded as safe) by The Food and Drug Administration based on findings of studies on human and animal (FDA, 2016). Numerous studies also declared that curcumin is safe even at high doses. Lao *et al.* (2006) reported from their study that increasing a high single dose of curcumin from 500 to 12,000 mg showed excellent tolerance. DiSilvestro et al. (2012) studied in healthy middle-aged people (40-60 years) that exposed by a low dose of curcumin of 80 mg/day showed potential health effect including lowering of plasma triglyceride levels, lowering of saliva amylase levels, raising of plasma catalase activities, etc. Moreover, according to Joint FAO/WHO Expert Committee on Food Additives (JECFA) and European Food Safety Authority (EFSA) reports, adequate daily intake (ADI) value of curcumin is 0–3 mg per kg body weight (Kocaadam and Sanlier, 2017).

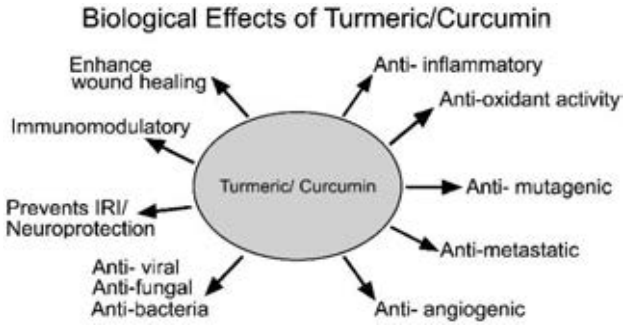

Figure 2. 2 The Schematic of several biological activities of turmeric/curcumin (Maheshwari et al., 2006)

2.1.3. Curcumin Limitations in Food System

Despite several advantages that curcumin has, it has several challenges that limit its application in food systems. Curcumin is a water insolubility compound due to its lipophilic nature (Bansal et al., 2011). The water insolubility of curcumin not only limits its dispersion into the food system since the food system is mainly composed of water but also its bioavailability within the gastrointestinal tract (Gómez-Estaca et al., 2015). The reason for low bioavailability of curcumin is related to low absorption, inactivity of the final product of metabolism, high rate of elimination and rapid metabolic degradation (Liu et al., 2016, Anand et al., 2007). To overcome the limitations of curcumin and to enhance the bioavailability during the gastrointestinal ingestion, several studies have reported that curcumin can be encapsulated using several delivery systems. Araiza-Calahorra et al. (2018) have reported that oil in water emulsion can be used as a vehicle to encapsulate curcumin to improve the stability and oral bioavailability of curcumin by controlling the bioaccessibility of curcumin in oil in water emulsion. Bioaccessibility is the percentage of a compound that is dissolved from food upon ingestion (Gómez-Estaca et al., 2015). Since curcumin has poor water solubility, dissolvation of curcumin plays an essential role to improve its bioaccessibility (Yu et al., 2012).

2.2. Konjac Glucomannan (KGM)

2.2.1. Source and structure

KGM is a neutral polysaccharide obtained from the tuber of *Amorphophallus muelleri* and cultivated in several parts of Asia. KGM has been used as food for nearly 2000 years in Asia (Hu et al., 2016, Yuan et al., 2018).

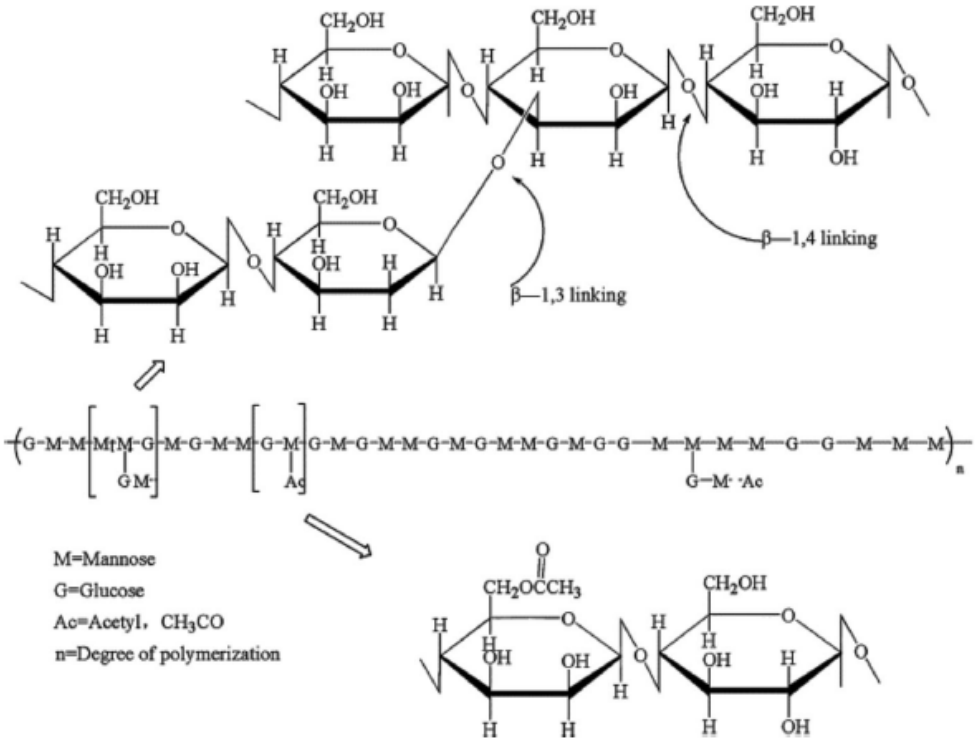

Figure 2. 3 Chemical structure of KGM (Lafarge and Cayot, 2018).

KGM is a random linear polymer, which is mainly composed of D-mannose and D-glucose linked by glucosidic bond β (1-4) at a molar ratio of 1.6:1 (Figure 2.3) (Chen et al., 2005). The distribution of D-mannose and D-glucose is mostly take turned with a small number of the chained residues, with the acetyl groups are present one in every 19 mannose residues, at 2, 3, or 6 positions (Lafarge et al., 2014, Lafarge and Cayot, 2018). Generally, the molecular weight of KGM ranges from 500,000-2,000,000, depends on the species, storage time, producing areas, and processing technologies (Zhang et al., 2014). KGM has been reported as a relatively homogenous polysaccharide by measuring its molecule weight (MW) using GPC spectrum, and the result showed that the MW distribution of KGM was found in a normal distribution (Wang et al., 2012)

2.2.2. Physicochemical properties

KGM has an excellent water solubility, yet it forms a very viscous solution in pH 5-7. The solubility can be increased by increasing temperature

and agitation rate (EU, 2012). However, KGM has poor solubility in organic solvents, including ethanol, methanol, or ether (Wang et al., 2015). As described before, KGM can form a highly viscous solution. The viscosity of 1% (w/w) KGM solution at room temperature is 31.6 Pa.s and increases exponentially with increasing KGM concentration. Shah et al. (2015) reported that the viscosity of a KGM water solution at 2% (w/w) was 12 times higher than a solution with 1% (w/w) KGM. The comparison of the viscosity of KGM solution with another hydrocolloid at the same concentration (1% w/w) such as guar gum 4.2 Pa s, κ-carrageenan 0.3 Pa.s, and xanthan 8.2 Pa.s (Yoshimura et al., 1998). The reason for this finding is due to the high water sorption properties that KGM has, >100 g of water per g of KGM. The water sorption of KGM decreases with the increase of the degree of acetylation of KGM chains (Zhang et al., 2014).

Moreover, the carbonyl and hydroxyl group that KGM contains in its molecular chain contributes to the high solubility of KGM in water (Kohyama et al., 1993). The solubility of a molecule in water is linked to the intermolecular hydrogen bonding, the higher the hydrogen bonding, the harder for the molecule to dissolve in water. Also, the important factor that mainly affects the solubility of KGM in water is the degree of acetylation. The acetyl groups potentially inhibit the intermolecular hydrogen bonds, which improve its solubility in water (Alonso-Sande et al., 2009). Furthermore, in a relatively short time and without heating, the highly hydrophilic KGM can be a poor water solubility macromolecule (Chen et al., 2011).

2.2.3. Utilization of KGM

KGM has been used for a long time in some part of Asia as food and medicine. KGM is used to make tofu, and noodles in eastern cuisine. In traditional Chinese medicine, KGM gel has been used for detoxification, tumor treatment, decreasing blood pressure, and for decades has been used in China to treat several diseases such as cough, asthma, breast pain, skin disease, and hernia (Chua et al., 2010). The introduction of KGM as a food additive and a small scale supplement to the United States and Europe was

established in the past two decades followed by discovery in the form of a capsule, food, and beverage system. Moreover, previous clinical studies have reported that introducing KGM to the diet can significantly contribute in decreasing plasma cholesterol, enhancing carbohydrate metabolism, colonic ecology, and bowel movement (Chua et al., 2010). As a food additive, KGM is authorized in Europe as E-425. KGM is declared as GRAS by the FDA. It also presents excellent functional properties and is claimed as a low-calorie ingredient due to its non-digestible fiber compound. Therefore, KGM has a great potential to be developed as a functional food with various health benefit (Jiménez-Colmenero et al., 2012). KGM is also reported that are largely used as emulsifier and stabilizer in food, drinks, and cosmetic products due to its gelling properties and incomparable rheological (Behera and Ray, 2017). Moreover, the ability of KGM as to be used as drug delivery has also been highly studied and used with controlled release properties, bioadhesive properties, and cellular therapy (Zhang et al., 2014).

2.3. Milk as a carrier delivery system

2.3.1. Milk properties

Milk is a major constituent of the daily diet for human in several countries of the world. Milk is an oil-in-water emulsion which the fat droplet is diluted in milk plasma and consists of a principal component that is shown in Table 2.1.

Table 2. 1 Approximate Milk Composition (Walstra et al., 1999)

Component	Average composition (% w/w)	Range (% w/w)
Water	87.1	85.3-88.7
Lactose	4.6	3.8-5.3
Fat	4.0	2.5-5.5
Protein	3.3	2.3-4.4
Casein	2.6	1.7-3.5
Mineral substances	0.7	0.57-0.83

Since milk is oil in water emulsion, water is the most largely component present in the system. Lactose is the special disaccharide in milk which composed two monosaccharides: galactose and glucose. The fat is composed of triglycerides which the fatty acid component vary in the chain length (2-20

carbon atoms) and saturation (0-4 double bonds). Milk also contains other lipids, including phospholipids, diglycerides, free fatty acids, and cholesterol (Walstra et al., 1999). Milk proteins consist of the group of caseins and the group of whey proteins. Casein is the main group of milk protein composed of around ten different kinds of proteins. Caseins consist of the hydrophobic and hydrophilic part, which play an important role in its emulsifying properties (Elzoghby et al., 2011). The remainder is whey proteins which are often called serum protein and are globular proteins. The mineral components in milk composed of K, Ca, NA, CI, Mg, and phosphate.

