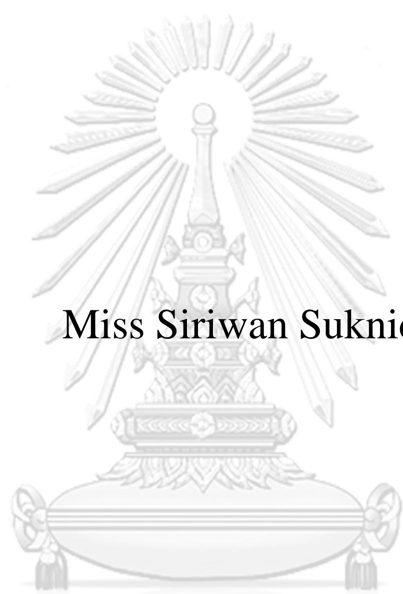
# PREPARATION OF ENCAPSULATED KONJAC GLUCOMANNAN-BASED FISH OIL AND ITS USE IN HIGH PRESSURE PROCESSED GOAT MILK



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A Dissertation Submitted in Partial Fulfillment of the Requirements
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# การเตรียมไมโครแคปซูลน้ำมันปลาจากบุกกลูโคแมนแนนและการใช้ในนมแพะที่ผ่านความดันสูง

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

น.ส.สิริวรรณ สุขนิคม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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การศึกษาครั้งนี้แบ่งออกเป็น 3 ส่วน โดยการศึกษาส่วนแรกมีวัตถุประสงค์เพื่อศึกษาผลกระทบของสารละลายบุกกลูโคแมนแนน (KGM) (0.02-0.5%, w/w) ที่ pH ต่างกัน (3, 5 และ 9) ต่อความคงตัวของอิมัลชันน้ำมันปลาความเข้มข้นร้อยละ 5 กับนมขาดมันเนยที่มีและไม่มีเคซีน การศึกษาส่วนที่สองมีวัตถุประสงค์เพื่อศึกษาผลกระทบของ KGM (0.02-0.5%, w/w) ที่ pH ต่างกัน (3-10) ต่อความคงตัวของอิมัลชันน้ำมันปลาความเข้มข้นร้อยละ 5 กับนมและการศึกษาส่วนที่สามมีวัตถุประสงค์เพื่อศึกษาผลกระทบของแรงดันสูง (400, 500 และ 600 MPa) ต่อการปรับปรุงความเสถียรของอิมัลชันน้ำมันปลากับนม โดยผลการศึกษาส่วนแรกแสดงให้เห็นว่าที่ระดับความเข้มข้นและ pH ของ KGM เท่ากัน ขนาดอนุภาค (particle size) ในอิมัลชันที่เตรียมจากนมขาดมันเนยที่มีเคซีนมีขนาดใหญ่กว่าอิมัลชันที่เตรียมจากนมขาดมันเนยที่ไม่มีเคซีน, ค่าศักย์ซีตา (Zeta potential) ของอิมัลชันที่เตรียมจากนมขาดมันเนยที่มีเคซีนมีค่าต่ำกว่าอิมัลชันที่เตรียมจากนมขาดมันเนยที่ไม่มีเคซีน และเมื่อเปรียบเทียบความคงตัวก็พบว่าอิมัลชันที่เตรียมจากนมขาดมันเนยที่มีเคซีนมีความคงตัวมากกว่าอิมัลชันที่เตรียมจากนมขาดมันเนยที่ไม่มีเคซีนในทุกระดับความเข้มข้นและ pH ของ KGM ส่วนผลการทดลองส่วนที่สองพบว่าขนาดอนุภาค (particle size) ในอิมัลชันลดลงเมื่อค่า pH ของสารละลาย KGM เพิ่มขึ้น ค่าศักย์ซีตา (Zeta potential) ของอิมัลชันมีค่าลดลงเมื่อ pH ของ KGM เพิ่มขึ้นในทุกๆความเข้มข้นของ KGM แต่อย่างไรก็ตามค่าการกระจายตัวของพอลิเมอร์ (Polydispersity index) ไม่เปลี่ยนแปลงเมื่อเพิ่มความเข้มข้นของ KGM และ pH โดยการเพิ่มขึ้นของค่า pH ของสารละลาย KGM จาก 3-10 ที่ความเข้มข้นของ KGM ที่เท่ากันสามารถเพิ่มความคงตัวของอิมัลชันได้ ภาพกล้องจุลทรรศน์แบบคอนโฟคอลสามารถยืนยันสมมติฐานที่ว่าโครงสร้างจุลภาคของอิมัลชันที่เสถียรของ KGM ถูกควบคุมโดย pH โดยจากภาพพบว่าค่า pHที่ต่ำกว่า ส่งผลให้ขนาดอนุภาคมีลักษณะที่ขยายออก โดยจะเห็นการรวมกลุ่มของอนุภาคชัดเจนในอิมัลชันที่เตรียมจาก KGM ที่ pH 7 ในขณะที่อิมัลชันที่เตรียมจาก KGM ที่ pH 10 พบว่ามีการกระจายของอนุภาคมากกว่าจากการศึกษานี้พบว่าความคงตัวของอิมัลชันจะเพิ่มขึ้นเมื่อลดความเข้มข้นของ KGM และเพิ่ม pH โดยอิมัลชันที่มีความเข้มข้นของ KGM ร้อยละ 0.04 ที่ pH 9 และ 10 มีความคงตัวสูงสุด โดยอิมัลชันที่สภาวะนี้มีความคงตัวโดยเกิดการแยกชั้นเป็นเวลา 10 วัน จากนั้นอิมัลชันสภาวะนี้จะถูกนำไปทดสอบด้วยความดันสูง ผลการศึกษาพบว่าขนาดอนุภาคจะลดลงเมื่อความดันเพิ่มขึ้น จากการศึกษานี้พบว่าความคงตัวของอิมัลชันที่ถูกทดสอบด้วยความดันสูงเพิ่มขึ้นจาก 10 วันเป็น 14 วันโดยไม่มีการแยกชั้น โดยความดันที่เพิ่มขึ้นส่งผลให้ความคงตัวออกซิเดชันของไขมัน ซึ่งแสดงได้จากผลการทดลองที่แสดงให้เห็นว่าค่าไฮโดรเปอร์ออกไซด์ (PV) และค่ากรดไธโอบาร์บิทูริก (TBARS) เพิ่มขึ้น อย่างไรก็ตาม ไม่พบความแตกต่างของความคงตัวออกซิเดชันของไขมันระหว่างการใช้ความดันสูงและการพาสเจอร์ไรส์ นอกจากนี้การใช้ความดันสูงสามารถช่วยลดจุลินทรีย์ทั้งหมด, โคลิฟอร์มแบคทีเรียและ *S. aureus* ในอิมัลชันลง แต่เช่นเดียวกับความคงตัวออกซิเดชันของไขมัน ซึ่งพบว่าไม่มีความแตกต่างในการลดลงของปริมาณจุลินทรีย์ระหว่างการใช้ความดันสูงกับการพาสเจอร์ไรส์

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# # 5772853923 : MAJOR FOOD TECHNOLOGY
KEYWORD: milk, casein, emulsion, fish oil, glucomannan, pH, stability, high pressure processing

Siriwan Suknicom : PREPARATION OF ENCAPSULATED KONJAC GLUCOMANNAN-BASED FISH OIL AND ITS USE IN HIGH PRESSURE PROCESSED GOAT MILK. Advisor: Assoc. Prof. CHALEEDA BOROMPICHAICHARTKUL, Ph.D. Co-advisor: Prof. Richard Archer, Ph.D.

This study was divided into 3 parts. The objective of first part aims to investigate the effect of konjac glucomannan (KGM) solution (0.02-0.5%, w/w) at different pH (3, 5 and 9) on the stability between 5% fish oil- skim milk with and without casein emulsion. The second part aims to study the effects of konjac glucomannan (KGM) solution (0.02-0.5%, w/w) at different pH (3-10) on the stability of 5%fish oil-milk emulsion. And the last part aims to study the effect of high-pressure (400, 500 and 600 MPa) on the improvement of the stability of 5%fish oil-milk emulsion. The results of first part show that, particle size of 5% fish oil- skim milk with casein emulsion was larger than % 5 fish oil- skim milk without casein emulsion at the same concentration and pH of KGM. Zeta potential of the emulsion from skim milk with casein was lower than emulsion from skim milk without casein at the same concentration and pH of KGM. Comparing the stability of emulsions, it was found that 5% fish oil- skim milk with casein were more stable than % 5 fish oil- skim milk without casein emulsion. The results from second part show that, particle size in emulsion was decreased with the increase in pH values of the KGM solution at all concentrations of KGM. Zeta potential of the emulsion was decreased when the pH of KGM solution was increased as well at all concentration of KGM. However, polydispersity index (PDI) was not changed when increase KGM concentration and pH. The increase in pH values of the KGM solution from 7-10 at the same concentration of KGM could increase the stability of the emulsion. Confocal laser scanning microscopy images confirmed an assumption that microstructures of KGM stabilized emulsions were controlled by pH. The images revealed that lowering the pH resulted in the expanded appearance of the aggregates. Moreover, the appearance of aggregates changed from isolated cluster to cluster networks as shown in emulsions at pH 7 vs emulsions at pH 10. Fish oil emulsions containing KGM in milk at different pH and concentration of KGM solution exhibited difference in stability. The mixture stability was enhanced when decreasing the KGM concentration in solution and increasing pH. The highest stability of the mixture was obtained with 0.04% KGM at pH 9 and 10. The emulsion at appropriate conditions was stable without separation for 10 days. After that, the emulsion at appropriate conditions was subjected to HPP. The results show that, particle size was decreased when increase pressure. Moreover, it was also found that the viscosity of the emulsion increased with increasing pressure. The stability of the HPP-treated emulsion increased from 10 days to 14 days without separation. The impact of HPP on the oxidative stability of lipids was increased when pressure was increased as the hydroperoxides values (PV) and thiobarbituric acid values (TBARS) were markedly enhanced by high pressure. However, no oxidative stability differences were observed between HPP and pasteurization. Moreover, HPP also significantly reduced the total plate count (TPC), coliform bacteria and *S. aureus* in emulsion. But as with oxidative stability, no differences result in reduce microbial between HPP and pasteurization.

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Siriwan Suknicom

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# CHAPTER 1
# INTRODUCTION

## 1.1 Research background

Nowadays, there are many studies suggest that long chain polyunsaturated fatty acids (PUFA) have beneficial effects on health, including prevention of cardiovascular diseases, decrease the risk of many types of cancer and prevention of many diseases. (Adkins & Kelley, 2010; Lorente-Cebrián et al., 2013; Papanikolaou, Brooks, Reider, & Fulgoni, 2014). Fish oil are the richest sources of long chain polyunsaturated fatty acids especially omega 3 PUFA. The main omega-3 PUFA present in fish oil are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Randomized clinical trials recommend consuming at least 250 mg per day of EPA+DHA can reduce the relative risk of cardiovascular disease (CHD) mortality by 36% when compared to no consume (Richter, Skulas-Ray, & Kris-Etherton, 2016). Fortification fish oil into various foods is an innovative choice of elevating the intake of omega-3 fatty acids without changes in eating habits. However, adding lipids such as omega 3 PUFA into food products gives rise to major formulation challenges (Jacobsen, 2010). Like many lipids, omega-3 fatty acids are sensitive to oxidative damage due to heat, light and oxygen (Albert, Cameron-Smith, Hofman, & Cutfield, 2013). Oxidation of fatty acids is a major cause of food deterioration which can effect on the properties of food such as aroma, flavor, color and shelf life. Besides producing undesirable properties, oxidation can reduce the bioavailability of lipid (Amaral, Silva, & Lannes, 2018). One way of reducing fatty acid oxidation of through lipid encapsulate to reduce its exposure to oxygen, metals and substances that attack the double bonds and other susceptible position of the lipid (Singh, Zhu, & Ye, 2015).

Encapsulation is an effective method of containing materials in microparticle and nanoparticle. It is commonly used as a process to cover substance which call “Active ingredient” inside other material which call “Wall material” (Jafari, Assadpoor, Bhandari, & He, 2008). In this case, oxidizable fatty acid have been encapsulated by substance including polysaccharides and protein, in the form of an oil-in-water emulsion (Miyagawa & Adachi, 2017).

Konjac glucomannan (KGM) is a polysaccharide used as one of the hydrocolloid materials. The hydrocolloid membrane encapsulating the liquid was a thickness capable of holding the liquid without exploding through a temperature range of about -20 $^{o}$C to about 90 $^{O}$C (Yingqing, Xie, & Gan, 2005). Konjac glucomannan (KGM) is a water-soluble polysaccharide with a molecular weight of 200 to 2000kDa (Zhang, Chen, & Yang, 2014). KGM can extracted from a tuber of konjac, which is a plant in the genus Amorphophallus (Weixun & Wu, 2004). KGM is made up of D-

mannose and D-glucose molecules, which linked by β-1,4 glycosidic bonds at a ratio of 1.6:1 and 5–10% acetyl group substitution. When dissolved in water, KGM can form a highly viscosity solutions (Yoshimura & Nishinari, 1999). In Europe and USA, KGM is often used as a food additive due to its gel-forming properties (Chua, Baldwin, Hocking, & Chan, 2010). In addition, KGM also lists several possible nutraceutical properties, such as being found to be able to lower blood cholesterol and blood sugar levels. It is beneficial for weight loss, and also has properties to promote intestinal and immune functions (Ying, Xie, & Gan, 2005).

In recent two decades, milk have shown possible to be fortified with omega 3 PUFA (Barrow, Nolan, & Holub, 2009; Bermúdez-Aguirre & Barbosa-Cánovas Gustavo V, 2011; Rasti, Erfanian, & Selamat, 2017). Milk is characterized as a food matrix of simple access and easy to eat, which contain many nutrients such as proteins, carbohydrates, vitamins and minerals. Goat milk is currently interest because of its specific composition, which has led to it being considered a high-quality raw material for production (Ceballos et al., 2009). The protein in goat milk is more digestible and at the same time less allergenic (Türkmen, 2017). The hypoallergenicity of goat milk compared to cow milk was found to be lower. This is because it is associated with absence or low alpha s1-casein (αs1-cn) levels in goat milk and this fraction has been shown to have allergenic potential (Ballabio et al., 2011). It is very important to keep milk at low temperatures to prevent deterioration before submitting it to heat treatments such as pasteurization and Ultra-High Temperature (UHT) process. The main goals of heat treatment are to reduce the number of microorganisms, to inactivate enzyme activities and to reduce the chemical reactions resulting in minimal physical properties changes. However, heat often has some effects of on certain quality and properties of milk. Pestana et al. (2015) suggested that heat processing influences lactose degradation to organic acids, whey protein denaturation, vitamins degradation and lipids hydrolysis. Other effects include altered cooked flavor and nutritional loss cause by new substances produced by the Millard reaction (Pestana, Gennari, Monteiro, Lehn, & Souza, 2015).

In recent years, non-thermal processing technologies such as high-pressure processing (HPP) have been developed as an alternative to pasteurization and sterilization (Mújica Paz, Valdez-Fragoso, Samson, Welti-Chanes, & Torres, 2011). Delgado et al. (2013) reported that HPP results in a similar reduction in microbial counts of olive jam compared to heating processing, while not changing sensory characteristics (Delgado et al., 2013). Liu et al. (2020) compared the effect of high-pressure processing (HPP: 600 MPa for 5 min) and thermal treatments, (low temperature long time: 63 °C for 30 min and high temperature short time: 72 °C for 15 s) in milk. They found that HPP at 600 MPa for 5 min was considered an alternative to raw milk processing. HPP able to maintain the original composition and sensory characteristics of raw milk compared to heat treatment (Liu et al., 2020).

However, the pressure exerted by HPP processes lead to degradation of whey and casein proteins, disruption of Milk Fat Globule Membrane (MFGM) and reduction in diameter (Sahu & Mallikarjunan, 2012). However, using extreme pressure, the formation of volatile compound is different from that under atmospheric pressure conditions. Thermal processing at high temperature promotes the formation of methyl ketones and aldehydes, while high pressure contributes to the formation of aldehydes. (Vazquez-Landaverde, Torres, & Qian, 2006).

After careful literature review over a wide range of research, there is a lacking in the use of konjac glucomannan on encapsulation of fish oil and its comprehensive description of the possible alteration in milk complexity during HPP. Hence, the goal of this study is to find the preparation process to stabilize fish oil under microencapsulation by using konjac glucomannan and mixed into goat milk and undergoes HPP processing.

## 1.2 Objective

1. study suitable preparation process of KGM to the right concentration and pH in order to prepare stable fish oil milk emulsion.
2. study effect of HPP of goat milk fortified with fish oil and its stability during storage.

## 1.3 Scope of the study

There are two main parts of this experiment.

1. Study effect between skim milk, skim milk without casein and KGM solution (0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4 and 0.5 %w/w) and pH (3, 5 and 9) in order to prepare stable fish oil milk emulsion
2. Study suitable condition for surface modification of KGM by varying concentration of KGM solution (0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4 and 0.5 %w/w) and pH (3, 4, 5, 6, 7, 8, 9 and 10) to prepare stable fish oil milk emulsion.

2\. Study effect of HPP (400, 500, 600 MPa, 3 min) of KGM milk -fish oil emulsion and its stability during storage.

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# CHAPTER 2
# LITERATURE REVIEW

## 2.1 Microencapsulation

Microencapsulation is a process using material to form wall to avoid chemical and physical reactions and to preserve the biological and physicochemical properties of core materials (Amr et al., 2015; Bakry et al., 2016). With the microencapsulation method, particles or droplets are surrounded by a coating material, or are encapsulated in a homogeneous or heterogeneous matrix, to form small capsules (Calvo, Castaño, Hernández, & González-Gómez, 2011). It can cover compound with different matter (solid, liquid and gaseous) within another substance in a very small capsule. The core material is continuously dispersed through the capsule walls, thereby offering controlled release properties under suitable conditions (Fang & Bhandari, 2010). Briefly, microencapsulation is the process of creating a barrier between a core and a wall material to prevent chemical and physical reactions and to provide the biological and physicochemical properties of the core materials. Thus, microencapsulation technology can be used to distribute bioactive substances and improve handling properties (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Typically, microcapsules consist of core material and wall material. Core material referred to as internal phase, which are also called coated material, core material, actives, fill and internal phase. Wall material referred external, which can call coating material, wall material, capsule, membrane, carrier or shell. Wall material controls the stability of the microcapsule, the process efficiency, and the degree of protection for the core. Mostly, wall material is made from sugars, gums, proteins, natural and modified polysaccharides, lipids and synthetic polymers (Amr et al., 2015; Gibbs, Kermasha, Alli, & Mulligan, 1999) (Figure 1).

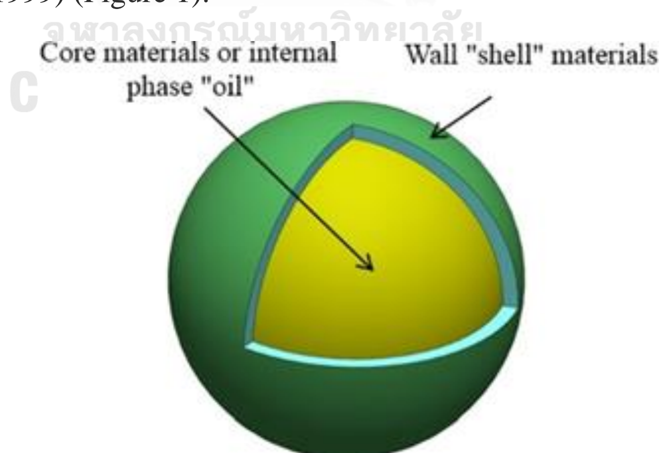


Figure 1 Composition of an oil microcapsule in simplified form (Bakry et al., 2016)

Microcapsules are vesicles or particulates that can range in size from a few microns to several millimeters (Dziezak, 1988)). The morphologies of microcapsule come in many forms, but two major morphologies can be seen commonly (Figure 2): mononuclear capsules, which have one core surrounded by a shell, while the other is

aggregated, which have many cores spread in a matrix (Schrooyen, Van der Meer, & De Kruif, 2001). Specific shapes of microcapsule are different due to the influence of process technologies, core and wall materials of the microcapsule (Fang & Bhandari, 2010).

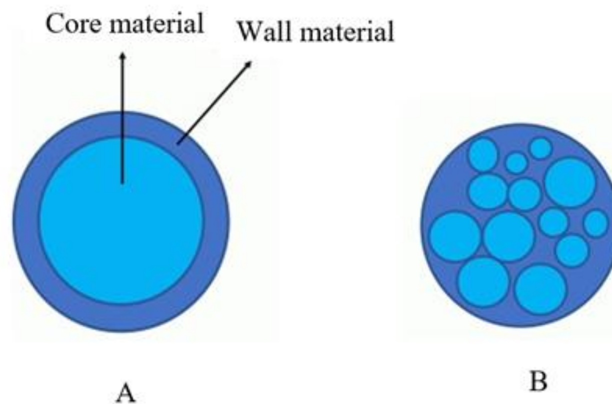


Figure 2 Major forms of encapsulation: mononuclear capsule(A) and aggregate(B) Adapted from (Fang & Bhandari, 2010)

In the food industry, microencapsulation process is used for a variety of reasons (Desai & Jin Park, 2005):

- Protect the core material change by reducing the reaction with the external environment (e.g., moisture, air, heat and light).
- Reduce or delay the evaporation or transfer rate of the core material to the external environment.
- The physical characteristics of the internal material are little or not changed, making them easy to handle.
- The core material of producing microcapsule can be customized to release slowly over time or at a certain time (i.e., to control the release of the core material to obtain a delayed property until stimulus is reached).
- It can mask the flavor of the core material.
- The core material can be distributed evenly in the host material, even in small quantities.
- They can be used to separate components within materials that may interact with each other.

Different techniques are used in making microcapsule. Typically, 3 steps involve encapsulation of bioactive compound (Mozafari et al., 2008):

- Creating the wall around the material to be wrapped.
- Make sure that undesired leakage will not occur.
- Make sure that unwanted materials are kept out.

The current encapsulation techniques include spray drying, spray cooling/chilling, extrusion, fluidized bed coating, coacervation, liposome entrapment,

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inclusion complexation, centrifugal suspension separation, lyophilization, co-crystallization and emulsion, etc. (Mary Ann Augustin & Hemmar, 2009)

## 2.2 Health benefits of fish oil and their encapsulation

### 2.2.1 Fish oil

Fish oils consist of essential PUFAs, including omega-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, it is estimated that fish oil contains approximately 43% of omega-3 PUFAs. The remaining 57% consists of saturated fat, omega-6 PUFAs linoleic (LA) and arachidonic (AA) acids, cholesterol, and other fats (Figure 3 and Figure 4). There are 3 unique characteristics of fish oil that make it different from other commercially available oils (Ackman & Lamothe, 1989).

1.The number of carbon atoms of the fatty acid molecule and high degree of unsaturation.

2.The high content of PUFAs and the long-chain omega-3.

3.There are many fatty acids and there are many different types of fatty acids present in the triacylglycerols

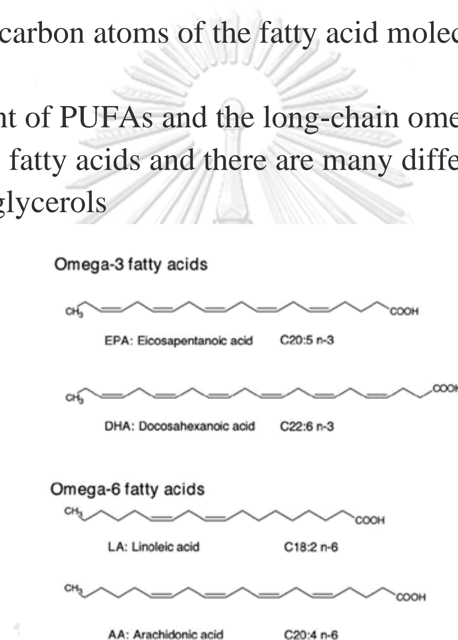


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Figure 3 Structure of omega(ω) -3 and omega (ω)-6 Fatty acid (Kashiwagi & Huang, 2012)

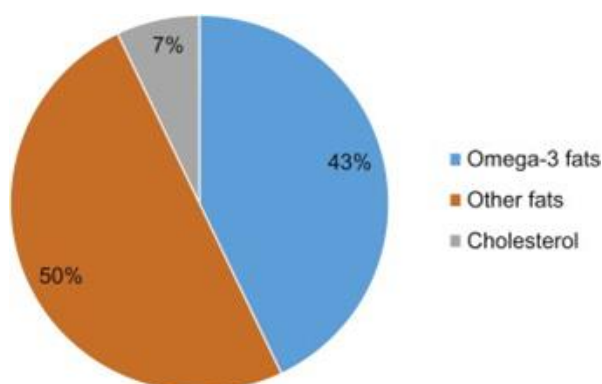


Figure 4 Constituents of fish oil (Lembke & Schubert, 2014)

2.2.2 Health benefits of fish oil

Intake of EPA and DHA has been found to be health beneficial such as the prevention of cardiovascular disease, reducing the symptoms in rheumatoid arthritis and reducing the progression stages of some types of cancer. Studies suggesting the effect of long-chain n-3 PUFAs 2-3 g/day was associated with lowering blood pressure in hypertension (Arab-Tehrany et al., 2012; Bimbo, 2013; Weitz, Weintraub, Fisher, & Schwartzbard, 2010). In addition, research suggested that omega 3 PUFA may be effective in treating depression and schizophrenia (Schram et al., 2007). DHA is also essential for the brain development, mammalian nervous system, fetus and infants eye development (Kolanowski, Jaworska, & Weißbrodt, 2007). Epidemiological studies suggest that high consumption of fish is associated with a decrease in cognitive impairment, helping to slow down the development of dementia or Alzheimer's disease (Larrieu, Letenneur, Helmer, Dartigues, & Barberger-Gateau, 2004). EPA has also been found to play a role in cardiovascular and immunological health (Ryan et al., 2010). In addition, intervention studies have shown that 6 months of intake EPA of Alzheimer-type and cerebrovascular dementia patients improves cognitive function (Otsuka & Ueki, 2001). Typical recommendations from the World Health and North Atlantic Treaty Organization recommends 0.3–0.5 g/day of EPA + DHA. Additionally, the UK Committee on Medical Aspect of Food and Nutrition Policy recommends that the intake of EPA and DHA should be 0.2 g/d or 1.5 g/week (Kris-Etherton, Harris, & Appel, 2002). The International Society for the Study of Fatty Acids and Lipids also recommends that pregnant and lactating women should adequate intake of EPA plus DHA at least 0.65 mg per day (Kolanowski, Jaworska, Weißbrodt, & Kunz, 2007).

2.2.3 Fish oil encapsulation

The major problems associated with adding fish oils in food products are insoluble in water, the sensitivity of PUFAs to oxidative which cause the development of undesirable rancid odor and flavors (Augustin, Sanguansri, & Bode, 2006).

- Oxidation of omega-3 PUFA

The oxidation of lipid in fish oil and other PUFA-rich foods are a major contributing factor to many problems, including loss of shelf-life, lower consumer acceptance, lower nutritional value, and safety (Dacaranhe & Terao, 2001). The oxidation lipid is caused by exposure to oxygen and light, which results in decreasing stability and shelf-life of products containing fish oil.

3 main problems of Lipid oxidation were (Jacobsen et al., 2001):

- The formation of an unpleasant taste and flavor.
- The nutritional value of food products which lipid-containing is reduced.
- Free radicals produced during oxidation may be involved in the development of atherosclerosis.