2.3.2. Milk protein

Despite its function in protecting the immunological system for infant, the components and properties of milk have a positive impact on its utilization in the food system (Ranadheera et al., 2016). Several approaches described the application of milk as a carrier of encapsulation bioactives and for its delivery to the human body due to the functional and structural properties that it has. In the delivery system, several mechanisms can describe the binding of milk protein to the hydrophobic molecules, dominantly hydrogen bonds, hydrophobic interaction, and van der Waals attraction (Livney, 2010). Tavares et al. (2014) reported the potential utilization of milk protein to encapsulate hydrophobic bioactive regard to its ability as excellent surface agents. The hydrophilic and hydrophobic structure of milk proteins contribute to the excellent interfacial properties, make it can attach to the oil-water surface and stabilize emulsions (Livney, 2010). The overview of protein in milk presents in Table 2.2.

Table 2. 2 Protein composition in Milk (Walstra et al., 1999)

Protein	Mmol/m^3 milk	g/kg milk	g/100g protein	Molar mass
Casein	1170	26	78.5	
α_{s1}-Casein	440	10.0	31	~23600
α_{s2}-Casein	110	2.6	8	~25200
β-Casein	400	9.3	28	23983
κ-Casein	180	3.3	10	~19550
γ-Casein	40	0.8	2.4	~20500
Serum protein	~320	6.3	19	
β-Lactoglobulin	180	3.2	9.8	18283
α-Lactoglobulin	90	1.2	3.7	14176
Serum albumin	6	0.4	1.2	66267
Proteose-peptone	~40	0.8	2.4	4000-40000
Immunoglobulins	~4	0.8	2.4	
IgG1, IgG2		0.65	1.8	~150000
IgA		0.14	0.4	~385000
IgM		0.05	0.2	~900000
Miscellaneous		0.9	2.5	
Lactoferrin	~1	0.1		86000
Transferrin	~1	0.1		76000
Membrane proteins		0.7	2	

Casein is the major group of protein in milk, which composed of several different kinds of proteins. The main components of casein are α_{s1}, α_{s2}, β-, κ-, and γ-Casein (Walstra et al., 1999). Caseins have the hydrophobic and hydrophilic structure, which play an important role in its emulsifying properties (Elzoghby et al., 2011). Caseins present in milk in an aggregated form called casein micelles (Walstra et al., 1999). Casein micelles have contents approximately 94% casein and the remainder 6% mineral; the most part is calcium phosphate. Whey proteins, or called as serum protein, present in milk serum in the form of the dissolved form (Tavares et al., 2014). The major serum protein in cow milk is α-lactalbumin and β-lactoglobulin. It is suggested that the serum proteins contribute to providing amino acids. Miscellaneous proteins contain various proteins, including lactoferrin, transferrin, membrane proteins, and various enzymes. The membrane proteins present in a small concentration in the plasma and the enzymes in milk proteins present in the fat globule (Walstra et al., 1999, Tavares et al., 2014).

The structural and functionality of milk proteins make them possible to be used as carriers of hydrophobic molecules. Milk protein has excellent interfacial properties which contribute to the emulsion formation and the stabilization of oil-in-water emulsions. The binding of milk protein with hydrophobic molecules can be by covalent or electrostatic complexes (Tavares et al., 2014).

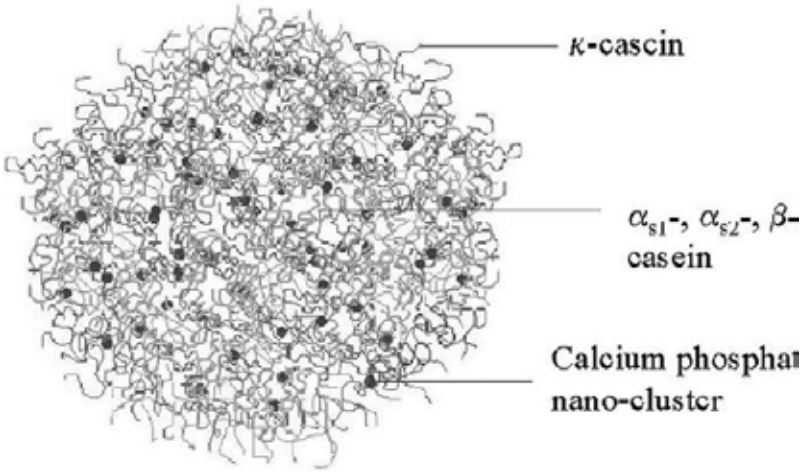

Figure 2. 4 Illustration of casein micelle (Hristov et al., 2016)

2.4. KGM-Milk protein stabilization mechanism

KGM is classified as a poor surface active polysaccharide. In many emulsion systems, the formation, functional properties, and stability of the emulsion can be improved using combinations of emulsifier (Lafarge and Cayot, 2018). Previous studies have shown that introducing polysaccharide into the water phase of protein stabilized emulsion can improve the stability of emulsion (Dickinson, 2011). The system with the combination of emulsifier can give a maximum advantage of proteins which are more surface active in the emulsion formation and polysaccharides, which can stabilize the emulsion by steric repulsion or viscosity enhancement. Indeed, both proteins and polysaccharides contribute to creating a better property of emulsion, improving stability and functionality, by their emulsifying and stabilizing ability (Bouyer et al., 2012a, Lafarge and Cayot, 2018).

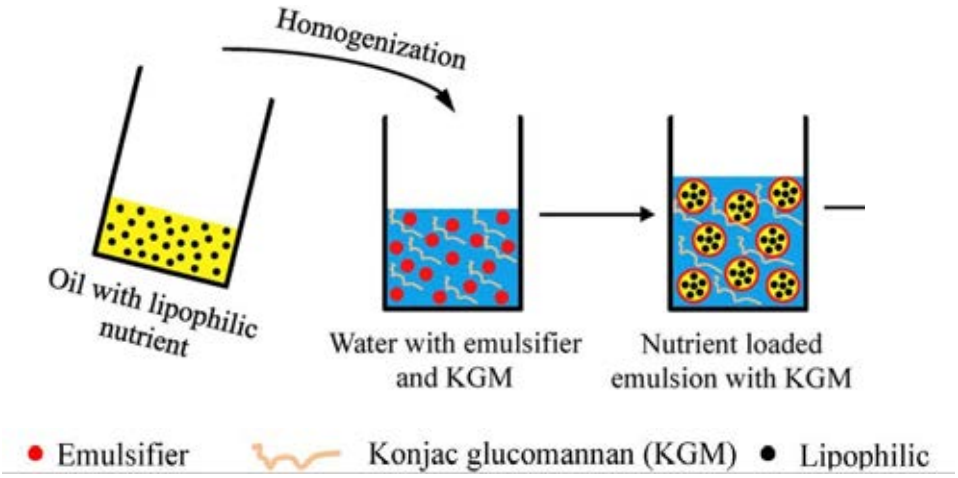

Figure 2. 5 Illustration of protein-stabilized emulsion with KGM (Lu et al., 2018)

The stabilization mechanism of polysaccharide-protein stabilized emulsion mostly contributed by complex formation by either covalent bonding or electrostatic interaction (Bouyer et al., 2012a). However, in KGM-protein stabilized emulsion the stabilization mechanism can be by polymeric stabilization (without complexation) since KGM is a non-ionic polysaccharide. The stabilization mechanism of KGM-protein stabilized emulsion consists of two-step of mechanisms (Bouyer et al., 2012a, Hu et al., 2016).

1. Protein adsorption

   The protein contributes to the small droplet formation in the emulsification process. The hydrophobic part of protein denatured at the oil-water interface and followed by fixing of the surface of the oil in the emulsion.

2. Polysaccharide stabilization (KGM)

   The hydrophobic part will interact with adsorbed protein by hydrophobic interaction and the predominant hydrophilic part will be oriented to the aqueous phase which generates an extended thickening structure, which promotes high viscosity at low shear rate, which then enhanced the steric hindrance and the further force of friction between droplets and the continuous phase, thus slowering the droplet motion of the system.

By the mechanism, both proteins and polysaccharides play a role by their emulsifying and stabilizing properties to improve a better property of emulsion with improving stability and functionality. The amphiphilic mixture enhanced the viscosity of water phase and attached well at the surface of oil-in-water with the hydrophobic side chains absorbed at the oil phase and the hydrophilic side chains oriented to the water

phase (Lafarge and Cayot, 2018). These properties indicated that introducing KGM to the water phase of emulsions could be possible to design curcumin emulsion in milk system containing MCT oil with potential emulsion stability and control release properties.

CHAPTER 3
MATERIALS AND METHODS

3.1. Materials and Instruments

3.1.1. Chemicals and reagents

No.	Chemicals and reagents	Sources
1.	Curcumin (purity >95%)	Vitajoy Biotech Co., Ltd, Sozhou, China
2.	Pasteurized and homogenized whole fat cow's milk (3.3% protein, 4.1% total fat)	Meiji Co., Ltd, Bangkok, Thailand
3.	Medium-chain triglyceride oil (MCT); Caprylic acid 60%; Capric acid 40%	Vicchi Enterprise Co., Ltd, Bangkok, Thailand
4.	Konjac Glucomannan powder	Jingyu Co., Ltd, Yunnan, China
5.	Ethanol AR-grade	Sigma-Aldrich, Singapore
6.	Methanol AR-grade	Sigma-Aldrich, Singapore
7.	Sodium azide	Sigma-Aldrich, Singapore
8.	2,2-diphenyl-1-picrylhydrazyl (DPPH)	Sigma-Aldrich, Singapore
9.	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)	Sigma-Aldrich, Singapore
10.	2,4,6-tripyridyl-s-triaze (TPTZ)	Sigma-Aldrich, Singapore
11.	Hydrochloric acid 37%	Sigma-Aldrich, Singapore
12.	Gastrointestinal track enzymes; Pepsin, Lipase, Bile salt	Sigma-Aldrich, Singapore
13.	Sodium phosphate dibasic (Na_2HPO_4)	Sigma-Aldrich, Singapore
14.	Sodium phosphate monobasic (NaH_2PO_4)	Sigma-Aldrich, Singapore
15.	Sodium Hydroxide Pellet (NaOH)	Sigma-Aldrich, Singapore

3.1.2. Instruments

No.	Chemicals and reagents	Sources
1.	Hot plate stirrer	IKA C-MAG HS7 digital, Staufen, German
2.	Ultra Turax Homogenizer	Ystral, Ballrechten-Dottingen, Germany
3.	Sonicator	Elmasonic E 70 H, Singen, Germany
4.	Spectrophotometer UV-Vis	Spectronic 20® Genesys™, Thermo Electron Corporation, Waltham, USA
5.	Centrifuge	Hettich-Zentrifugen, Universal 32 R, Germany