When fish oils are oxidized, it creates unstable intermediate compounds, such as free radicals and hydroperoxides, which are vulnerable to degradation products such as aldehydes and ketones (Valero, Villamiel, Miralles, Sanz, & Martínez-Castro, 2001). During polyunsaturated fatty acid peroxidation, a complex substance of secondary lipid oxidation products (alkanes, alkenes, aldehydes, ketones and etc.) are created (Vichi, Pizzale, Conte, Buxaderas, & López-Tamames, 2003). The oxidation activity of food can be assessed by measuring the formation of primary oxidation product (hydroperoxides) and the formation of secondary products (low molecular weight aldehydes) (Romeu-Nadal, Chávez-Servín, Castellote, Rivero, & López-Sabater, 2007). The oxidation of fish oil, as other fats, which containing PUFA lipids, involves the reaction of polyunsaturated fatty acids with oxygen and takes place in 3 phases (Figure 5):

- an initiation phase
- a propagation phase
- a termination phase

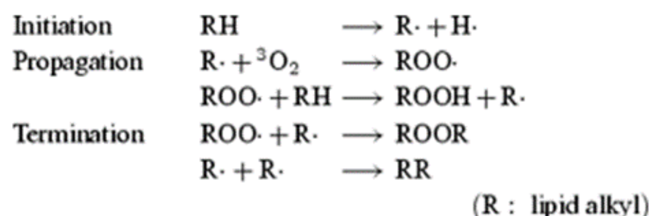


Figure 5 Oxidation of oil (Choe & Min, 2006)

At the beginning, the initiation phase starts by reacting with additional lipid molecule generate reactive products. This reaction, the addition of further oxidation by propagation phase products, gives rise to the term “autoxidation” (Kolanowski, Jaworska, & Weißbrodt, 2007). The autoxidation or peroxidation of lipids (lipid hydroperoxide: LH) in uniform solution is a free radical reaction (Lagarde, 2010). This is a major mechanism for lipid oxidation, an autocatalytic process introduced by generated of radicals in unsaturated lipids followed by oxygen attack (Frankel, 2014). The primary oxidation products (hydroperoxides) are developed and further oxidation, molecule decomposition and polymerization lead to create a secondary oxidation product which contain many compounds (Dobarganes & Márquez-Ruiz, 2003). The oxidation reaction is caused by a variety of internal and external factors, such as the fatty acid composition, the concentration of antioxidant, enzymes, pH, temperature, ionic strength, and exposure to oxygen (Figure 6).

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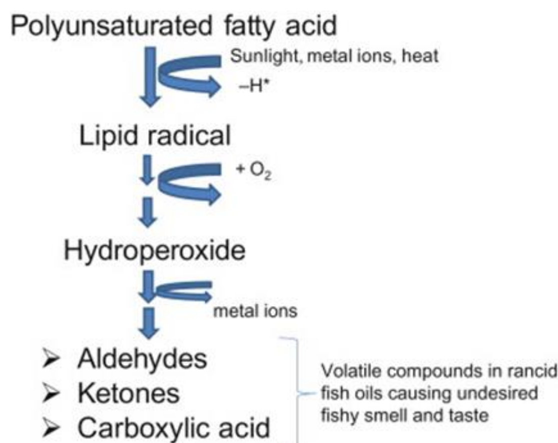


Figure 6 A simplified oxidation pathway of a fish oil (Lembke & Schubert, 2014)

- Stabilization of omega-3 PUFA in foods with encapsulation technique

Lipid oxidation is a process of deterioration foods that greatly affects unacceptable for customers and a loss in nutritional value. Additionally, oxidation can lead to health disorders such as atherosclerosis and carcinogenesis. Therefore, the use of fish oil in food matrix can be improved through encapsulation technology. Gelatin and sodium carboxymethylcellulose were used as wall material to protect EPA and DHA in fish oil by using the complex coacervation method (Patrick KE, Abbas S, Lv Y, Ntsama ISB, & X, 2013). The production of tuna oil microcapsules with gelatin–sodium hexametaphosphate complex particles was also found to be more stable compared to the nonencapsulated oil (Wang, Adhikari, & Barrow, 2014). A stable emulsion includes menhaden oil was created by using sodium caseinate (SC) and soluble rice bran fiber (SRBF), and SRBF provided better oxidative stability during storage and spray-drying (Wan, Bankston Jr, Bechtel, & Sathivel, 2011). According to a study by Augustin et al., (2011), microencapsulation of fish oil, tributyrin, and resveratrol in oil-in-water emulsions, stabilized with a mixture of caseinate, glucose, and modified starch can increase the levels of radioactivity from the bioactive in the blood and liver, thereby enhancing their absorption (Marry Ann Augustin et al., 2011).

## 2.3 Microencapsulation based on emulsification technique

The emulsion is a suitable system for delivering nutritional supplements, but instability limits the use of the emulsion. To increase emulsion stability, adding stabilizers is an effective way to improving emulsion stability.

### 2.3.1 Explanation of emulsions

An emulsion is defined as a dispersion of two or more liquids, which are generally incompatible, with one liquid being dispersed as small droplets (0.1–100 µm) in another (Friberg, Larsson, & Sjoblom, 2003).

In general, emulsions can be classified into the following 3 classes: water-in-oil; oil-in-water; and multiple emulsions.

Water-in-oil emulsions include water droplets in an oil continuous phase,

Oil-in-water emulsions are formed by oil droplets in a water continuous phase.

Multiple emulsions are more complex, such as water-in-oil-in water, which are made of droplets in another droplets, which disperse in a continuous phase.

The production of the 3 types of emulsions depends on many factors such as the oil/water ratio and temperature. In terms of quantity, when compared, it is found that the dispersed phase has less volume than the continuous phase. However, when the ratio of the two phases is the same, the type of emulsion is controlled by other factors, such as temperature or interfacial properties of the phases.

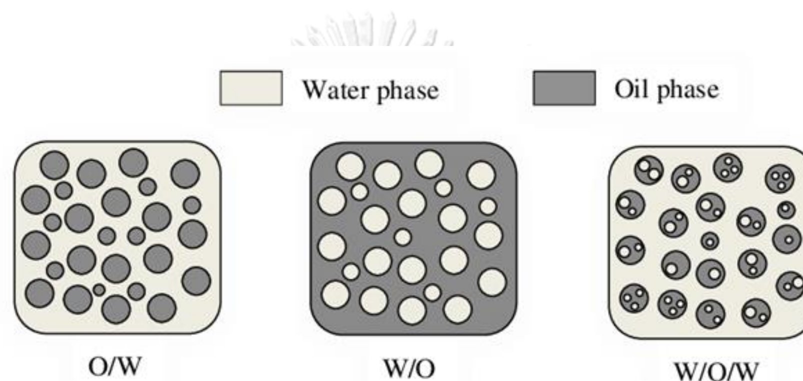


Figure 7 Classification of emulsions (Akbari, 2018)

In addition to the classification of emulsions from the ratio of the dispersed and continuous phases, the emulsions can also be classified according to the droplet size of the dispersed phase (Janssen, Noik, & Dalmazzone, 2001).

- Macroemulsions: droplet size of dispersed phase usually ranges from 0.1–5 μm.
- Microemulsions: droplet size of dispersed phase usually ranges from of 5–2 nm.
- Nanoemulsions: droplet size of dispersed phase usually ranges from 20–100 nm.

2.3.2 Emulsion formation

To form an emulsion, an emulsifier is required, emulsifier consists of both hydrophobic and hydrophilic parts that combined at the oil–water or water–oil interface to lower the interfacial tension (Bos & Van Vliet, 2001). There are many forms of emulsifiers, such as low molecular weight synthetic (*e.g.,* monoglycerides (Goldstein & Seetharaman, 2011), sucrose esters (Tual et al., 2006), polyglycerol esters (Su, Flanagan, Hemar, & Singh, 2006)or naturally molecules (*e.g.,* soy or egg

lecithin (Palacios & Wang, 2005), or large macromolecules, such as proteins (Damodaran, 2005). The emulsifier function is to form a viscous film around the dispersed phase droplets to stabilize the emulsion over time. In addition to using emulsifiers to increase emulsion stability, stabilizers are used. It improves the stability of the emulsion as well. The use of stabilizers is the most convenient and effective approach for improving emulsion stability. Stabilizers are divided into 3 categories: surface modifiers, ripening inhibitors and weighting agents (McClements, 2011). Although these stabilizers often do not have emulsifying properties, their interactions with emulsifiers creates a stable film at the oil and water junction to improve the stability. In addition, the stabilizers can prevent flocculation by creating a gel network structure in the emulsion system. (Anal, Shrestha, & Sadiq, 2019).

2.3.3 Emulsion stability

- Factors affecting emulsion stability

Emulsion instability can easily occur under various conditions during preparation and storage. Therefore, it is necessary to understand the factors influencing the stability of the emulsion. In general, there are 2 types of interacting factors affecting emulsion stability: static interaction and electrostatic interaction (Figure 8).

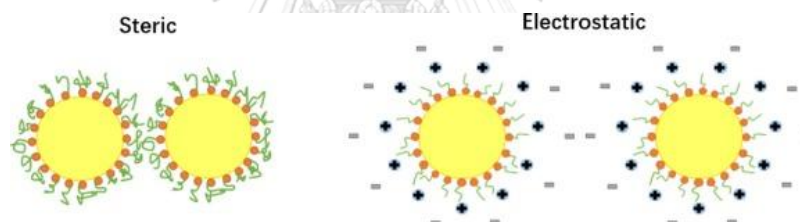


Figure 8 The different interactions between droplets that affect the stability of the emulsion (Shao et al., 2020).

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*Steric interactions*

Steric interactions are important factor for emulsion stability which is related to the interfacial properties of an emulsion system. During emulsion generation, the emulsifier is adsorbed on the oil–water interface of the droplets to form a stable emulsion (Van Oss, 2007). Different emulsifiers will result in different interfacial layer thicknesses that influence the strength of the interactions between the droplets. When the interfacial layer formed by the emulsifier is of the appropriate thickness, the emulsion is stabilized by strong spatial repulsive force (Tadros, 2015). When the interface layer between the droplet is not thick enough, it’s likely to make the emulsion to become unstable (Cai et al., 2018) (Figure 9). An example of the phenomenon of emulsion instability caused by the improper of the interfacial layer is the occurrence of creaming and Oswald ripening. Creaming occurs when droplet density in the dispersed phase is lower than that the continuous phase (Suriyarak & Weiss, 2014). Ostwald ripening occurred when droplets in the emulsion system,

increasing in size to form larger droplets. The process is due to the higher solubility of the oil molecules in the proximity of the smaller droplets compared to the larger droplets due to the effect of curvature (Kabalnov & Shchukin, 1992).

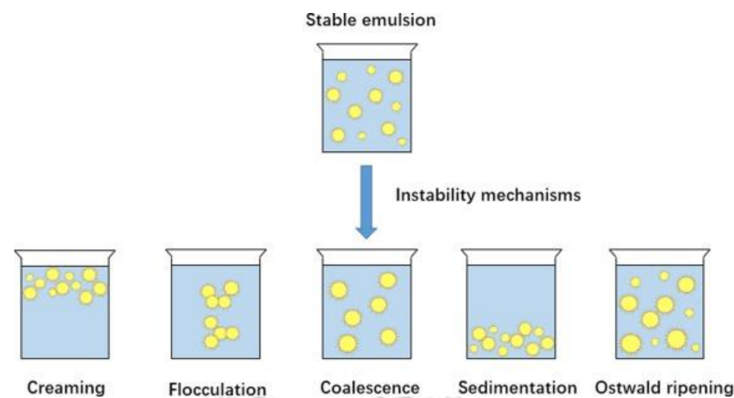


Figure 9 Emulsion instability (Creaming, Flocculation, Coalescence, Sedimentation and Ostwald ripening) (Shao et al., 2020).

*Electrostatic interactions*

The electrostatic interactions play a role in the stability of the emulsion when the strength of interfaces between the droplets is not enough. The electrostatic repulsion intensity is related to the electric charge density and the ionic strength of the droplets. The emulsion is unstable as the electrostatic interaction weakens. In this case, the emulsion instability is expressed in the form of coalescence and flocculation. Both phenomena are similar, characterized by the general nature that the electrostatic repulsion between droplets is not strong enough to withstand the attractive interaction (Van der Waals interaction) (Delahaije, Hilgers, Wierenga, & Gruppen, 2017).

2.3.4 Methods for improving emulsion stability

From various factors that affect the stability of the emulsion, this has led researchers to develop an effective method to improve the stability of the emulsion. Developing the emulsion production process is the most commonly used methods to increase emulsion stability. Nowadays, emulsions are often prepared by high pressure homogenization, ultrasonic technology, or micro-channel emulsion (MCE) (Khalid, Shu, Kobayashi, Nakajima, & Barrow, 2017). In addition to improving the emulsion production method, one effective way to improve emulsion stability is to add a stabilizer to help compensate for the emulsifier defects (McClements, Bai, & Chung, 2017). An example of the interaction of emulsifiers and stabilizers that will increase the stability of the emulsion, both substances with the same negative charge increase the total negative charge at the oil–water interface, preventing the coalescence phenomenon (Zhao et al., 2015).

Many researchers believe that polysaccharides are the preferred stabilizer. Xu et al., (2017) used the soybean polysaccharide to assist sodium caseinate to stabilize

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the emulsion, which formed a dense interfacial film through electrostatic and hydrophobic interactions, thereby improving emulsion stability (Xu, Wang, & Yao, 2017).

**2.4 Glucomannan in improving emulsion stability**

Konjac (Amorphophallus konjac) is a perennial in the genus Amorphophallus. Konjac plants grow in Southeast Asia and Africa (Chua et al., 2010). Konjac Glucomannan (KGM) is a dietary fiber and a source of Glucomannan with approximately 40% glucomannan content of the plant corm (Fank & Wu, 2004). KGM is often used as a food additive because of its water-absorbing properties, film forming, thickening and emulsifying properties (Zhou et al., 2013). KGM is also used as a nutritional supplement for treating obesity, and diabetes (Huang et al., 1990).

KGM is a non-ionic hydrocolloidal dietary fiber consisting of d-mannose and d-glucose linked by β-1,4 glycosidic bonds at 1:1.6–1:1.4 M ratio, depending on the genotypes (Zhang et al., 2014) (Figure 10). In addition, KGM also has an acetyl group at C-6 position of the saccharide units and has side chains link to mannose (Katsuraya et al., 2003). Degree of branching is approximately 8% (Zhang et al., 2014). The molecular weight of KGM ranges from 500 k to 2000 k. The difference in molecular weight depends on the plant source and extraction process (Fan, 2018).

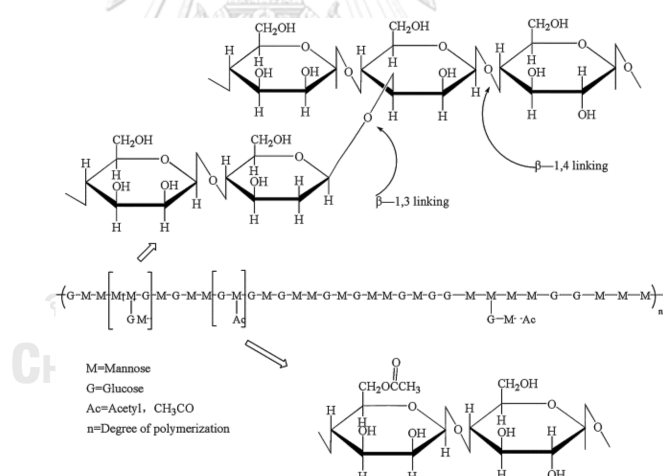


Figure 10 Chemical structure of KGM (Zhang et al., 2014).

KGM is water soluble with high viscosity even when used in low concentrations. It can also form a gel network structure as a result of extensive hydrogen bonding and entanglement. Based on these properties, KGM is used to stabilize the emulsion. The emphasis was mainly on improving the stability of the protein stabilized emulsion. As the only protein-based emulsion with excellent properties, it is more suitable for use in the food industry. However, protein-only systems are prone to environmental damage. The use of polysaccharides is therefore an alternative to compensating protein defects to prevent or slow this situation. The quantification of KGM (0.25, 0.5, 0.75 and 1%) was analyzed for the stability of the

oil-water sodium caseinate emulsion. Increasing the concentration of KGM increases the strength of the gel emulsion, resulting in the prevention of emulsion cream formation which causes phase separation (Belitz, Grosch, & Schieberle, 2009). In addition, the function of KGM in combination with other polysaccharides has been studied to promote emulsion stability. The effect of 2% wt. inulin and KGM content (0 wt.%, 0.1 wt.%, 0.2 wt.% or 0.4 wt.%) on the physical stability of the oil-in-water emulsion stabilized by sodium caseinate (CAS)was studied. The results showed that at high concentrations of inulin and KGM could strengthen the network structure of continuous phases. The increased amount of KGM (inulin/KGM>2:0.1) was able to significantly reduce the droplet size, thereby increasing the creaming stability of the emulsion (Wei et al., 2020). Other uses of KGM are also being studied to help stabilize the emulsion, such as deacetylation. Williams et al. (2000) studied the deacetylation and aggregation kinetics, the results indicated that the alkali showed the role of deacetylation and solubilization, and the deacetylation reduced the solubility and negated the alkali-induced polyelectrolytic nature of the polysaccharide chain (Williams et al., 2000). Luo, He & Lin (2013) studies show that KGM gelation occurs when alkali is added to the solution (Luo, He, & Lin, 2013). For this reason, the use of deacetyl KGM is of interest to enhance the stability of the emulsion. However, there are still few studies.

*Deacetylation of KGM*

When NaOH is added to KGM solution, the following phenomena occur (Doyle, Lyons, & Morris, 2009):

Firstly, the KGM deacetylation occurred, that caused in the decreasing water-soluble and the self-aggregation, and the expansion of the molecular chains became smaller. Second, the negative charge density of the molecular chains is much larger than in distilled water for the inductive effect of the alkali. The alkali was seen as a kind of nucleophilic substance, and it convince the electrons moving near to the oxygen atom of the hydroxyl group, even generated oxy anion, so the force of the electrostatic repulsion between the ionized molecular chain and hydroxide ion delay the expansion of the molecular chain in solution. As a result, there was a competition relationship between the inductive effect of alkali and the hydration of water. Thirdly, as an electrolyte, the alkali damaged the hydrogen bonding of the hydration between the molecular chain and the water molecule, resulting in the expansion-suppression.

**2.5 Milk emulsion**

Milk is the fluid from female mammals which secreted by the mammary glands. It contains almost all the nutrients needed to sustain life. Since ancient times, humans use the milk of cows, sheep and goats as food. In milk, milk fat is in the form of spherical droplets that are surrounded by a membrane and emulsified in the milk serum (or whey). The fat globules (or creams) separate after prolonged storage or

after centrifugation. The homogenization of the milk helps the fat globules not separate even after prolonged due to the reduction of globules (Belitz et al., 2009).

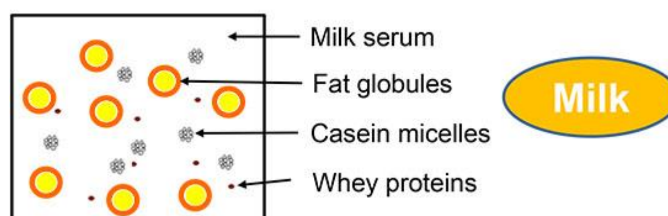


Figure 11 Milk contains emulsified fat globules and colloidally dissolved caseins and whey protein (Moreno-Villares & Germán-Díaz, 2019).

In milk serum, protein which various sizes are dispersed. They are called micelles and consist mostly of calcium salts of casein molecules. Two main fractions of milk protein were casein (80%) and whey protein (20%). The two fractions were difference in structure and functional properties allow them to be used in a wide range of applications (Haug, Høstmark, & Harstad, 2007).

The main structural elements and the properties of milk are listed in table 1

Table 1 Main structural elements of milk

| Name | Type of dispersion | Percentage |
|---|---|---|
| Fat globules | Emulsion | 3.8 |
| Casein micelles | Suspension | 2.8 |
| Whey proteins | Colloids | 0.6 |
| Lipoprotein | Colloids | 0.01 |

From: (Belitz et al., 2009)

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It is well known that milk-based proteins are good emulsifiers due to their amphiphilic properties, which permit them to adsorb and spread in the oil/water interface during homogenization (Dalgleish, 1996). Food emulsions prepared with milk proteins can divided into two groups according to type of protein: caseins, which exhibit flexible disordered conformations with little secondary structure and whey proteins, which have a considerably ordered secondary structure with a compact tertiary structure linked together by intramolecular disulfide bonds (Dickinson, 1998).

2.5.1 Emulsifying properties of milk protein

*Casein micelles*

In the aqueous environment, there are four types of casein: αS1-, αS2-, β-, and κ-casein. Only κ-casein has a hydrophilic fraction (Vilgis, 2020). The structure of a casein micelle is shown in figure 12.

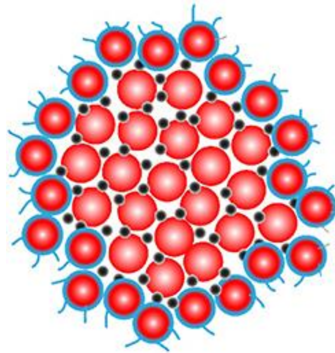

Figure 12 The casein micelle structure. Red spheres represent αS1-, αS2- and β-caseins. Black dot represents calcium phosphate bridging bonds. Blue surface represents the κ-caseins (Hege, Palberg, & Vilgis, 2020).

*Whey proteins*

The whey protein consists of β-lactoglobulin which makes 56% of the whey protein fraction and α-lactalbumin with 21%. Furthermore, whey protein contains immunoglobulins, serum albumin and lactoferrin (Hege et al., 2020). The globular proteins will be unfolding, denaturation and aggregation when heated at above 70 °C. The final structure of whey protein-stabilized emulsions can be tuned between liquid-like and solid-like by changing the conditions of heat treatment, pH and ionic strength (Sliwinski, Roubos, Zoet, Van Boekel, & Wouters, 2003).

2.5.2 Interactions between casein and polysaccharide

The reasons for using polysaccharide such ingredients might include viscosity control, general stabilization of an emulsion. When the casein stabilized emulsion comes in contact with the polysaccharide, many things will happen depending on the selected polysaccharide. If the polysaccharide is a neutral (no electrical charge on the backbone) the phase separation will occur. The mixture separates into two liquid phases, one containing fat and casein and the other containing the polysaccharide. If the polysaccharide is positively charged, then precipitation of casein will occur. (O'Kennedy, 2011). Hege et al. (2020) study the interactions between caseins and xanthan gum, guar gum, gellan gum as well as iota-carrageenan. It was found that, separation was occurring in xanthan gum and guar gum. The case of xanthan gum, the upper phase were the xanthan gum molecules, whereas the casein was in the lower phase. In the case of guar gum, the guar gum molecules are located in the upper phase and the casein are located in the lower phase. In the cases of iota-carrageenan and gellan gum, no phase separation was observed (Hege et al., 2020).

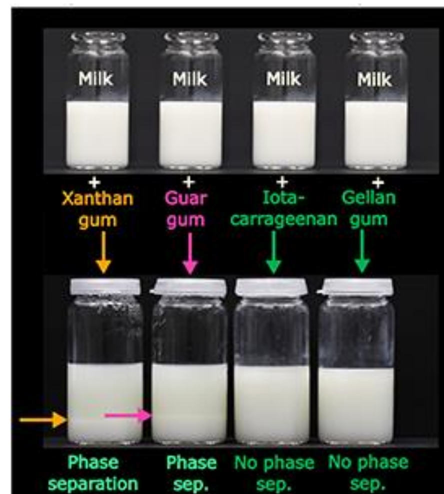


Figure 13 Appearance of the four different hydrocolloids dissolved in milk (0.1 wt% hydrocolloid) (Hege et al., 2020).

**2.6 Applications of high-pressure processing (HPP) in food emulsions**

Over the past decade, HPP has been used as an alternative to heat treatment in the food industry to improve quality and safety (Barba, Terefe, Buckow, Knorr, & Orlien, 2015; Mújica Paz et al., 2011) HPP is also highly effective in modifying the functionality of protein and polysaccharides making it possible to improve the hydrophobic and electrostatic interactions (Qin et al., 2013).

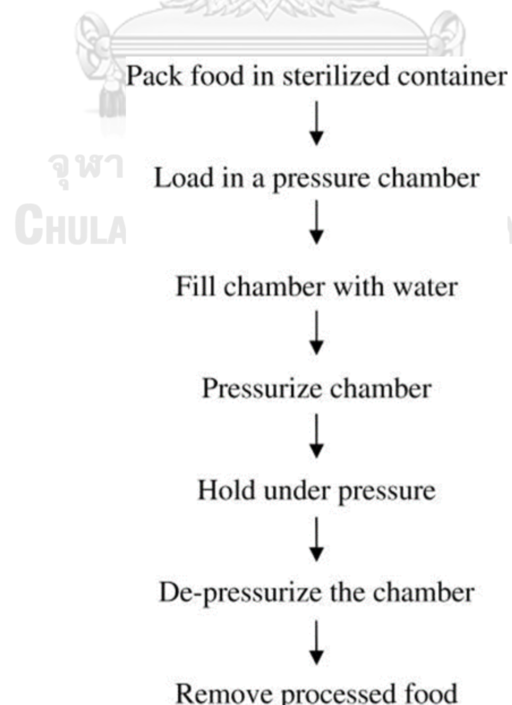


Figure 14 Flowchart Working of HPP (Chawla, Patil, & Singh, 2011)

In general, the medium used for pressure transmission is water. The pressure is applied isostatically. Therefore, the pressure in the product is uniform throughout the product. High pressure is non-thermal in principle, but the pressure increase causes small rise in temperature (Ohlsson & Bengtsson, 2002).

2.6.1 Basic principles of high-pressure processing

The basic principles that rule the behavior of foods under pressure are:

- Le Chatelier's principle: reaction, conformational, phase transition, attend by a decrease in volume is enhanced by pressure (Ledward, 1995);

- Microscopic ordering: an increase in pressure at stable temperature, increases the degrees of ordering of molecules of a given substance. Therefore, pressure and temperature exert antagonistic forces on molecular structure and chemical reactions (Balny & Masson, 1993);

- Isostatic principle: The product is compressed with consistent pressure from all directions and will return to its original shape when the pressure is released. The products are compressed independently of the product size and geometry because transmission of pressure to the core is not mass/time dependent thus the process is minimized (Olsson, 1995). This change in pressure causes a new structural change, such as protein denaturation or water dissociation (Smelt, 1998).

2.6.2 High pressure equipment

HPP system consists of a vessel and pressure-generation system, temperature control device and handling system (Mertens & Gould, 1995). The product is loaded into the chamber. The vessel is sealed and filled with pressure transmitting agent (Earnshaw, 1996). High pressure was applying to product by compress the water surrounding the product. When the desired pressure is reached, the pump is stopped. After holding the product for the desired time, the vessel is decompressed by releasing the pressure-transmitting fluid (Farkas & Hoover, 2000).

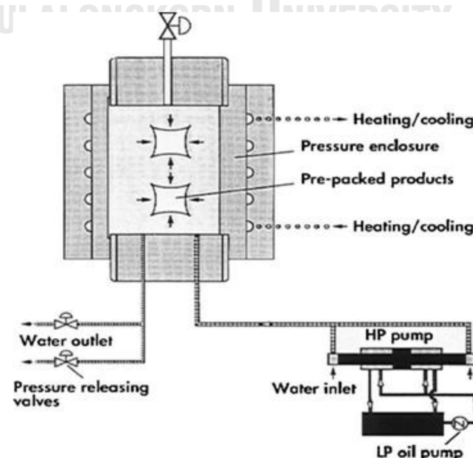


Figure 15 High-pressure processing system (Chawla et al., 2011)

Studies have shown that the use of HPP can improve the functionality of polysaccharides and protein molecules which can it improves the stability of the food grade emulsion system (Mohamad et al., 2018). The reason why HPP can increase the stability of food grade emulsions is because HPP can cause structural, morphological and physicochemical modifications of polysaccharides and proteins, such as starch gelatinization, denaturation of proteins, increasing interactions between food components and improving viscosity (Gharibzahedi et al., 2019). Yuan, Xu, Qi, Zhao, and Gao (2013) studied the effects of HPP (0–600 MPa for 10–30 min) on the stability of O/W emulsions stabilized by WPI and chitosan mixtures at pH = 4.0. The stability of emulsion was improved as a result of a three-fold decrease in the droplet size, increasing droplet size distribution homogeneity and increasing the creaming stability (Yuan, Xu, Qi, Zhao, & Gao, 2013). Emulsion treated at 600 MPa had smallest droplet sizes and stable without creaming separation after 30 days of storage at room temperature. The authors concluded that HPP had the potential to improve the stability of a protein polysaccharide emulsion.