6.	Zetasizer	Malvern Instruments, Worcestershire, UK
7.	Oven	
8.	Fumehood	Mastap, Thailand
9.	Vortex	Vel Scientifica, China
10.	Micropipette	Mettler Toledo Rainin, Thailand
11.	Viscometer	Premium Series, Fungilab, S.A., Barcelona, Spain
12.	Minolta® chroma meter	-
13.	Incubator	Innova Incubator Shaker, Model 4080, New Brunswick, NJ, USA

3.2. Methods

3.2.1. Investigation on the formulation of curcumin emulsion in milk system with various KGM concentration

3.2.1.1. Emulsion preparation

Oil phase was prepared by the method described by Kharat et al. (2018) with modification in curcumin concentration, by dissolving 5 mg/ml powder curcumin into MCT oil. The mixture was heated to 80 °C and stirred for 2 h, and then sonicated for 20 mins. Milk-KGM suspension was prepared by adding KGM into the milk in several concentration. The three concentration of KGM was added to the milk consisted of 0%, 0.1%, 0.2%, and 0.3%, and were prepared by stirring for 4h and keeping at 4ºC overnight. The oil phase 10%, 20%, and 30% (v/v) was then mixed with the water phase using high-speed mixer at 24,000 rpm for 15 minutes. The final concentration of curcumin are 0.5 mg/ml in 10% oil, 1 mg/ml in 20% oil and 1.5 mg/ml in 30% oil. The emulsion will be subjected to the following analyses.

a. Creaming Index

Creaming index of emulsions was measured visually as reported by Akhtar et al. (2014) by measuring the creaming separation height at room temperature over 14 days. The creaming separation height can be assessed visually, and the height will be measured using the ruler. The total height of the emulsion sample was HE. HS expressed the height

of serum layer present at the bottom of the tube. The extent of creaming was calculated ed by the creaming index CI (%) (Eq. (1)):

$$CI\ (\%) = \frac{HS}{HE} \times 100 \qquad (1)$$

b. Curcumin Loading Capacity

Curcumin concentration was measured according to a calibration formula listed below:

$$Y = 0.1638X + 0.028,\ R^2 = 0.9997 \quad (2)$$

Where Y was the absorption at 425 nm, and X was the concentration of curcumin (mg/ml). The calibration curve was conducted by reading the absorption at the maximum wavelength of 425 nm of the standards curcumin in ethanol solutions.

Curcumin concentration in emulsion samples was measured using the method by Kharat et al. (2017). Curcumin emulsion samples (100 μL) were dissolved in ethanol (5,900 μL), and the mixtures were mixed well. The mixtures were then centrifuged at 2,000 rpm for 15 min, and the serum layer was collected. The absorption was measured spectrophotometer UV-Vis at 425 nm. Loading efficiency was calculated by the ratio of curcumin measured in final emulsion samples and the initial curcumin added.

c. Color Stability

The color of curcumin emulsions was analyzed by using Minolta® chroma meter. The colors were expressed in CIE units in the parameters of b^* (yellowness). Three replicate measurements were conducted.

d. Apparent viscosity

Viscosity measurements were performed to all emulsion samples at different KGM concentration and oil concentration using a digital rotational Viscometer (Premium Series, Fungilab, S.A., Barcelona,

Spain). Spindle No. R2 rotating at 200 rpm was used to give dial readings between 0 to 100 cP. A 110 ml emulsion samples were used for all sample measurements to ensure the sample was enough to cover the immersion groove on each spindle shaft. The measurements were conducted in three replications.

3.2.2. Investigation on physicochemical properties of curcumin emulsion in milk system during the storage period

3.2.2.1. Particle size analysis and zeta potential

The particle size and zeta potential of curcumin emulsion were measured by a laser particle analyzer (Zetasizer, Malvern Instruments, Worcestershire, UK). Emulsion was diluted by Phosphate Buffer Saline (PBS) pH 6.5 before testing. The refractive index (RI) of samples were at 1.334. Each sample was measured in triplicate, and the average numbers were used.

3.2.2.2. Antioxidant activity

The antioxidant activity of curcumin emulsions was analyzed by two methods: DPPH (hydrogen atom transfer and single electron transfer mechanisms) and FRAP (Ferric reducing antioxidant power). DPPH procedure was conducted according to the method of Brand-Williams et al. (1995) with some modifications. The DPPH solution was prepared by dissolving 2.5 mg in 100 ml methanol (0.025g/l). One ml samples were diluted by 2 ml ethanol. A volume of 0.1 ml of pigment was mixed with 5 ml of DPPH solution and followed by incubation for 30 minutes in the dark room. The absorbance was recorded at 517 nm using UV-vis spectrophotometer. Results were reported mg of Trolox equivalents. Trolox equivalents per g of solution (mg TE/g) were calculated using a standard curve of Trolox.

FRAP procedure was conducted according to the method of Benzie and Strain (1996). FRAP solution was prepared by mixing acetate buffer: 0.3g sodium acetate trihydrate and1.6 ml acetic acid glacial in water to be 100

ml (pH 3.6), ferric chloride ($FeCl_3$): 0.054 g FeCl3 in 10 ml deionized water, and TPTZ (tridyltriazine): 0.0312 g in 0.04 M HCl 10 ml in the ratio of 10:1:1, respectively. A hundred ml samples were diluted by 5900 ml ethanol. The 210 µL of the sample solution is mixed with 3.99 ml FRAP solution and stored for 30 min in the dark condition. The absorbance was recorded at 593nm using UV-vis spectrophotometer. The results will be reported as mg of Trolox equivalents per g of solution (mg TE/g) using a standard curve of Trolox.

3.2.2.3. Curcumin concentration and loading capacity of curcumin

Curcumin concentration and loading capacity of curcumin in the emulsion were measured as described in 3.2.1.1.b

3.2.3. Investigation on bioaccessibility of curcumin emulsion milk system

Bioaccessibility was tested to all of the treatment by simulated gastrointestinal digestion using the method as reported by (Zou et al., 2016). Emulsion was passed through two steps of gastrointestinal track model that simulated the condition of the stomach, and small intestine. The curcumin emulsion was preheated in a shaking incubator at 37 °C for 10 min before testing.

a. Stomach Phase

Simulated gastric fluid (SGF) was previously prepared by adding 2 g NaCl and 7 ml HCl into 1 L distilled water. The 20 ml sample was mixed with 20 ml SGF containing 0.0032 g/ml pepsin preheated to 37ºC at a 1 : 1 (20ml:20ml) mass ratio. The mixture was adjusted to pH 2.5 and placed in a shaker at 100 rpm for 2 hours to mimic stomach digestion.

b. Small Intestine Phase

Small intestine fluid (SIF) was prepared by mixing 5.5 g $CaCl_2$ and 43.8321g NaCl into a volumetric flask (100 ml), and then adding distilled water to 100 ml. The 30 ml chyme solution from the stomach were added with 30 ml phosphate buffer solution (10 mM pH 6.5), followed by incubation in a water bath for 10 min and the PH was adjusted to pH 7.0. The 3 ml of SIF (containing 0.5 M $CaCl_2$ and 7.5 M NaCl) was added to

60 ml digesta solutions. The 7 ml bile extract (containing 375.0 mg bile extract), was added and the pH was adjusted back to 7.0. The 5 ml of lipase suspension, containing 120 mg of lipase (pH 7.0, PBS), was added to the sample and monitored the pH to a fixed value (pH 7.0) by adding 1 N NaOH solutions into the digesta solution every 30 min for 2 h.

c. Determination of bioaccessibility

After passing through the simulated digestion, 30 ml raw digesta of each mixture was centrifuged 8,500 rpm form 30 min at 25 °C. The clear supernatant was assumed to be the ‘‘micelle’’ fraction in which the curcumin was solubilized. The 1000 μL sample micelle or raw digesta were added to ethanol 5000 μL, mixed well and centrifuged 2000 rpm for 15 min, collect the supernatant. The concentration of curcumin in the raw digesta (without centrifugation) and micelle phase (after centrifugation) was then calculated from the measured absorbance using the standard curve of curcumin. The bioaccessibility of curcumin was calculated using the following equation:

$$\text{Bioaccessibility} = 100 \times (C_{micelle} / C_{Raw\ Digesta}) \quad (3)$$

where, $C_{Micelle}$ and $C_{Raw\ Digest}$ are the concentrations of curcumin in the micelle fraction and in the raw digesta, respectively.

CHAPTER 4
RESULT AND DISCUSSION

4.1. Investigation on emulsion stability of curcumin emulsion with various concentration of KGM

4.1.1. Creaming stability

Creaming is one of instability mechanism which arises by the effect of droplet gravity where the droplets move upward because they have a lower density than the continuous phase (McClements, 2007). The creaming index (CI) in this study were determined visually by measuring the serum separation height at room temperature over 14 days of storage. A higher CI value indicates lower creaming stability of the emulsion. The photograph of emulsion after 14 days of storage is shown in the figure 4.1.

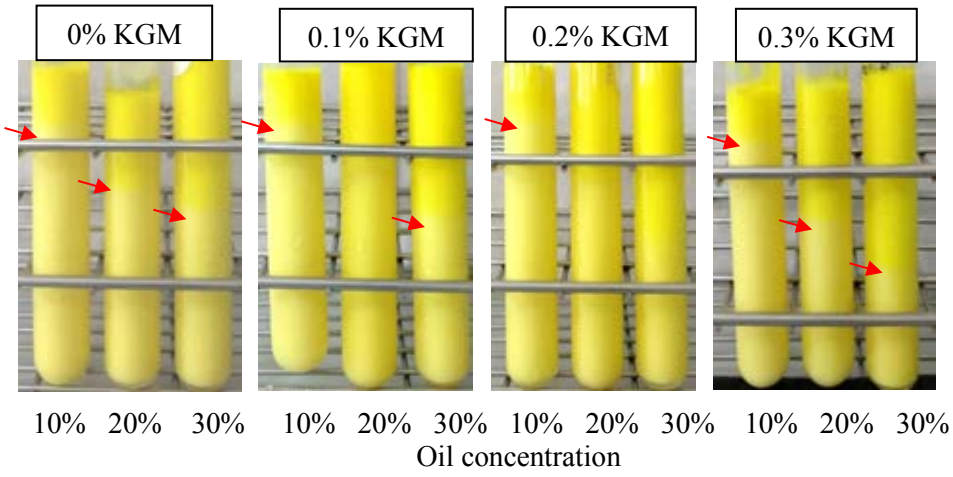

Figure 4. 1 Creaming separation of different KGM concentration and oil content after 14 days of storage

The creaming effect occurred at the top layer of emulsion with the turbid cream layer and the bottom opaque emulsion layer were observed in emulsion both in the present of KGM and without KGM. Increasing KGM concentration from 0.1% to 0.2% can retard the creaming separation of the emulsion containing 20% and 30% oil up to 14 days of storage. The influence of KGM on the creaming index of the emulsion is presented in Figure 4.2.