Lipid oxidation is considered as the main deteriorative factor in foods containing high-fat levels such as emulsion. Zhu, Ye, Teo, Lim, and Singh (2014) evaluated the effects of HPP (300, 400, 500, 600 and 700 MPa at 20 or 50 °C for 15 min) on lipid oxidation in fish oil emulsions stabilized by whey protein isolate or sodium caseinate (0.5 wt%) (Xiangqian, Aiqian, Jiahan, Jeanne, & Harjinder, 2014)). They found that when the pressure was increased at low temperatures, the thiobarbituric acid and hydroperoxide value increased.

Besides, HPP can be used to pasteurize or sterilize emulsion systems to inhibit the growth or destroy microorganisms. Oh et al. (2017) found that the emulsion stabilized with 1% chitosan treated with HPP (138 MPa, 1 cycle) had a potential to inhibit the growth of *Salmonella typhimurium*, total mesophilic aerobes, yeasts, and molds during the storage time (Oh et al., 2017).

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# CHAPTER 3
# MATERIALS AND METHODS

## 3.1 Materials

### 3.1.1 Raw materials

Fresh goat's milk was purchased from a local farm in Phra Nakhon Si Ayutthaya province. The composition of goat milk containing 3.5% protein, 3.0% total fat and 4.3% lactose. The KGM powder was purchased from Yunnan Genyun Konjac Resource Corp., Kunming (Yunnan, PR China). Fish oils (food grade, DHA+EPA approximately 70%) were bought from TC Union Agrotech Co., Ltd., Thailand.

Skimmed milk (containing 3.0% protein, 0.3% total fat and 4.3% lactose) was made by separating the fat from the whole goat milk using cream separator. Skimmed milk without casein (containing 1% protein, 0.3% total fat and 4.3% lactose) was made by coagulation of casein using 0.03% rennet enzyme) according to Holzmüller, Gmach, Griebel & Kulozik (2016) (Holzmüller, Gmach, Griebel, & Kulozik, 2016).

### 3.1.2 Reagents and instruments

Table 2 Reagents for preparation emulsion

| Name | Company | Country |
|---|---|---|
| Sodium hydroxide (ACS Reagent, ≥97%) | Sigma-Aldrich | USA |
| Hydrochloric acid (ACS Reagent, ≥37%) | Sigma-Aldrich | USA |
| Sodium azide (ACS Reagent, ≥99.5%) | Sigma-Aldrich | USA |

Table 3 Reagent for confocal laser scanning microscopy

| Name | Company | Country |
|---|---|---|
| Nile Red (for fluorescence, ≥98.0%) | Sigma-Aldrich | USA |
| Fluorescein-5-isothiocyanate (for fluorescence ≥98.0%) | Sigma-Aldrich | USA |
| Ethanol (Absolute ≥99.8%) | Sigma-Aldrich | USA |

Table 4 Reagent for thiobarbituric acid (TBARS) and peroxide value (PV) analysis

| Name | Company | Country |
|---|---|---|
| Ethanol (Absolute ≥99.8%) | Sigma-Aldrich | USA |
| 2-Thiobarbituric acid (ACS | Sigma- | USA |

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| | | |
|---|---|---|
| Reagent, ≥98%) | Aldrich | |
| Cyclohexanone (AR grade, ≥99.5%) | Loba | India |
| 1,1,3,3-Tetraethoxypropane (AR grade, ≥96%) | Sigma-Aldrich | USA |
| Ethyl acetate (ACS Reagent, ≥99.5%) | Sigma-Aldrich | USA |
| Hexane (Anhydrous, 95%) | Sigma-Aldrich | USA |
| Methanol (Anhydrous, 99.8%) | Merk | Germany |
| 1-Butanol (ACS Reagent, ≥99.4%) | Sigma-Aldrich | USA |
| Ammonium thiocyanate (ACS Reagent, ≥97.5%) | Sigma-Aldrich | USA |
| Barium chloride (ACS Reagent, ≥99%) | Sigma-Aldrich | USA |
| Iron(II)sulfate heptahydrate (ACS Reagent, ≥99%) | Sigma-Aldrich | USA |
| Cumene hydroperoxide (Technical grade, 80%) | Sigma-Aldrich | USA |

Table 5 Reagent for microbial analysis

| Name | Company | Country |
|---|---|---|
| 3M Petrifilm E. coli/Coliform Count Plate | 3M Food safety | USA |
| 3M Petrifilm Staph Express Count Plates | 3M Food safety | USA |
| 3M Petrifilm Aerobic Count Plate | 3M Food safety | USA |
| Peptone (Bacteriological) | Himedia | India |

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Table 6 Instruments and apparatus for skim milk preparation

| Instrument | Mode | Country |
|---|---|---|
| Milk separator | 80L/H Creampurifier, WINN | China |

Table 7 Enzyme for skim milk without casein preparation

| Name | Company | Country |
|---|---|---|
| Rennet enzyme (EC 3.4.23.4) | NATUREN® Premium 145 | Demnark |

Table 8 Instruments and apparatus for preparation emulsion

| Instrument | Model | Country |
|---|---|---|
| Ultra-Turrax homogenizer | T25, IKA | Malaysia |
| pH meter | Docu-pH$^+$, Satorius | Germany |

| High pressure homogenizer | APV 2000, APV | Denmark |
|---|---|---|
| Hot plate with stirrer | SLR, SI Analytics | Germany |
| Centrifuge Tubes 50 ml | Thermo Fisher Scientific | USA |
| High-pressure unit | HPP 600/5L, TSUS Febix Foodtech | Thailand |

Table 9 Instruments and apparatus for physical properties analysis (particle size, polydispersity index, zeta potential, precipitation percentage and confocal laser scanning microscopy)

| Instrument | Model | Country |
|---|---|---|
| Zetasizer | Nano ZSP, Malvern | United Kingdom |
| Folded Capillary Zeta Cell | Malvern | United Kingdom |
| Confocal laser scanning microscope | FLUOVIEW FV10i, Olympus | Japan |

Table 10 Instruments and apparatus for chemical properties analysis (thiobarbituric acid, peroxide value)

| Instrument | Model | Country |
|---|---|---|
| Centrifuge | 2-16PK, Satorius | Germany |
| Waterbath | Memmert | Germany |
| Spectrophotometer | Genesys10S UV-VIS, Thermo Fisher | USA |
| Centrifuge Tubes 50 ml | Thermo Fisher Scientific | USA |
| Quartz cuvette | Mettler Toledo | Singapore |

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Table 11 Instruments and apparatus for Microbiological analysis

| Instrument | Model | Country |
|---|---|---|
| Incubator | INB 500, Memmert | Germany |
| Vortex Mixer | G560E, Scientific Industries | USA |
| Colony Counter | Galaxy 230, WIGGENS GmbH | China |

## 3.2 Preparation of emulsions

KGM powder was dissolved in water at a concentration of 0.1 - 0.5% (w/w) by continuously stirring for 4 hours at room temperature. The pH of the KGM solution was then adjusted to 3.0, 4.0, 6.0, 7.0, 8.0, 9.0 and 10.0 using 0.1 M NaOH and 0.1 M HCl (pH of native KGM was 5.0). The amount of NaOH or HCl added to the KGM solution for pH adjustment was very low and had little effect on the

concentration of the KGM solution. The pH-adjusted solution was kept at room temperature for 12 hours to allow the KGM to swell and pH unchanged.

After that, skim milk, skim milk without casein, whole milk, fish oil and prepared KGM solution were mixed using overhead homogenizer for 1 minute. The final composition of mixture was 5% fish oil, KGM content varied between 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4 and 0.5% at pH of 3, 4, 5, 6, 7, 8, 9 and 10 and 3% milk protein. The coarse emulsions were immediately homogenized by passing in a single stage high pressure homogenizer at 40 MPa (400 bar) for 2 times. The freshly emulsion was added with sodium azide (0.02% v / v), as antimicrobial agent.

**3.3 High-pressure treatment**

For high-pressure processing, 50 ml samples were treated using a high-pressure unit (HPP 600/5L, TSUS Febix Foodtech, Thailand) at Food Innovation Service Plant, Thailand Institute of Scientific and Technological research, Pathum thani province. The samples were treated at 400, 500 and 600 MPa with a hold time at pressure of 3 min. The samples were cooled immediately after high pressure processing in an ice bath and stored at 4 °C until further analysis. The control variables in the study of the effect of high pressure on the stability (particle size, zetapotential and oxidative stability) of the KGM- fish oil, milk emulsion was emulsion not treated by pressure but treated for pasteurization condition (72$^{O}$C, 15 second).

**3.4 Emulsion analyses**

3.4.1 Particle size analysis

The particle size and polydispersity index (PDI) of all emulsions was determined by dynamic light scattering using a Zetasizer (Nano ZSP, Malvern, UK). The emulsions were dispersed with distilled water before measurement and the particle size is measured in cuvette styrene. The Z-average of all emulsion exhibited the size of the particle.

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3.4.2 Zeta-potential measurements

The zeta potential values of all emulsions were measured using a Zetasizer instrument (Nano ZSP, Malvern, UK). The emulsions were diluted 500 times with deionized water prior to the measurement.

3.4.3 Precipitation percentage

Precipitation percentage of all emulsions were examined following Ye & Harte (2014) method with some modifications (Ye & Harte, 2014). Visual observation was performed after the emulsion had been staying at 4 $^{O}$C. When the phase separation was observed, the precipitation of the suspension was calculated by the following formula:

$$\text{Separation \%} = (Vp/Vt) \times 100$$

Where: *Vp* represents the volume (ml) of the layer which the precipitation occurs and *Vt* represents the total volume (ml) of the emulsion.

Particle size, zeta potential and precipitation percentage were analyzed immediately after emulsion preparation and after high pressure treatment for every 2 days during storage at 4 $^{O}$C.

3.4.4 Confocal laser scanning microscopy

Confocal laser scanning microscopy, CLSM (FLUOVIEW FV10i, Olympus, Japan) was examined according to Li & Shah (2015) with some modifications (Li & Shah, 2015). The polysaccharide / protein staining was achieved by mixing 1 mL emulsion with 10 ml of Nile Red (1 mg/mL in ethanol), for 5 minutes. Then adding 10 mL of fluorescein isothiocyanate (FITC; 1 mg/mL in ethanol) 10 ml, mixing it for 5 min. To prepare CLSM samples, 1 mL of the stained emulsion were transferred onto microscope slide and cover slip were used to cover emulsion. These CLSM samples were examined using a confocal laser scanning microscope. FITC and Nile Red were excited by 488 nm laser and 534 nm laser, respectively. The emitted lights of FITC and Nile Red were set at 493-538 nm and 586-753 nm, respectively. CLSM was analyzed at day 0 and day 10 during storage at 4 $^{O}$C.

3.4.5 Viscosity measurements

The emulsion viscosity was determined by means of the viscometers using standard cones. The temperature was kept at 25 °C during measurement. After equilibrating the samples to 25 °C, viscosity was collected after subjecting the sample to shear for 60 s. The shear rate used was 31.10 $s^{-1}$ (Chivero, Gohtani, Yoshii, & Nakamura, 2015).

**3.5 Oxidative stability of emulsion**

3.5.1 Determination of Thiobarbituric acid (TBARS)

Thiobarbituric assay (TBARS) was determined by mixing 0.5 mL emulsion with 2.0 mL TBA reagent and then put it in boiling water for 10 min. After heating, the mixture was cooled, then 3 ml of cyclohexanone was added. After that, mixture was centrifuged (1000 g) for 5 min. Subsequently, the mixture was centrifuged (1000 g) for 5 min to separate the supernatant for analysis. The supernatant was measured the absorbance at 532 nm. The amount of TBARS was determined using 1,1,3,3-tetraethoxypropane as a standard curve (Xiangqian et al., 2014).

3.5.2 Determination of Peroxide value (PV)

Peroxide value (PV) was determined using a method from Zhu et al. (2014) with some modification. 0.5 mL emulsion was mixed with 2.5 mL of extracted solvent (ethanol/ethyl acetate/hexane, 1:1:1 (v/v/v)) for 20 min at room temperature. After that, the supernatant was separated. Next, 0.2 mL of supernatant was mixed with 4 mL of methanol/butanol (2:1 (v/v)), 25 µl of ammonium thiocyanate (3.94 M) and 25 µl of ferrous ion solution. When all chemicals were added, the mixture was kept in the dark for 20 min at room temperature. The mixture was measured the absorbance at 510 nm. The amount of lipid hydroperoxides was determined using cumene hydroperoxide as a standard curve (Xiangqian et al., 2014).

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Oxidative stability of emulsion was analyzed immediately after high pressure treatment and day 14 during storage at 4 $^{O}$C.

**3.6 Microbiological analysis**

For the determination of total bacterial count in emulsion and survivors after high pressure processing, serial dilutions (in sterile 0.1% peptone water) of samples were done. The total plate count (TPC), Coliform bacteria and Staphylococcus were determined using the 3M Petrifilm Aerobic Count Plate, 3M™ Petrifilm™ coliform count plate and 3M™ Petrifilm™ Staph Express Count Plates, respectively. Total bacteria count was incubated in at 32 °C under aerobic conditions for 48 h. The 3M™ Petrifilm™ coliform count plate was incubated at 32 °C, for 24 h. The 3M™ Petrifilm™ Staph Express Count Plates was incubated at 37 °C, for 24 h. The control variables in the study of the effect of high pressure on the stability (particle size, zetapotential and oxidative stability) of the KGM- fish oil, milk emulsion was emulsion not treated by pressure but treated for pasteurization condition (72$^{O}$C, 15 second) and mixture of milk and oil (not added KGM).

Microbial were analyzed immediately after high pressure treatment for every 5 days during storage at 4 $^{O}$C.

**3.7 Data Analysis**

All experiment were performed in triplicate. Results were report as mean values ± standard deviation (SD). The data were analyzed by one-way analysis of variance with Duncan's multiple range tests through SPSS Statistics Version 22 (SPSS Inc, Chicago, USA) and significant differences accepted at $p < 0.05$.

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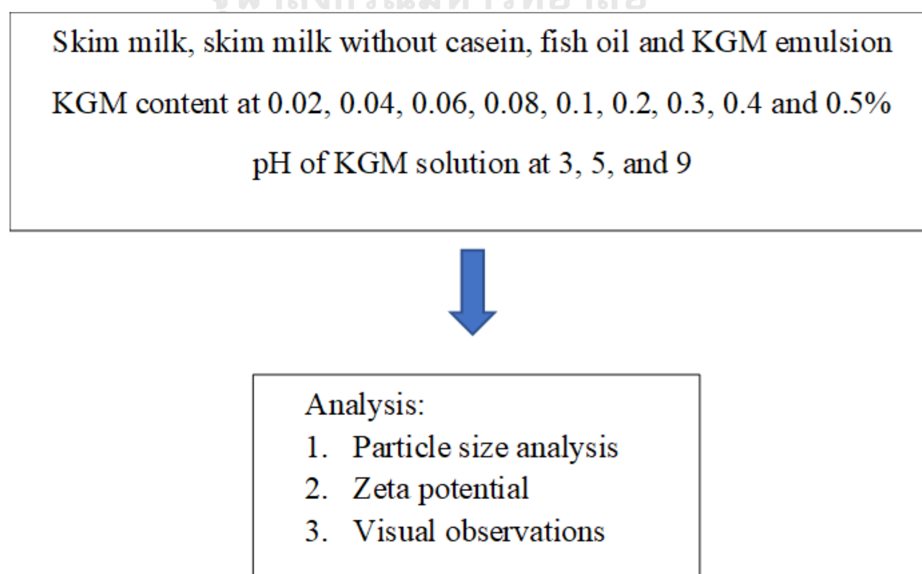

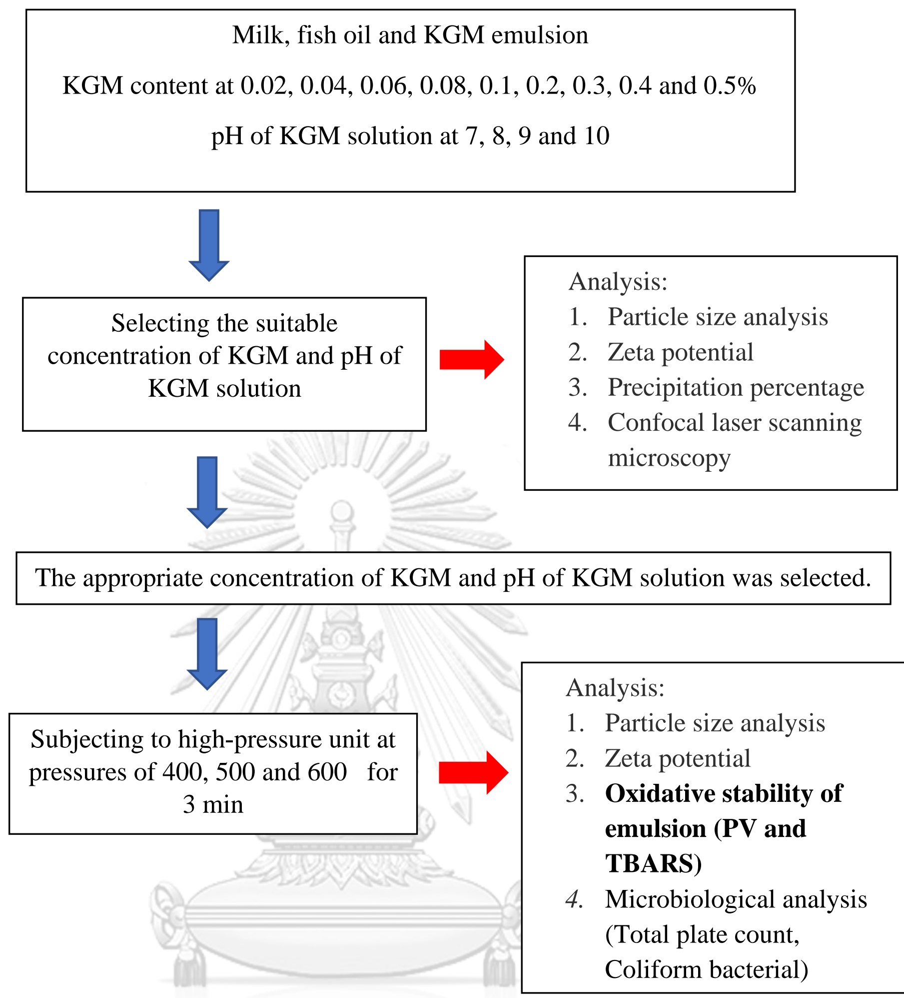


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Figure 16 Experimental flow chart of the study

# CHAPTER 4
# RESULTS AND DISCUSSION

## 4.1 Effect of pH and KGM concentration on stability of the KGM- fish oil skim milk with casein, skim milk without casein and milk emulsion

### 4.1.1 Effect of pH and KGM concentration on stability of the KGM- fish oil skim milk and skim milk without casein emulsion

#### 4.1.1.1 Particle size analysis

In these studies, the emulsions were prepared using a high-pressure homogenizer at 40 MPa. Studies have shown that the particle size of the mixtures and emulsions varied with concentration and pH of the KGM solution. Table 12 showed the effects of KGM concentration and pH values on the particle size. The control of this study was an emulsion prepared from skim milk and skim milk without casein without KGM added. The precipitation of the control emulsion was occurred within 1 hours. The results indicated that pH and the concentration of KGM were affected the particle size. However, the concentration was more effect than pH. For example, at the same concentration (0.02%) and different pH (3, 5, 9) of KGM, the particle size of skim milk- KGM fish oil emulsion was 640.20, 656.29 and 647.06 nm, respectively. While, at the same pH (5) of and different concentration (0.02 – 0.5%) of KGM, the particle size of skim milk- KGM fish oil emulsion particle size was varied from 640.20-786.40 nm.

This section was a comparative study of the particle size of the mixture and emulsion between the presence and absence of casein. The results indicated that, particle size (average diameter) of mixture and emulsions with presence of casein at all concentration and pH of KGM were higher than mixture and emulsions with presence of casein. Casein proteins are highly effective emulsion stabilizing agents. Under optimal conditions, casein loops and tail surround the surface of the oil drop, providing steric stability against flocculation and coalescence (Dickinson, 2008). The surface coverage of casein on the surface of the lipid droplets resulted in larger particle sizes when compared with emulsions with absence of casein. Moreover, the change in particle size of emulsions and mixtures is partly due to the structure of the KGM, which may be caused by various mechanisms. At low pH (pH 3), the decrease in particle size was probably caused by acidic degradation of glycoside linkage; at high pH (pH 9.0), the slight decrease in particle size was probably a result of deacetylation (Jian, Siu, & Wu, 2015).

Table 12 The particles sizes (average diameter, nm) obtained from the use of different pH (3, 5, 9) and KGM concentration

| pH/KGM concentration | Average diameter, nm | | | |
|---|---|---|---|---|
| | Skim milk+KGM | Skim milk+KGM +5% fish oil | Skim milk (No casein) +KGM | Skim milk (No casein) +KGM+5% fish oil |
| pH3 0.02% KGM | 344.63±15.68$^{i}$ | 640.20±35.25$^{i}$ | 204.30±12.68$^{e}$ | 462.50±25.30$^{f}$ |
| pH3 0.04% KGM | 366.62±5.59$^{h}$ | 651.44±12.25$^{h}$ | 254.68±14.74$^{d}$ | 469.43±14.74$^{e}$ |
| pH3 0.06% KGM | 399.20±12.59$^{g}$ | 652.22±10.63$^{h}$ | 298.30±10.63$^{c}$ | 495.94±37.33$^{d}$ |
| pH3 0.08% KGM | 409.27±16.59$^{f}$ | 659.78±10.67$^{g}$ | 300.67±29.53$^{c}$ | 499.39±11.85$^{d}$ |
| pH3 0.1% KGM | 439.39±19.68$^{e}$ | 698.25±20.61$^{f}$ | 300.70±11.50$^{c}$ | 500.05±13.77$^{cd}$ |
| pH3 0.2% KGM | 478.30±15.08$^{d}$ | 702.77±20.22$^{e}$ | 305.64±14.40$^{b}$ | 502.56±21.63$^{cd}$ |
| pH3 0.3% KGM | 494.27±9.89$^{c}$ | 725.69±19.57$^{d}$ | 309.20±10.25$^{a}$ | 506.33±19.43$^{c}$ |
| pH3 0.4% KGM | 503.26±16.20$^{b}$ | 746.03±19.49$^{c}$ | 310.45±12.22$^{a}$ | 519.44±11.48$^{b}$ |
| pH3 0.5% KGM | 525.30±11.50$^{a}$ | 786.40±26.60$^{b}$ | 311.28±10.09$^{a}$ | 522.91±8.35$^{a}$ |
| pH 5 0.02% KGM | 355.20±15.52$^{hi}$ | 656.29±15.60$^{gh}$ | 205.27±1.28$^{e}$ | 463.60±14.50$^{f}$ |
| pH 5 0.04% KGM | 395.03±3.60$^{g}$ | 667.37±29.50$^{fg}$ | 255.10±18.10$^{cd}$ | 468.39±11.33$^{e}$ |
| pH 5 0.06% KGM | 403.40±10.50$^{f}$ | 679.30±38.77$^{fg}$ | 288.11±19.40$^{dc}$ | 497.26±10.66$^{d}$ |
| pH 5 0.08% KGM | 496.29±10.69$^{c}$ | 698.69±25.3 6$^{f}$ | 300.20±11.20$^{c}$ | 503.02±14.59$^{cd}$ |
| pH 5 0.1% KGM | 498.10±19.39$^{c}$ | 700.57±23.55$^{e}$ | 309.31±10.22$^{a}$ | 502.64±18.45$^{cd}$ |
| pH 5 0.2% KGM | 502.50±13.36$^{b}$ | 712.50±15.04$^{e}$ | 309.20±11.02$^{a}$ | 505.21±10.47$^{c}$ |
| pH 5 0.3% KGM | 506.45±10.44$^{b}$ | 752.64±6.65$^{bc}$ | 310.02±13.10$^{a}$ | 507.35±14.63$^{c}$ |
| pH 5 0.4% KGM | 510.30±14.35$^{h}$ | 776.46±35.50$^{b}$ | 311.52±19.28$^{a}$ | 513.91±13.46$^{bc}$ |
| pH 5 0.5% KGM | 516.24±19.60$^{b}$ | 798.33±13.67$^{a}$ | 311.33±17.31$^{a}$ | 517.30±17.41$^{b}$ |
| pH9 0.02% KGM | 354.64±29.35$^{hi}$ | 647.06±27.60$^{h}$ | 204.51±11.15$^{e}$ | 474.68±9.40$^{de}$ |
| pH9 0.04% KGM | 379.30±12.05$^{gh}$ | 657.70±25.90$^{gh}$ | 225.20±18.09$^{de}$ | 469.36±18.59$^{e}$ |
| pH9 0.06% KGM | 399.36±11.30$^{g}$ | 656.04±33.56$^{gh}$ | 228.30±13.44$^{de}$ | 478.55±13.50$^{de}$ |
| pH9 0.08% KGM | 409.40±13.45$^{f}$ | 660.35±15.37$^{g}$ | 287.29±11.37$^{cd}$ | 497.25±9.28$^{d}$ |
| pH9 0.1% KGM | 449.55±30.45$^{e}$ | 699.56±12.50$^{f}$ | 299.48±3.36$^{c}$ | 505.98±11.59$^{c}$ |
| pH9 0.2% KGM | 498.33±21.42$^{c}$ | 700.45±11.58$^{e}$ | 302.54±17.53$^{c}$ | 507.20±17.50$^{c}$ |
| pH9 0.3% KGM | 500.34±23.50$^{b}$ | 733.95±13.05$^{d}$ | 309.35±11.20$^{a}$ | 514.29±15.66$^{b}$ |
| pH9 0.4% KGM | 499.35±19.35$^{c}$ | 757.59±15.04$^{bc}$ | 305.98±21.05$^{b}$ | 518.67±12.73$^{b}$ |
| pH9 0.5% KGM | 511.54±23.40$^{b}$ | 790.49±12.50$^{a}$ | 312.50±17.20$^{a}$ | 516.33±14.36$^{b}$ |

The particle size of skim milk was 286.50±11.40

The particle size of skim milk without casein was 129.48±18.07

All values are presented as the mean ± SD.

Mean values followed by different superscripted letters in the same column (a–h) are significantly different, according to Duncan's multiple range test ($p < 0.05$).

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4.1.1.2 Zeta potential analysis

The effects of KGM concentration and pH values on the zeta potential are shown in table 13. Studies have shown that the zeta potential of the mixtures and emulsions varied with concentration and pH of the KGM solution. The same pH, but different concentrations of KGM, the zeta potential values were quite similar. For example, at pH5 and KGM concentration vary from 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4 and 0.5% the zeta potential of skim milk- KGM fish oil emulsions were -35.30, -35.65, -35.70, -35.60, -36.94, -36.54, -36.94, -37.02 and -37.24 mV, respectively. And when the concentration of KGM was the same, but the pH was different, it was found that the zeta potential values were quite similar too. For example, at 0.04% KGM, pH 3, 5, and 10 zeta potential of skim milk- KGM fish oil emulsions were -36.65, -36.54, and -36.38 mV, respectively. The zeta potential of emulsions and mixtures were exhibited negative zeta potentials. According to the DLVO theory, the absolute of zeta potential less than 30 mV indicates an unstable dispersion which related to the stability of emulsions and mixtures by visual

observation from this study (Bhattacharjee, Elimelech, & Borkovec, 1998). The negative zeta potential of emulsions and mixtures mostly comes from two component: casein and KGM. Casein is highly charged as their average zeta potential is close to −20 mV (Bouzid et al., 2008). Therefore, when comparing mixture and emulsion with and without casein, under the same KGM concentration and pH conditions, the different zeta potential was found. In addition, the difference in zeta values between the casein and absence emulsions was due to KGM prepared at different pH conditions. Wenjie Jian, Ka-Chai Siu & Jian-Yong Wu (2015) studies the effect of pH on colloidal properties and molecular characteristics of KGM, they found that, the KGM solution showed negative zeta potentials at pH 3.0–10.0, and the negative zeta potential increased with the pH increase (Jian et al., 2015). As a result, the zeta potential of emulsion and mixture with and without casein with different KGM concentrations and pH were different.