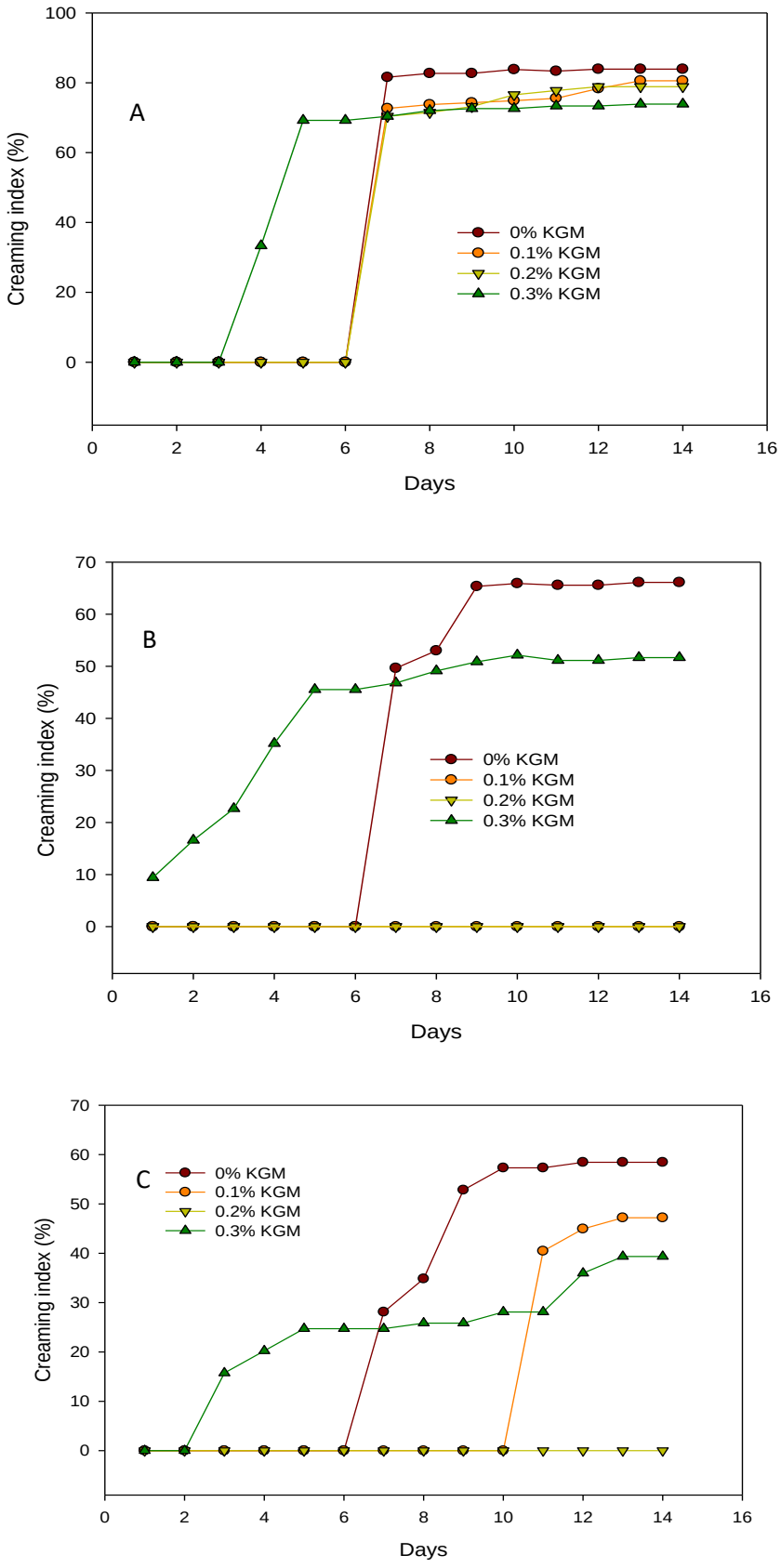

Figure 4. 2 Changes of creaming index of different KGM concentration and oil content: A) 10%, B) 20%, and C) 30% during storage.

The emulsions without KGM had a distinct creaming separation, and the creaming index gradually increased with the extension of time, starting from the sixth day of storage in all oil concentration. The result correlated with a study by Lu et al. (2018) that the creaming separation of the emulsion can be retarded by adding KGM as a stabilizer. The result could be explained by the reported fact by Bouyer et al. (2012b) and Hu et al. (2016) that hydrophilic parts of KGM oriented and formed an extended thickening network within the water phase which increased the viscosity of system, thereby slowing down the movement of droplet due to gravity or Brownian motion. Moreover, the result also suggested that KGM at these concentrations (0.1% and 0.2%) can potentially structure the water phase with the chain structures. The results were related to Lu et al. (2018) that the chain-structure of KGM can retard the creaming of oil droplet due to the enhanced steric hindrance and force of friction between droplets and the continuous phase. However, in emulsions containing 0.3% KGM, the higher creaming index was observed in all oil concentration. The creaming phenomena occurred may due to the depletion flocculation of emulsion droplet when KGM concentration exceeds a critical value (Lu et al., 2018). The excess biopolymer in the continuous phase can generate an attractive force between the droplet by osmotic effect and stimulate the exclusion of biopolymer chains from the depletion region surrounding two droplets. Increasing polymer concentration can increase the osmotic force until it reaches the limit to defeat the repulsive force between the droplet and cause droplet flocculation. The result also suggested that increasing oil-phase volume fraction can potentially prevent the oil droplet from creaming separation in the emulsion containing 0.1% and 0.2% KGM up to 14 days of storage. Sun and Gunasekaran (2009) reported that the stability of emulsion could be improved by increasing oil-phase volume fraction due to the increase in packing fraction of oil droplets, which then increase the viscosity and thus lowering the creaming rate. The study also stated that in the low oil-phase volume fraction, the creaming rate was rapid due to the poorly flocculated network that may collapse under its weight. This may also explain why emulsion with 10% oil showed poor stability compared to the higher oil concentration, as described above.

4.1.2. Apparent Viscosity

Viscosity measurements were performed to all emulsion samples using a digital rotational Viscometer at various KGM level containing different oil concentration. The viscosity of emulsion can be potentially influenced by several factors, such as water phase, oil content, particle size, or surface properties. In this study, the viscosity of emulsion significantly increased with the increasing KGM concentration followed by increasing oil-phase volume fraction. The result showed that the viscosity of emulsions without KGM is lower than that in the present of KGM and the viscosity increased with increasing KGM concentration. The results also correlated with Lu et al. (2018) that the increased viscosity of KGM emulsion can be associated with two factors: (i) the increased viscosity of water phase and (ii) droplet flocculation by depletion force generated by the non-absorbed KGM. Figure 4.3 suggested that increasing KGM concentration can significantly increase the viscosity of the water phase and then increase the viscosity of the final emulsions. The higher the viscosity of the system, the greater the creaming stability. The result is related to the Stoke's law that stated the velocity of a droplet movement is inversely related to the viscosity of the water phase. Therefore, the stability of an emulsion can be improved by increasing viscosity of the water phase (Taherian et al., 2008). However, in the emulsions with a higher level of KGM, a depletion force can be potentially induced and thus generate flocculation between the droplets, which then increased the viscosity of the emulsion. This effect correlated with the creaming stability phenomena in the highest KGM concentration (0.3% w/v) which discussed above.

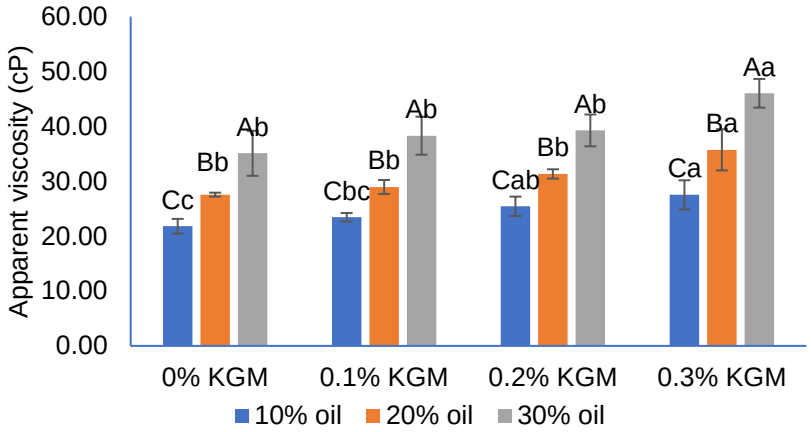

Figure 4. 3 Impact of KGM concentration on viscosity of curcumin emulsion containing different oil volume fraction
([a-c] Different lowercase superscript indicate significant different in apparent viscosity between means for KGM concentration, [A-C] different uppercase superscript indicate significant for oil concentration (P<0.01).

4.1.3. Curcumin Loading Capacity

The loadings of curcumin in the emulsion were investigated by measuring the curcumin concentration in final emulsion after homogenization and after 14 days of storage. Decreasing curcumin content related to the chemical degradation that might be occurred either during emulsion preparation or during storage. Figure 4.4 showed the concentration of curcumin in the emulsions after homogenization. The concentration of curcumin after homogenization was ranging from 0.42-1.38 mg/ml. As the oil volume fraction increased, the amount of curcumin in the final emulsion increased with the increase in the amount of added curcumin into the system.

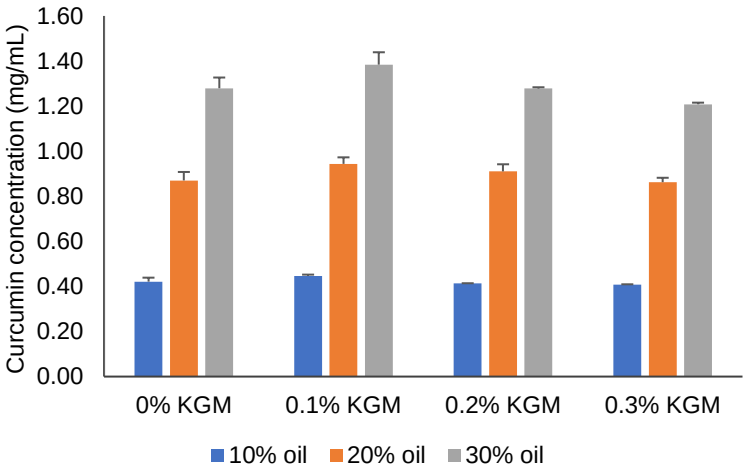

Figure 4. 4 The curcumin concentration in the emulsions after homogenization.