Table 13The zeta potential (mV) obtained from the use of different pH (3, 5, 9) and KGM concentration

| pH/KGM concentration | Zeta potential (mV) | | | |
|---|---|---|---|---|
| | Skim milk+KGM | Skim milk+KGM +5% fish oil | Skim milk (No casein) +KGM | Skim milk (No casein) +KGM+5% fish oil |
| pH3 0.02%KGM | -35.30±0.48$^{a}$ | -35.98±0.78$^{a}$ | -15.30±0.95$^{b}$ | -15.63±1.52$^{bc}$ |
| pH3 0.04%KGM | -35.65±1.30$^{ab}$ | -35.88±0.59$^{a}$ | -15.43±1.40$^{d}$ | -15.69±1.22$^{c}$ |
| pH3 0.06%KGM | -35.70±1.56$^{b}$ | -36.02±1.40$^{b}$ | -15.49±0.15$^{d}$ | -15.96±2.18$^{d}$ |
| pH3 0.08%KGM | -35.60±1.40$^{ab}$ | -36.26±2.58$^{c}$ | -15.94±0.85$^{de}$ | -16.14±0.15$^{e}$ |
| pH3 0.1%KGM | -36.94±2.34$^{c}$ | -36.34±1.50$^{c}$ | -15.50±1.14$^{de}$ | -15.59±0.52$^{b}$ |
| pH3 0.2%KGM | -36.58±1.49$^{d}$ | -36.55±0.46$^{cd}$ | -15.95±0.66$^{e}$ | -16.02±2.45$^{de}$ |
| pH3 0.3%KGM | -36.94±2.01$^{c}$ | -37.06±1.05$^{e}$ | -16.57±0.54$^{de}$ | -16.70±1.45$^{fg}$ |
| pH3 0.4%KGM | -37.02±1.34$^{ef}$ | -37.08±0.56$^{e}$ | -16.49±0.38$^{d}$ | -16.58±1.34$^{f}$ |
| pH3 0.5%KGM | -37.24±1.40$^{f}$ | -37.70±1.60$^{g}$ | -16.20±1.57$^{fg}$ | -16.64±0.69$^{fg}$ |
| pH 5 0.02%KGM | -36.40±1.54$^{d}$ | -36.48±1.98$^{d}$ | -15.39±0.46$^{bc}$ | -15.47±1.59$^{a}$ |
| pH 5 0.04%KGM | -36.54±1.40$^{d}$ | -36.98±1.59$^{e}$ | -15.06±0.47$^{a}$ | -15.48±1.40$^{a}$ |
| pH 5 0.06%KGM | -36.94±1.59$^{c}$ | -36.47±1.76$^{d}$ | -15.59±1.69$^{de}$ | -15.95±0.36$^{d}$ |
| pH 5 0.08%KGM | -36.04±2.53$^{bc}$ | -36.87±0.48$^{e}$ | -15.55±1.06$^{de}$ | -16.03±1.40$^{de}$ |
| pH 5 0.1%KGM | -36.43±1.99$^{cd}$ | -37.04±0.46$^{e}$ | -15.65±0.27$^{e}$ | -16.10±1.28$^{de}$ |
| pH 5 0.2%KGM | -36.99±1.30$^{ef}$ | -36.99±0.60$^{e}$ | -15.97±0.47$^{ef}$ | -16.08±1.58$^{de}$ |
| pH 5 0.3%KGM | -37.40±2.55$^{fg}$ | -37.17±0.22$^{ef}$ | -15.85±0.56$^{ef}$ | -15.97±1.39$^{d}$ |
| pH 5 0.4%KGM | -37.34±1.48$^{fg}$ | -37.56±1.43$^{g}$ | -16.04±1.62$^{f}$ | -16.21±0.93$^{ef}$ |
| pH 5 0.5%KGM | -37.45±1.57$^{fg}$ | -37.78±0.46$^{g}$ | -16.33±0.68$^{fg}$ | -16.29±0.45$^{ef}$ |
| pH9 0.02%KGM | -36.30±1.45$^{e}$ | -36.09±0.36$^{bc}$ | -16.25±1.50$^{fg}$ | -15.98±2.42$^{de}$ |
| pH9 0.04%KGM | -36.38±1.04$^{e}$ | -36.39±0.79$^{c}$ | -16.49±0.59$^{g}$ | -16.47±1.89$^{ef}$ |
| pH9 0.06%KGM | -36.85±1.50$^{c}$ | -36.98±0.48$^{e}$ | -16.09±1.30$^{f}$ | -16.14±1.96$^{e}$ |
| pH9 0.08%KGM | -37.98±1.49$^{g}$ | -37.92±0.48$^{gh}$ | -16.07±0.68$^{f}$ | -16.35±1.49$^{ef}$ |
| pH9 0.1%KGM | -37.03±1.56$^{ef}$ | -37.34±2.96$^{f}$ | -16.09±2.02$^{f}$ | -16.29±1.05$^{ef}$ |
| pH9 0.2%KGM | -37.22±0.98$^{f}$ | -37.30±1.85$^{f}$ | -16.43±1.56$^{fg}$ | -16.46±0.48$^{ef}$ |
| pH9 0.3%KGM | -37.50±2.60$^{fg}$ | -37.85±0.48$^{gh}$ | -16.88±0.58$^{h}$ | -16.80±1.34$^{g}$ |
| pH9 0.4%KGM | -37.98±1.55$^{g}$ | -37.90±0.74$^{gh}$ | -16.59±2.67$^{gh}$ | -16.98±0.33$^{h}$ |
| pH9 0.5%KGM | -37.95±2.48$^{g}$ | -38.03±1.03$^{h}$ | -16.89±1.50$^{h}$ | -16.94±2.48$^{h}$ |

The zeta potential of skim milk was -24.69±0.25

The zeta potential of skim milk (No casein) was -10.53±1.43

All values are presented as the mean ± SD.

Mean values followed by different superscripted letters in the same column (a–h) are significantly different, according to Duncan's multiple range test ($p < 0.05$).

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4.1.1.3 Visual observations

In general, the stability of most emulsions can be observed directly with the human eye. By visual observation, the gravitational separation of the emulsion is assessed (Hu, Ting, Hu, & Hsieh, 2017). The effects of KGM concentration and pH values on the stability of mixtures and emulsions using visual observation are shown in table 14.

Table 14 Stability (Day) by visual observation obtained from the use of different pH (3, 5, 9) and KGM concentration

| pH/KGM concentration | Stability (day) | | | |
|---|---|---|---|---|
| | Skim milk+KGM | Skim milk+KGM +5% fish oil | Skim milk (No casein) +KGM | Skim milk (No casein) +KGM+5% fish oil |
| pH3 0.02% KGM | 6 | 6 | 2 | 1 |
| pH3 0.04% KGM | 6 | 6 | 2 | 1 |
| pH3 0.06% KGM | 6 | 6 | 2 | 1 |
| pH3 0.08% KGM | 6 | 6 | 2 | 1 |
| pH3 0.1% KGM | 6 | 6 | 2 | 1 |
| pH3 0.2% KGM | 6 | 6 | 2 | 1 |
| pH3 0.3% KGM | 4 | 4 | 2 | 1 |
| pH3 0.4% KGM | 2 | 2 | 2 | 1 |
| pH3 0.5% KGM | 2 | 2 | 2 | 1 |
| pH 5 0.02% KGM | 6 | 6 | 2 | 1 |
| pH 5 0.04% KGM | 6 | 6 | 2 | 1 |
| pH 5 0.06% KGM | 6 | 6 | 2 | 1 |
| pH 5 0.08% KGM | 6 | 6 | 2 | 1 |
| pH 5 0.1% KGM | 4 | 4 | 2 | 1 |
| pH 5 0.2% KGM | 2 | 2 | 2 | 1 |
| pH 5 0.3% KGM | 2 | 2 | 2 | 1 |
| pH 5 0.4% KGM | 2 | 2 | 2 | 1 |
| pH 5 0.5% KGM | 2 | 2 | 2 | 1 |
| pH9 0.02% KGM | 8 | 8 | 2 | 1 |
| pH9 0.04% KGM | 8 | 8 | 2 | 1 |
| pH9 0.06% KGM | 6 | 6 | 2 | 1 |
| pH9 0.08% KGM | 6 | 6 | 2 | 1 |
| pH9 0.1% KGM | 6 | 6 | 2 | 1 |
| pH9 0.2% KGM | 6 | 6 | 2 | 1 |
| pH9 0.3% KGM | 4 | 4 | 2 | 1 |
| pH9 0.4% KGM | 2 | 2 | 2 | 1 |
| pH9 0.5% KGM | 2 | 2 | 2 | 1 |

All values are presented as the mean ± SD.

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For skim milk- KGM fish oil emulsions and skim milk- KGM mixtures, when the concentration of KGM in the emulsion increased, the separation occurred more rapidly. For example, when KGM concentration was 0.02% (w/w) at pH 9, separation by visual observation of skim milk- KGM fish oil emulsions occurred at day 8. Meanwhile, at KGM concentration was 0.5% (w/w) at pH 9 separation by visual observation of skim milk- KGM fish oil emulsions occurred at day 2. Moreover, pH values were affecting the separation too. The increase in pH values, at the same concentration of KGM led to an increase of the stability of the skim milk- KGM fish oil emulsions and skim milk- KGM mixtures. For example, at pH 3, 5 and

9, 0.04% KGM concentration, separation by visual observation of skim milk- KGM fish oil emulsions occurred at day 6, 6 and 8. On the other hand, instability of skim milk without casein- KGM fish oil emulsions and skim milk without casein- KGM mixtures were observed. Separation of emulsions and mixtures by visual observation was occurred at day 2 for skim milk without casein- KGM fish oil emulsions and day 1 for skim milk without casein- KGM mixtures at all KGM concentration and pH. The results indicated that, KGM and casein may improve the emulsion stability.

4.1.2 Effect of pH and KGM concentration on stability of the KGM- fish oil milk emulsion

4.1.2.1 Particle size and polydispersity index (PDI) analysis

The particle size and PDI of emulsion are important physical characteristics to be considered when creating emulsion products. These characteristics can affect the stability and appearance of the final product (M. Danaei & Mozafari, 2018). In these studies, the emulsions were prepared using a high-pressure homogenizer at 40 MPa. Studies have shown that the particle size of the emulsions varied with concentration and pH of the KGM solution. However, the difference in emulsion particle size is small. Table 15 showed the effects of KGM concentration and pH values on the particle size. The control of this study was an emulsion prepared from milk and fish oil without adding KGM. On Day 0, the particle size of emulsion with a KGM concentration from 0.02% (w/w) to 0.5% (w/w) at pH 3,4,5,6,7,8,9 and 10, including control was approximately 900 nm. However, precipitation of the control emulsion was occurred within 4 hours. After day 2, the increase of particle size was noticeable when KGM concentration was higher than 0.3% (w/w). For example, the increase of particle size after 2 days at pH 7 was 69.04% when KGM concentration was 0.3% (w/w) while the increase of particle size was 3.45% when KGM concentration was 0.02% (w/w). An increase in pH from 7-10 results in a slower rate of particle size increase across all KGM concentrations. For example, the increase of particle size after 4 days was 37.59% at pH 7 while at pH 10 it was 27.29% (with 0.04% KGM concentration). Table 16 showed the effects of KGM concentration and pH values on the polydispersity index (PDI). PDI is a measure of the heterogeneity of a sample based on size. PDI can occur due to size distribution in a sample or agglomeration or aggregation of the sample during isolation or analysis (Mudalige et al., 2019). The PDI values of all controllers and emulsions ranged from 0.12-0.65, indicating that the emulsion possesses monodisperse properties. However, when the emulsion was kept, the PDI value tended to increase in all emulsion conditions. The results from table 10 and 11 showed that, the highest stable emulsion was emulsion prepared from KGM at a concentration of 0.04% (w/w) at pH 9 and 10. Similar results were also found in chitosan/casein complexes (F. Zhang, Cai, Ding, & Wang, 2021) and glucomannan/whey protein complex (Lu, Zheng, & Miao, 2018).