The loadings of curcumin in emulsion were calculated by the ratio of curcumin determined in emulsion samples and curcumin initially added. The comparison between curcumin concentration after 14 days of storage and curcumin initially added are shown in the Table 4.1. The loadings of curcumin in emulsions after homogenization and after storage are ranging from 82-95% and 78-93%, respectively. The results were correlated with Kharat et al. (2017) that investigated the curcumin retention under different pH value. They found that in the acidic condition, curcumin was relatively stable with the curcumin remaining ranging from 85-95%. In this study, due to the relatively acidic pH condition (6.6-6.7), curcumin remained stable during emulsion preparation (Figure 4.4), but there might be a little chemical degradation occurred during 14 days of storage due to the fact that the curcumin retention decreased to be 78-93% (Figure 4.5). However, the final pH condition of emulsions which is relatively close to neutral pH, make it possible for the hydrolytic degradation occurred in which hydroxyl ion interact to the carbonyl group of curcumin, forming derivative products of curcumin such as ferulic acid and feruloyl methane (Wang et al., 1997). This condition is also correlated with the low curcumin loading in the low oil volume fraction (10% v/v). In the 10% (v/v) oil, the higher concentration of water phase (90% v/v) in the system leads to the presence of more hydroxyl ions that potentially interact to the carbonyl group of curcumin, resulting in degradation of curcumin in the droplet. The emulsions containing 20% v/v oil significantly showed the best loading capacity compared to 10% v/v and 30% v/v oil, suggesting that the concentration of the native emulsifier in milk (milk protein) and KGM are sufficient to cover and protect oil droplet containing curcumin in 20% v/v oil. It is suggested from the result that in the higher oil fraction (30% v/v), the poor loading capacity can be possibly due to the amount of emulsifiers are not sufficient to cover the higher amount of oil droplets which are formed after homogenization. This effect may cause coalescence between the droplet, resulting in the release of curcumin from the droplet (Dalgleish, 1997). In the presence of 0.1% and 0.2% (w/v) KGM, the loading capacity of curcumin was significantly increased compared to the system without KGM. However, in the presence of 0.3% (w/v) KGM, the loading capacity of curcumin was lower due to the depletion flocculation, and the results are

correlated with the higher creaming index in 20% v/v oil-phase volume fraction which described above.

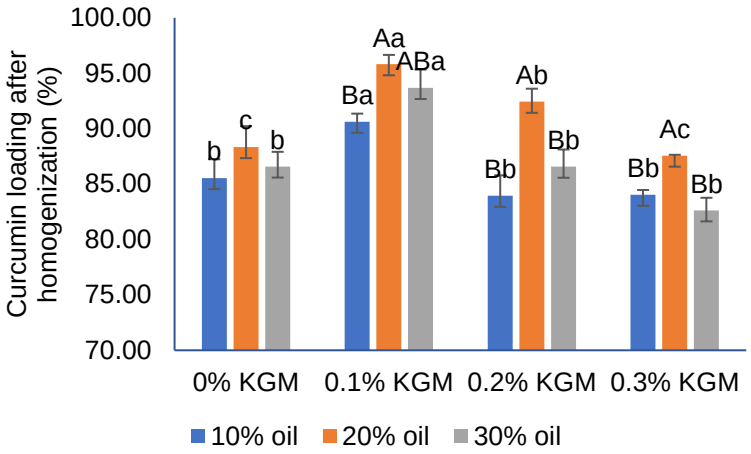

Figure 4. 5 The curcumin loading in the emulsions after homogenization. ([a-c] Different lowercase superscript indicate significant different in Curcumin loading between means for KGM concentration, [A-C] different uppercase superscript indicate significant for oil concentration (P<0.01).

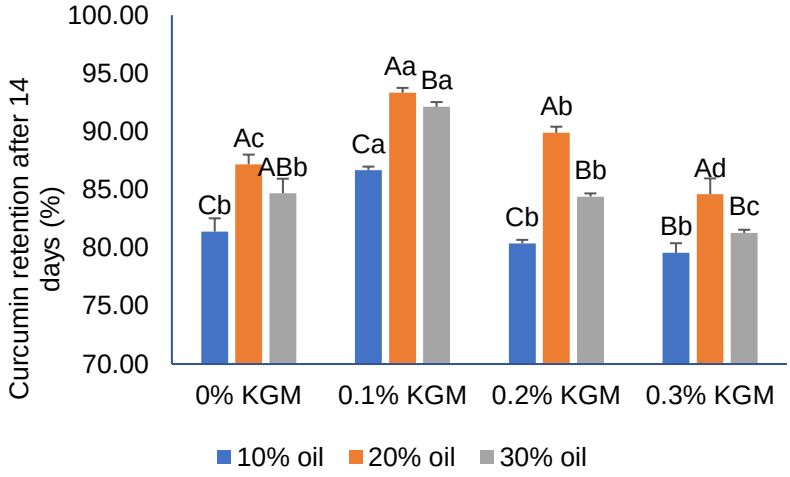

Figure 4. 6 The curcumin retention in the emulsions after 14 days. ([a-c] Different lowercase superscript indicate significant different in Curcumin retention between means for KGM concentration, [A-C] different uppercase superscript indicate significant for oil concentration (P<0.01).

Table 4. 1 Comparison between curcumin concentration after 14 days of storage and curcumin initially added

Conc. of KGM	10% Oil		20% Oil		30% Oil	
	Curcumin initially added (mg/ml)	Curcumin after 14 days (mg/ml)	Curcumin initially added (mg/ml)	Curcumin after 14 days (mg/ml)	Curcumin initially added (mg/ml)	Curcumin after 14 days (mg/ml)
0%	0.50	0.41	1.00	0.87	1.50	1.27
0.10%	0.50	0.43	1.00	0.93	1.50	1.38
0.20%	0.50	0.40	1.00	0.90	1.50	1.27
0.30%	0.50	0.40	1.00	0.85	1.50	1.22

4.1.4. Color Stability

The instrumental color measurement was used to support the observed differences in color as the visual appearance of the emulsion. In this study, b^* value was used to represent the yellowness of emulsion containing curcumin, since emulsion appeared predominantly yellow to the eye. The results showed that the increased of KGM concentration had no significant impact on the yellowness of emulsion, while the increased oil volume fraction significantly increased the b^* value of emulsions (Figure 4.7). The increased oil volume fraction indicated the increasing of curcumin content that added into the emulsions, resulting in increasing of the yellowness. Therefore, the results suggested that the b^*value (yellowness) correlated with the curcumin concentration in the final emulsion (Figure 4.4). Change in yellowness of curcumin emulsions after 14 days of storage are shown in Table 4.1. The intensity of yellowness (b^* value) remained stable during storage in almost all conditions. However, in some conditions, there are a slight decreased of curcumin after storage which is correlated with the report by Kharat et al. (2017) that a slightly decreased of yellow color intensity indicated that little chemical degradation of curcumin occurred in the emulsions. The formation of derivative products of curcumin such as ferulic acid and feruloyl methane by hydrolytic mechanism, contribute to the color fading effect.

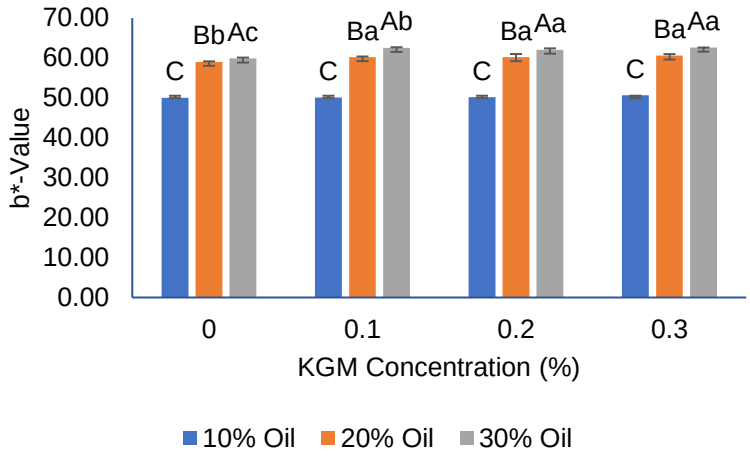

Figure 4. 7 Impact of KGM concentration on the yellowness (*b**value) of curcumin emulsion containing different oil volume fraction
([a-c] Different lowercase superscript indicate significant different in apparent viscosity between means for KGM concentration, [A-C] different uppercase superscript indicate significant different between means for oil concentration (P<0.01)).

Table 4. 2 Change in yellowness (*b** value) of curcumin emulsion stored at 4ºC after 14 days

Conc. of KGM	10% Oil		20% Oil		30% Oil	
	day 1	day 14	day 1	day 14	day 1	day 14
0%	50.03±0.12[a]	48.42±0.43[b]	59.04±0.24[a]	57.51±0.05[b]	59.86±0.13[a]	58.58±0.16 [b]
0.10%	50.11±0.16[a]	49.78±0.47[a]	60.22±0.17[a]	58.38±0.12[b]	62.53±0.09[a]	61.77±0.14b
0.20%	50.18±0.81[a]	49.82±0.39[a]	60.2±0.32[a]	55.83±0.04[b]	62.09±0.005[a]	62.073±0.04[a]
0.30%	50.69±0.36[7a]	50.52±0.24[a]	60.61±0.02[a]	58.95±0.68[b]	62.60±0.18[a]	59.80±0.27[b]

note: different superscripts indicate significant difference (p<0.05)

4.2. Investigation on physicochemical properties of curcumin emulsion during storage

This study investigated the physicochemical properties of curcumin emulsion during storage. Emulsions that reached the physical stability until 14 days of storage including 0.1% KGM 20% oil, 0.2% KGM 20% oil, and 0.2% KGM 30% oil were subjected to further analysis including antioxidant, particle size, and zeta potential and were compare to control (0% KGM).

4.2.1. Antioxidant Activities

The antioxidant activity of curcumin emulsions was determined by two methods: DPPH (hydrogen atom transfer and single electron transfer mechanisms) and FRAP (Ferric reducing antioxidant power). The antioxidant

activities of DPPH and FRAP assay expressed as mg of Trolox equivalents (TE) per g of emulsion are presented in Figure 4.8 and Figure 4.9, respectively.

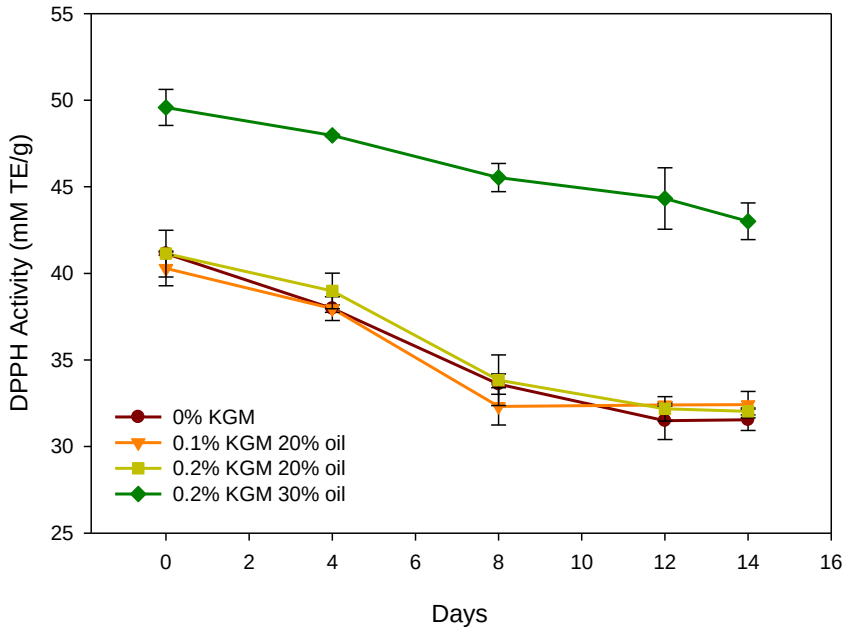

Figure 4. 8 Antioxidant activity (mM of Trolox equivalent per g of emulsion) of curcumin emulsion containing different oil volume fraction in DPPH assay.

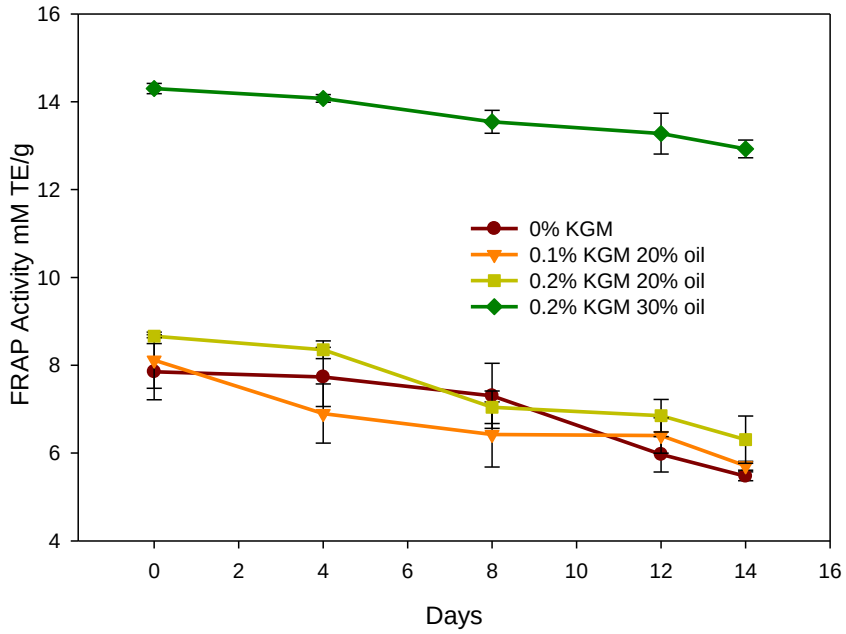

Figure 4. 9 Antioxidant activity (mM of Trolox equivalent per g of emulsion) of curcumin emulsion containing different oil volume fraction in FRAP assay.

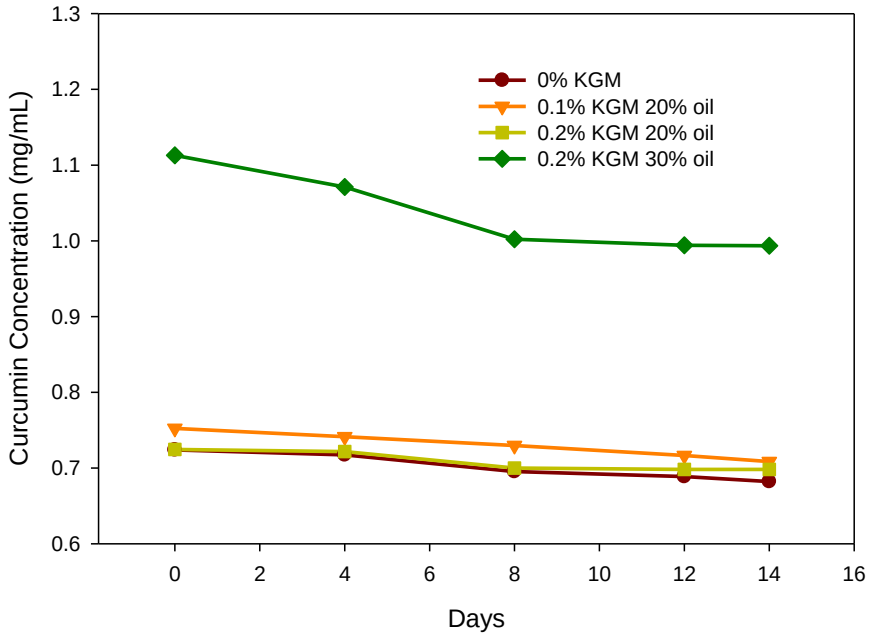

Figure 4. 10 The concentration of curcumin (mg/ml) in emulsion containing different oil volume fraction

The antioxidant activity value for both measured by DPPH assay and FRAP were correlated with the concentration of curcumin in the emulsion system (Figure 4.4) since the curcumin has a major contribution of antioxidant activity in the system. The antioxidant activity measured by DPPH assay did not present the significant difference between the samples regardless of the concentration of KGM in different oil concentration. However, antioxidant activity value obtained by DPPH assay was higher than that by FRAP. The results were also correlated with Artiga-Artigas et al. (2018), which reported that due to the major contribution of curcumin, the antioxidant activity measured by DPPH assay was higher than FRAP. Curcumin has three ionizable protons contributed by the enolic proton and two phenolic OH- groups. In acidic and neutral conditions (i.e., pH 3–7), the major constituents present are curcumin molecules in bis-keto form, where curcumin acts as a potent hydrogen atom donor (Wiggers et al., 2017a). Moreover, the scavenging activity in DPPH assay which is based on both hydrogen atom transfer (HAT) and single electron transfer (SET), suggested that antioxidant activity contributed by curcumin is dominated by HAT mechanism rather than SET. The antioxidant activity remains stable during storage time, but there was significantly decreased in day-4 of storage. This can be explained that some insoluble curcumin may exclude from the droplet resulting in the degradation of curcumin in the surrounding water phase by the hydrolytic

mechanism. The hydrolytic degradation occurred in which hydroxyl ion interact to the carbonyl group of curcumin, forming derivative products of curcumin such as ferulic acid and feruloyl methane (Wang et al., 1997).

4.2.2. Particle size and zeta potential

The particle size and zeta potential of curcumin emulsion were measured by integrated light scattering analyzer. Particle size and droplet charge of curcumin emulsion after homogenization, day-7, and day-14 of storage are listed in Table 4.2. The result showed that the particle size decreased with increasing of KGM concentration from 0.1% to 0.2% (w/v) and increased with increasing of oil volume fraction from 20% to 30% (v/v). Increasing KGM concentration increases the viscosity of the aqueous phase, which slows down droplet mobility and minimizes the number of collisions. Therefore, there is enough time for protein to adsorb on the interface and stabilized the droplet from coalescence. A study by Krstonošić et al. (2009) which investigated the effect of xanthan gum on the stability of tween 80-stabilized emulsion, also reported that the ability of xanthan to increase the viscosity of continuous aqueous phase could prevent the droplet from coalescence by minimizing the mobility of droplet and decreasing collision number of the droplets. The low droplet mobility provides enough time for the surfactant to attach on droplets and prevented the droplet coalescence. These results are also confirmed by the study by Samavati et al. (2012), which reported that whey protein-stabilized emulsion containing xanthan gum had the highest viscosity and smaller droplet size. The increased viscosity and the small droplet size contribute to the better stability of the emulsion.

Table 4. 3 Particle size and zeta potential of curcumin emulsion at day-0, day-7, and day-14.

KGM Conc. (%)	Oil Conc. (%)	Particle size (nm)			Zeta potential (mV)		
		Day-0	Day-7	Day-14	Day-0	Day-7	Day-14
0	20	306.6±9.0a	273.7±4.9b	433.5±11.7a	-27.1±0.4	-29±0.3	-24.40±0.4b
0.1	20	290.5±6.4b	289.1±3.5ab	347.7±4.5c	-27.3±0.5	-29.2±0.7	-28.07±0.5
0.2	20	271.5±4.8c	300.5±13.3a	398.1±7.8b	-25.8±0.4	-28.4±1.8	-27.20±0.2
0.2	30	263.6±0.9d	274.2±3.4b	290.7±5.1d	-27.2±0.8	-28.4±1.8	-27.00±1.2

*NA: the result is not available

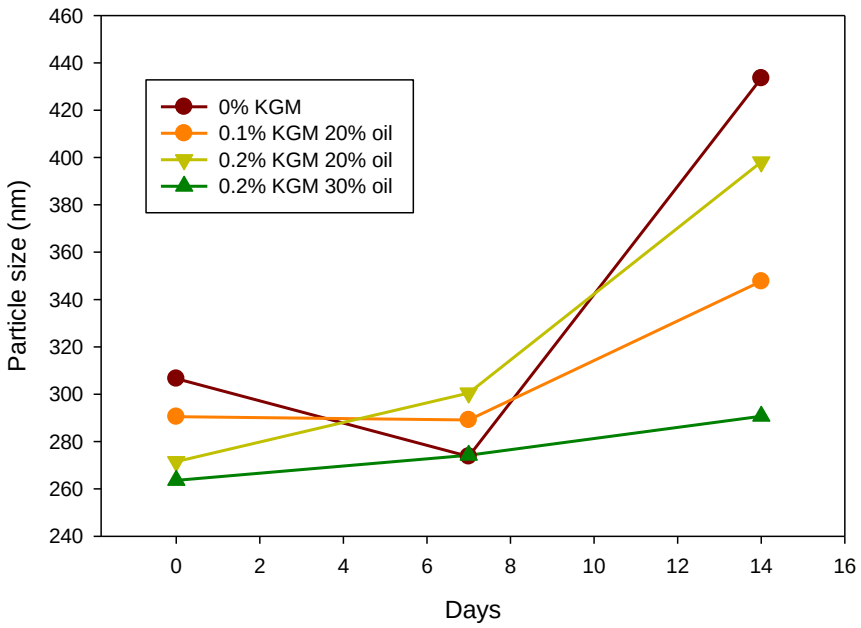

Figure 4. 11 The changes in average droplet size during storage.

All emulsion samples exhibited the zeta potential value below -30, and no significant difference between different emulsion was observed (Table 4.2). The result shows that KGM concentration, along with different oil phase volume fraction, did not have a significant effect on the surface charge of emulsion. By the presence of milk protein, the negative charge values were obtained in all emulsion samples. Generally, KGM is a nonionic polysaccharide and binding of KGM to the surface of curcumin emulsion droplet stabilized by milk protein should lead to the significantly reduced surface charge. However, the surface charge from all emulsions containing KGM showed almost no difference from that of emulsion without KGM. The result was correlated with Lu et al. (2018), which reported that there is no significant difference between emulsion containing KGM and without KGM in WPI-stabilized emulsion. The result suggested that there is very limited binding of KGM to the oil droplet interface.

4.3. Investigation on bioaccessibility of curcumin emulsion during storage

Bioaccessibility is defined as the percentage of a bioactive compound that is dissolved from food upon ingestion (Gómez-Estaca et al., 2015). Before it is absorbed, a hydrophobic bioactive compound has to be released from the droplet and solubilized within mixed micelles that present in the small intestine. To measure the

amount of bioaccessible curcumin in the system, simulated GIT was conducted to measure the curcumin concentration present in the raw digesta and the curcumin concentration present in the mixed micelle and in the raw digesta containing stomach and small intestine phase. The bioaccessibility of curcumin emulsion within 14 days of storage shows in Figure 4.12.

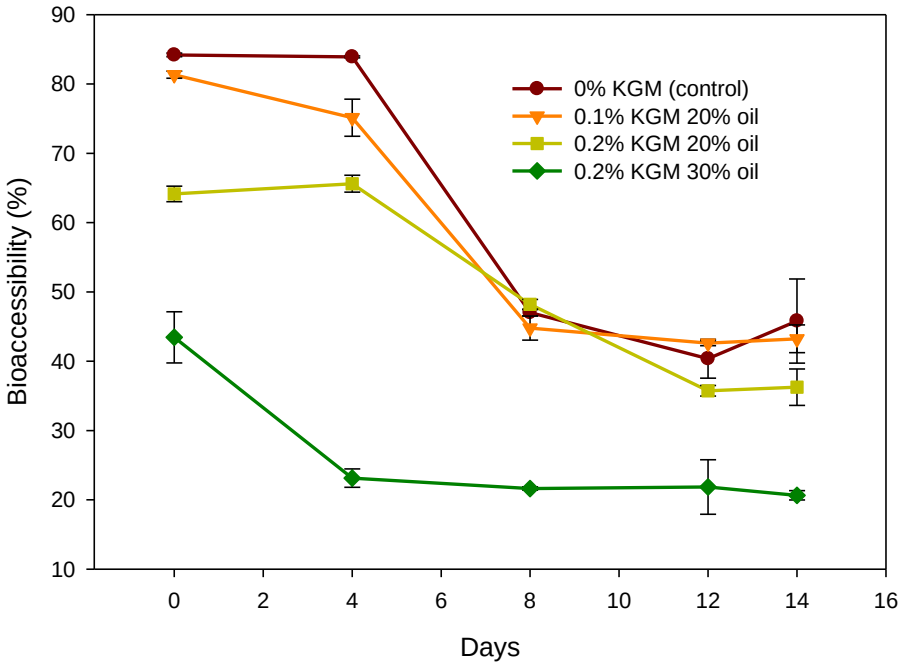

Figure 4. 12 Bioaccessibility of curcumin emulsion during storage.

The result showed that the bioaccessibility of curcumin emulsions containing 0.1% and 0.2% KGM (w/v) was lower than that of emulsion without KGM. The low bioaccessibility means that there are a small concentration of curcumin present in the mixed micelle compare to the concentration of curcumin in the raw digesta. The results indicated that introducing KGM to the emulsion system contribute to slower the release of curcumin from emulsion droplet during small intestine phase. The previous study by Lu et al. (2018) reported that adding KGM significantly modified the release of β-carotene from emulsion droplet after passing through GIT. The release of β-carotene was more inferior than that of emulsion without KGM, and the release rate lowered with increasing KGM level. Another study by Mao et al. (2015) also showed that, adding polysaccharide, maltodextrin, to structure the water phase reported significantly different release condition of hydrophobic food flavor by modifying droplet mobility within the continuous phase. The higher viscosity and a

chain like structure that showed in the emulsion with the present of KGM can contribute to interfere the hydrolysis of the oil phase and the surface layer of protein by steric hindrance effect (Lu et al., 2018). Also, KGM is also non-degradable in the small intestine but degradable by β-mannanase, an enzyme generated by colon bacteria (Zhang et al., 2014). In fact, KGM is a promising candidate for the development of controlled bioactive delivery systems in the upper part of the gastrointestinal tract (GIT) to the colon part. This mechanism may explain why emulsions with the present of KGM in this study showed the lower release of curcumin from emulsion droplet. The result indicated that introducing KGM to the water phase of emulsions is feasible to achieve a controlled release of curcumin from emulsions. It can be proved by the result of concentration curcumin in the micelle (Figure 4.13) which suggested that addition the low concentration of KGM potentially control the loss of curcumin during emulsion formation and within the GIT. The concentration of curcumin in the micelle represents the amount of curcumin that ready to be absorbed in the final curcumin emulsion after passing through the upper part of GIT. The concentration of curcumin in the micelle was higher in 0.1% KGM compared to 0.2% KGM and without KGM. The result suggested that structuring water phase with KGM can protect the curcumin within the droplet during formulation as well as in the digestion process, and this related to the higher loading capacity that described above.

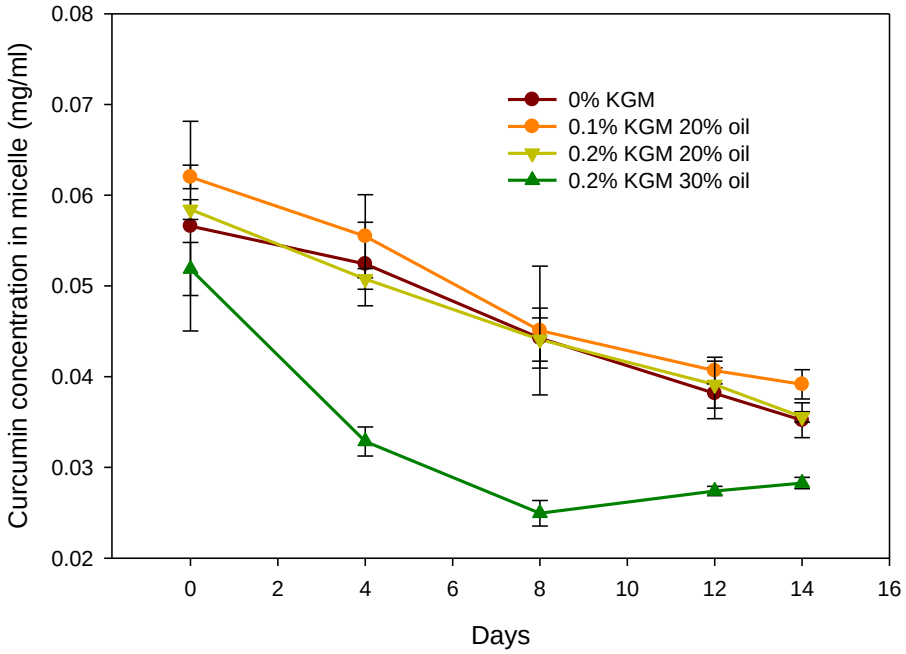

Figure 4. 13 The concentration of curcumin in the micelle after passing through the GIT during storage.

Moreover, the low bioaccessibility also showed in increasing the oil volume fraction from 20% to 30% (v/v). Ahmed et al. (2012) reported that the bioaccessibility of curcumin increased with the increase of lipid level due to the increase of the total amount of mixed micelle that available to solubilize curcumin. The result in this study may have been due to the higher lipid level; there was an amount of lipid which was not digested. Therefore some curcumin may not have been released from the emulsion droplets. In this condition, there are two possible physiochemical mechanisms that describe the correlation of curcumin bioaccessibility and oil level. First, bioaccessibility increase with the increase of lipid level, because more mixed micelle will be formed. Second, bioaccessibility decrease with the increase of lipid level due to a higher fraction of oil content remains non-hydrolyzed, resulting in some curcumin will not be released from the emulsion droplet into the surrounding mixed micelle. In this study, it is expected that the non-hydrolyzed oil content will be potentially further absorbed in the colon. Since the KGM will be degradated by a colonic enzyme, β-mannanase, it promotes the access of oil to be absorbed, and the release of curcumin from the oil droplet. Jeppesen and Mortensen (1998) reported that there are two reasons that can explain the MCT absorption in the colon. First, a MCT can be possibly absorbed because it has the ability to soluble in water. The absorption of water soluble fatty acid in colon would be related to the report that colon can

completely absorb C8:0 and almost all of C10:0 at physiological pH. Second, MCT can be absorbed due to the backward effect of the colon on fat absorption in the small intestine.

During the storage period, the bioaccessibility of curcumin emulsion significantly decrease after 4 days of storage and remain stable from the day-8 to day 14. The results were related to the concentration of curcumin during storage, which has been described above. It can be explained by the mechanism of hydrolytic degradation in which some insoluble curcumin may exclude from the droplet resulting in the degradation of curcumin in the surrounding water phase. The hydrolytic degradation occurred when hydroxyl ion interacts with the carbonyl group of curcumin, forming derivative products of curcumin such as ferulic acid and feruloyl methane (Wang et al., 1997).

CHAPTER 5
CONCLUSION

5.1. Conclusion

Addition of KGM in the water phase can significantly increase the creaming stability of emulsion containing 0.1% and 0.2% w/v KGM until 14 days due to the increase of viscosity which induces the enhanced steric hindrance and force of friction between droplets and the continuous phase. The curcumin loading also suggested that the presence of 0.1% and 0.2% (w/v) KGM have significantly increased the curcumin loading compared to the system without KGM. Moreover, the presence of KGM gave no impact on antioxidant activities both in DPPH and FRAP analysis, however, due to the relatively stable curcumin loading capacity, the antioxidant activities of curcumin emulsion remain stable over 14 days of storage. The particle size and zeta potential value indicated that the addition of KGM had a significant impact on decreasing droplet size but had no significant impact on the zeta potential. Lastly, introducing KGM to the emulsion system contribute to slow down the release of curcumin from emulsion droplet during small intestine phase due to the presence of KGM structure in the water which interfere the hydrolysis of the oil droplet. However, the result of concentration curcumin in the micelle suggested that addition the low KGM level (0.1% KGM w/v) potentially control the loss of curcumin during emulsion formation and within the GIT.

The results suggested that the 0.1% w/v KGM concentration containing 20% v/v oil-phase volume fraction is the optimal condition to achieve the 14-days emulsion stability of curcumin with higher loading capacity and higher final curcumin concentration in the micelle. Finding in this study denoted that structuring water phase with the low concentration of KGM could be possible to design the curcumin in milk system containing MCT oil with potential emulsion stability and controlled release of curcumin from emulsions.

5.2. Recommendation

1. A detailed study on oxidative stability such as TBARS assay on curcumin emulsion should be considered.
2. Further insight on sensory properties and consumer acceptance need to be studied.

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APPENDICS

APPENDIX A

Emulsion formulation

Table A1. The formulation of curcumin emulsion

No.	Conc. Of KGM solution (%)	KGM (ml)	Conc. Of protein in milk (%)	Milk (ml)	Final composition in emulsion				Oil:water ratio (%)
					%KGM in final emulsion	%protein in final emulsion	Oil phase (ml)	Water phase (ml)	
1	0	11.7	3.3	105.3	0	2.97	13	117	10:90
2	0	10.4	3.3	93.6	0	2.64	26	104	20:80
3	0	9.1	3.3	81.9	0	2.31	39	91	30:70
4	0.1	11.7	3.3	105.3	0.01	2.67	13	117	10:90
5	0.1	10.4	3.3	93.6	0.01	2.38	26	104	20:80
6	0.1	9.1	3.3	81.9	0.01	2.08	39	91	30:70
7	0.2	11.7	3.3	105.3	0.02	2.67	13	117	10:90
8	0.2	10.4	3.3	93.6	0.02	2.38	26	104	20:80
9	0.2	9.1	3.3	81.9	0.01	2.08	39	91	30:70
10	0.3	11.7	3.3	105.3	0.03	2.67	13	117	10:90
11	0.3	10.4	3.3	93.6	0.02	2.38	26	104	20:80
12	0.3	9.1	3.3	81.9	0.02	2.08	39	91	30:70

*On basis of total 130 ml

APPENDIX B

The maximum wavelength of curcumin

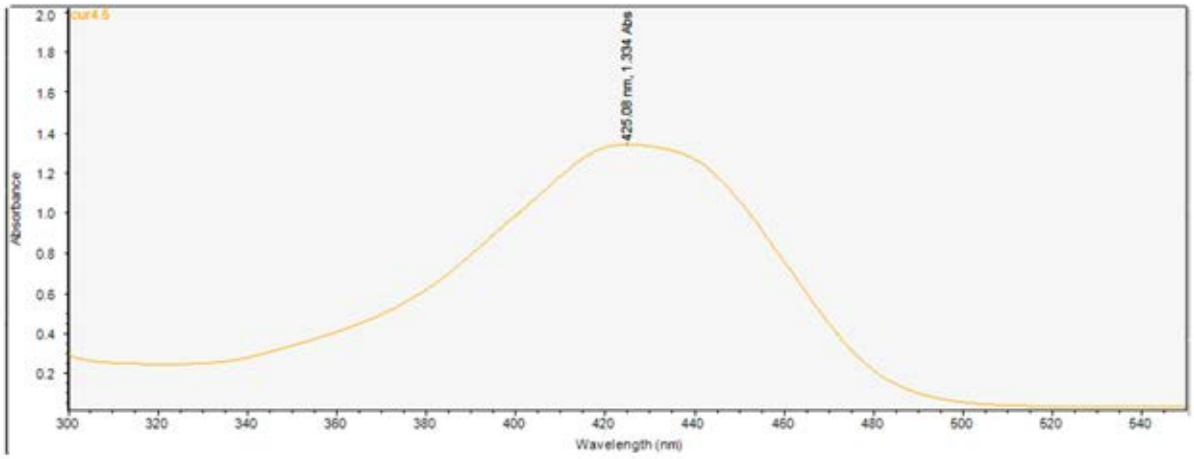

Figure B1. The maximum absorption of standard curcumin ethanol solutions

APPENDIX C

Standard curve of curcumin

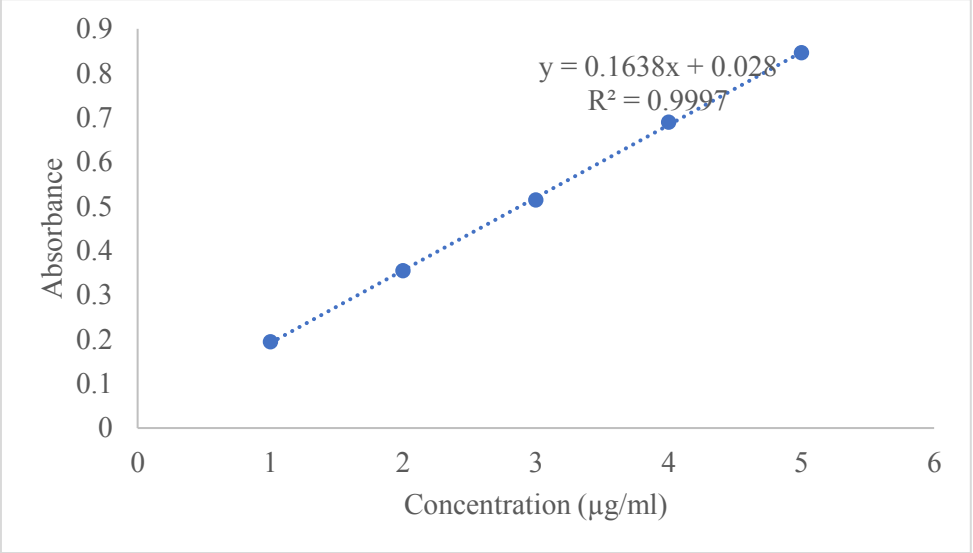

Figure C1. Relationship between concentration of curcumin (µg/ml) and absorbance value

APPENDIX D

Standard curve of DPPH

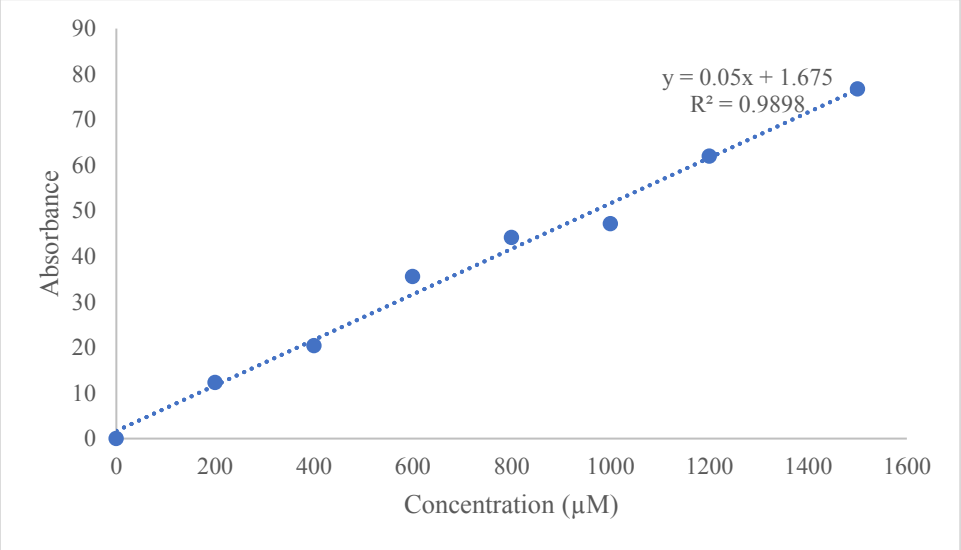

Figure D1. Relationship between concentration of trolox (µM) and the absorption reading (absorbance)

APPENDIX E

Standard curve of FRAP

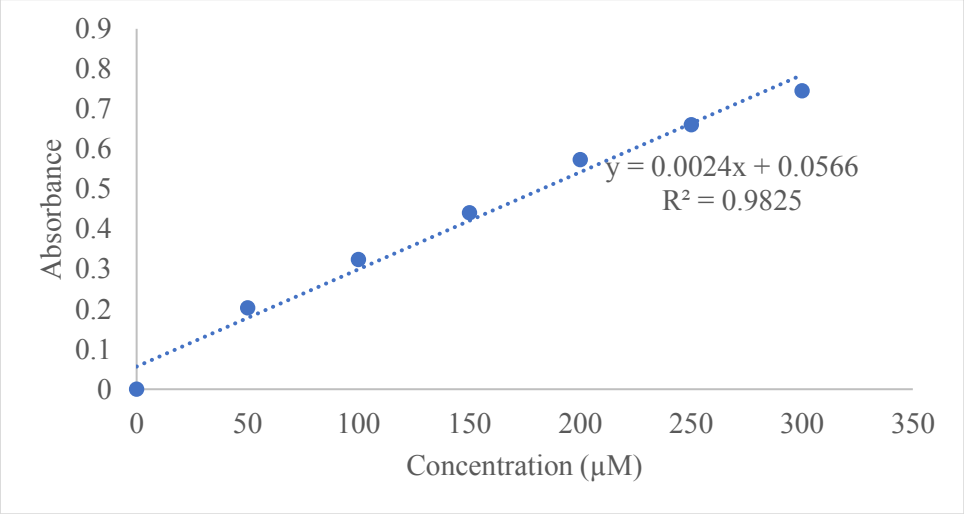

Figure E1. Relationship between concentration of trolox (µM) and the absorption reading (absorbance)

APPENDIX F

Simulated Fluid Preparation (Zou et al., 2016)

F1. Simulated Gastric Fluid (SGF

Dissolve 1g NaCl and 3.5 ml HCl in 500 ml distilled water. Mixed thoroughly without heat.

F2. Simulated Small Intestine Fluid (SIF)

Dissolve 0.5 M $CaCl_2$ and 7.5 M NaCl in 100 ml distilled water. Mixed thoroughly without heat.

APPENDIX G

Table G.1 Comparison of the curcumin concentration initially added, after homogenization and after 14 days of storage.

Conc. of KGM	10% Oil			20% Oil			30% Oil		
	Curcumin concentration (mg/ml)			Curcumin concentration (mg/ml)			Curcumin concentration (mg/ml)		
	Initial	Homo geniza tion	14 days	Initial	Homo geniza tion	14 days	Initial	Homo geniza tion	14 days
0%	0.50	0.42	0.41	1.00	0.87	0.87	1.50	1.28	1.27
0.10%	0.50	0.45	0.43	1.00	0.94	0.93	1.50	1.38	1.38
0.20%	0.50	0.41	0.40	1.00	0.91	0.90	1.50	1.28	1.27
0.30%	0.50	0.41	0.40	1.00	0.86	0.85	1.50	1.22	1.22

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