Table 15 The particles sizes (average diameter, nm) obtained from the use of different pH (3, 4, 5, 6, 7, 8, 9, 10) and KGM concentration.

| KGM concentration (%) | pH | Day | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 |
| 0.02 | 3 | $913\pm13^{Bdef}$ | $998\pm15^{Bcfg}$ | $1011\pm15^{Acde}$ | - | - | - |
| | 4 | $927\pm15^{Bcde}$ | $1107\pm18^{Afg}$ | $1124\pm17^{Acde}$ | - | - | - |
| | 5 (Native) | $937\pm10^{Ccde}$ | $982\pm21^{Bcd}$ | $1005\pm11^{Bl}$ | $1021\pm9^{Ai}$ | - | - |
| | 6 | $969\pm9^{Bcde}$ | $983\pm12^{Bcd}$ | $1024\pm7^{Al}$ | $1114\pm15^{Ai}$ | - | - |
| | 7 | $960\pm14^{Bcde}$ | $993\pm11^{Bfg}$ | $1012\pm1^{Bl}$ | $1253\pm137^{Aef}$ | $1309\pm16^{Abc}$ | - |
| | 8 | $909\pm11^{Edef}$ | $1007\pm9^{Dfg}$ | $1172\pm51^{Cjk}$ | $1273\pm40^{Bef}$ | $1331\pm6^{Ab}$ | - |
| | 9 | $908\pm17^{Ddef}$ | $968\pm54^{Cfg}$ | $1161\pm46^{Bjk}$ | $1296\pm8^{Acde}$ | $1297\pm5^{Ac}$ | $1332\pm8^{Ac}$ |
| | 10 | $904\pm17^{Def}$ | $956\pm48^{DC}$ | $1017\pm4^{Cl}$ | $1135\pm63^{Bh}$ | $1196\pm7^{Be}$ | $1307\pm22^{Ad}$ |
| 0.04 | 3 | $923\pm13^{Bcde}$ | $998\pm15^{Bcde}$ | $1011\pm15^{Bcde}$ | - | - | - |
| | 4 | $937\pm15^{Bcde}$ | $1107\pm18^{Bcde}$ | $1124\pm17^{Bcde}$ | - | - | - |
| | 5 (Native) | $946\pm11^{Ccde}$ | $987\pm19^{Bcd}$ | $1053\pm18^{Al}$ | $1104\pm15^{Ah}$ | - | - |
| | 6 | $955\pm7^{Ccde}$ | $998\pm12^{Bcd}$ | $1058\pm10^{Al}$ | $1106\pm17^{Ah}$ | - | - |
| | 7 | $962\pm15^{Cbc}$ | $999\pm1^{BCfg}$ | $1331\pm52^{Bl}$ | $1306\pm48^{Acde}$ | $1331\pm52^{Ab}$ | - |
| | 8 | $920\pm1^{Dcde}$ | $1001\pm1^{Cfg}$ | $1270\pm33^{Bhij}$ | $1305\pm15^{Bcde}$ | $1417\pm27^{Aa}$ | - |
| | 9 | $938\pm30^{Ecde}$ | $1006\pm11^{Dfg}$ | $1215\pm81^{Cijk}$ | $1296\pm6^{Bcde}$ | $1306\pm5^{Bbc}$ | $1439\pm15^{Aa}$ |
| | 10 | $931\pm47^{Ecde}$ | $1011\pm7^{Dfg}$ | $1201\pm3^{Cijk}$ | $1143\pm48^{Bh}$ | $1208\pm14^{Be}$ | $1419\pm35^{Ab}$ |
| 0.06 | 3 | $916\pm21^{Bdef}$ | $989\pm11^{Acd}$ | $1105\pm18^{Ajk}$ | - | - | - |
| | 4 | $936\pm13^{Ccde}$ | $1008\pm12^{Bfg}$ | $1205\pm12^{Aijk}$ | - | - | - |
| | 5 (Native) | $987\pm10^{Bab}$ | $1103\pm25^{Afg}$ | $1206\pm22^{Aijk}$ | - | - | - |
| | 6 | $989\pm12^{Bab}$ | $1196\pm24^{Ad}$ | $1216\pm21^{Aijk}$ | - | - | - |
| | 7 | $918\pm23^{Ccde}$ | $1034\pm57^{CBfg}$ | $1123\pm101^{Bkl}$ | $1285\pm30^{Adef}$ | - | - |
| | 8 | $937\pm41^{Ccde}$ | $1014\pm3^{Bfg}$ | $1302\pm4^{Aghi}$ | $1342\pm57^{Acd}$ | - | - |
| | 9 | $936\pm32^{Dcde}$ | $1012\pm5^{Cfg}$ | $1260\pm33^{Bhij}$ | $1307\pm4^{Acde}$ | $1335\pm6^{Ab}$ | - |
| | 10 | $928\pm22^{Dcde}$ | $1013\pm4^{Cfg}$ | $1189\pm8^{Bijk}$ | $1202\pm6^{Bg}$ | $1226\pm3^{Ad}$ | - |
| 0.08 | 3 | $937\pm20^{Ccde}$ | $1034\pm12^{Bfg}$ | $1294\pm18^{Agh}$ | - | - | - |
| | 4 | $949\pm12^{Ccde}$ | $1042\pm22^{Bfg}$ | $1298\pm25^{Agh}$ | - | - | - |
| | 5 (Native) | $988\pm15^{Bab}$ | $1004\pm35^{Bfg}$ | $1293\pm32^{Agh}$ | - | - | - |
| | 6 | $977\pm23^{Cbc}$ | $1103\pm28^{Bfg}$ | $1301\pm11^{Agh}$ | - | - | - |
| | 7 | $950\pm5^{Bcde}$ | $1001\pm3^{Bf}$ | $1259\pm57^{Ahij}$ | $1329\pm47^{Abcd}$ | - | - |
| | 8 | $929\pm9^{Ccde}$ | $1047\pm47^{Bfg}$ | $1366\pm56^{Aefg}$ | $1372\pm10^{Ab}$ | - | - |
| | 9 | $931\pm29^{Dcde}$ | $1045\pm57^{Cfg}$ | $1306\pm1^{Bghi}$ | $1343\pm38^{Bbc}$ | $1419\pm25^{Aa}$ | - |
| | 10 | $942\pm34^{Dcde}$ | $1020\pm1^{Cfg}$ | $1201\pm3^{Bijk}$ | $1235\pm53^{Bfg}$ | $1404\pm60^{Aa}$ | - |
| 0.1 | 3 | $944\pm9^{Ccde}$ | $1205\pm45^{Bd}$ | $1496\pm21^{Agh}$ | - | - | - |
| | 4 | $967\pm15^{Ccde}$ | $1295\pm16^{Be}$ | $1394\pm32^{Agh}$ | - | - | - |
| | 5 (Native) | $992\pm39^{Bab}$ | $1294\pm13^{Ae}$ | $1293\pm11^{Ahij}$ | - | - | - |
| | 6 | $995\pm28^{Bab}$ | $1164\pm28^{Ad}$ | $1244\pm17^{Ahij}$ | - | - | - |
| | 7 | $929\pm3^{Bcde}$ | $1029\pm3^{Bfg}$ | $1454\pm179^{Ade}$ | - | - | - |
| | 8 | $858\pm24^{Bf}$ | $1492\pm11^{Aabc}$ | $1539\pm129^{Abc}$ | - | - | - |
| | 9 | $940\pm18^{Ccde}$ | $1206\pm74^{Bd}$ | $1414\pm101^{Aef}$ | $1425\pm56^{Aa}$ | $1429\pm34^{Aa}$ | - |
| | 10 | $961\pm22^{Ccde}$ | $1189\pm54^{Bd}$ | $1248\pm41^{Bhij}$ | $1363\pm47^{Ab}$ | $1431\pm25^{Aa}$ | - |
| 0.2 | 3 | $926\pm14^{Bcde}$ | $1106\pm12^{Ad}$ | $1207\pm45^{Aijk}$ | - | - | - |
| | 4 | $966\pm32^{Ccde}$ | $1140\pm55^{Bd}$ | $1392\pm42^{Agh}$ | - | - | - |
| | 5 (Native) | $995\pm46^{Ccde}$ | $1105\pm65^{Bd}$ | $1398\pm16^{Agh}$ | - | - | - |
| | 6 | $993\pm19^{Ccde}$ | $1159\pm35^{Bd}$ | $1397\pm32^{Agh}$ | - | - | - |
| | 7 | $969\pm25^{Bab}$ | $1169\pm147^{Bde}$ | $1543\pm188^{Abc}$ | - | - | - |
| | 8 | $904\pm43^{Bef}$ | $1571\pm18^{Aab}$ | $1636\pm84^{Aa}$ | - | - | - |
| | 9 | $911\pm86^{Bcde}$ | $1211\pm13^{Ad}$ | $1305\pm5^{Aghi}$ | - | - | - |
| | 10 | $964\pm13^{Bbc}$ | $1240\pm37^{Ad}$ | $1326\pm70^{Afgh}$ | - | - | - |

Table 15 The particles sizes (average diameter, nm) obtained from the use of different pH (3, 4, 5, 6, 7, 8, 9, 10) and KGM concentration. (continued)

| KGM concentration (%) | pH | Day | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 |
| 0.3 | 3 | 973±13$^{Bbc}$ | 1295±13$^{Ad}$ | - | - | - | - |
| | 4 | 988±43$^{Bab}$ | 1206±13$^{Ad}$ | - | - | - | - |
| | 5 (Native) | 1029±11$^{Ba}$ | 1335±42$^{Ad}$ | - | - | - | - |
| | 6 | 1003±2$^{Ba}$ | 1396±135$^{Ad}$ | - | - | - | - |
| | 7 | 965±21$^{Bbc}$ | 1632±40$^{Aa}$ | - | - | - | - |
| | 8 | 958±41$^{Bcde}$ | 1525±147$^{Aabc}$ | - | - | - | - |
| | 9 | 954±6$^{Bcde}$ | 1487±144$^{Abc}$ | - | - | - | - |
| | 10 | 952±40$^{Bcde}$ | 1419±87$^{Aef}$ | - | - | - | - |
| 0.4 | 3 | 950±17$^{Bcde}$ | 1294±15$^{Ad}$ | - | - | - | - |
| | 4 | 996±12$^{Aab}$ | 1197±123$^{Ad}$ | - | - | - | - |
| | 5 (Native) | 937±47$^{Acde}$ | 1289±121$^{Ad}$ | - | - | - | - |
| | 6 | 1003±34$^{Aa}$ | 1296±115$^{Ad}$ | - | - | - | - |
| | 7 | 967±15$^{Bbc}$ | 1433±59$^{Abc}$ | - | - | - | - |
| | 8 | 940±32$^{Bcde}$ | 1540±125$^{Aab}$ | - | - | - | - |
| | 9 | 991±7 $^{Bab}$ | 1525±60$^{Aabc}$ | - | - | - | - |
| | 10 | 954±25$^{Bcde}$ | 1388±62$^{Ac}$ | - | - | - | - |
| 0.5 | 3 | 938±113$^{Bcde}$ | 1285±121$^{Ad}$ | - | - | - | - |
| | 4 | 948±114$^{Bcde}$ | 1293±13$^{Ad}$ | - | - | - | - |
| | 5 (Native) | 992±13$^{Bab}$ | 1267±13$^{Ad}$ | - | - | - | - |
| | 6 | 1001±11$^{Ba}$ | 1295±142$^{Ad}$ | - | - | - | - |
| | 7 | 970±12$^{Bab}$ | 1570±12$^{Aab}$ | - | - | - | - |
| | 8 | 965±23$^{Bbc}$ | 1532±43$^{Aabc}$ | - | - | - | - |
| | 9 | 956±4$^{Bcde}$ | 1536±123$^{Aabc}$ | - | - | - | - |
| | 10 | 954±14$^{Bcde}$ | 1420±42$^{Abc}$ | - | - | - | - |

The particles sizes of milk and oil (not added KGM) was 906±6 nm
All values are presented as the mean ± SD.
Mean values followed by different superscripted letters in the same row (A–D) or column (a–k) are significantly different, according to Duncan's multiple range test ($p < 0.05$).

Table 16 polydispersity index (PDI) obtained from the use of different pH (3, 4, 5, 6, 7, 8, 9, 10) and KGM concentration

| KGM concentration (%) | pH | Day | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 |
| 0.02 | 3 | 0.25±0.33$^{Ca}$ | 0.32±0.12$^{Bbc}$ | 0.47±0.15$^{Acd}$ | - | - | - |
| | 4 | 0.23±0.12$^{Cbc}$ | 0.26±0.18$^{Bcd}$ | 0.34±0.17$^{Ae}$ | - | - | - |
| | 5 | 0.37±0.10$^{Da}$ | 0.32±0.21$^{Cbc}$ | 0.37±0.11$^{Be}$ | 0.41±0.09$^{Af}$ | - | - |
| | 6 | 0.29±0.09$^{Da}$ | 0.33±0.12$^{Cbc}$ | 0.34±0.07$^{Be}$ | 0.44±0.15$^{Af}$ | - | - |
| | 7 | 0.22±0.03$^{DEcd}$ | 0.25±0.04$^{DEcd}$ | 0.34±0.11$^{Ce}$ | 0.55±0.08$^{Bc}$ | 0.62±0.23$^{Abc}$ | - |
| | 8 | 0.23±0.06$^{CDbc}$ | 0.26±0.03$^{Ccd}$ | 0.35±0.07$^{BBe}$ | 0.57±0.13$^{ABCb}$ | 0.59±0.09$^{Ac}$ | - |
| | 9 | 0.24±0.05$^{EFab}$ | 0.26±0.04$^{Ecd}$ | 0.32±0.05$^{De}$ | 0.46±0.80$^{Cf}$ | 0.54±0.03$^{Bd}$ | 0.62±0.09$^{Aa}$ |
| | 10 | 0.19±0.06$^{EFf}$ | 0.22±0.05$^{Ee}$ | 0.34±0.02$^{De}$ | 0.42±0.02$^{Cf}$ | 0.51±0.23$^{Bf}$ | 0.59±0.14$^{Ab}$ |
| 0.04 | 3 | 0.23±0.13$^{Bbc}$ | 0.38±0.15$^{Abc}$ | 0.38±0.13$^{Ae}$ | - | - | - |
| | 4 | 0.27±0.12$^{Ba}$ | 0.47±0.34$^{Abc}$ | 0.47±0.17$^{Acd}$ | - | - | - |
| | 5 | 0.26±0.11$^{Ca}$ | 0.37±0.19$^{Bbc}$ | 0.53±0.18$^{Abc}$ | 0.54±0.15$^{Ad}$ | - | - |
| | 6 | 0.25±0.07$^{Ca}$ | 0.38±0.12$^{Bbc}$ | 0.58±010$^{Aab}$ | 0.56±0.17$^{Ad}$ | - | - |
| | 7 | 0.24±0.09$^{DEab}$ | 0.26±0.08$^{Dcd}$ | 0.32±0.09$^{Ce}$ | 0.50±0.09$^{Bde}$ | 0.61±0.18$^{Abc}$ | - |
| | 8 | 0.22±0.06C$^{cd}$ | 0.24±0.06$^{Ce}$ | 0.40±0.05$^{Be}$ | 0.58±0.06$^{Ab}$ | 0.59±0.03$^{Ac}$ | - |
| | 9 | 0.25±0.06$^{EFa}$ | 0.27±0.09$^{Ecd}$ | 0.34±0.07$^{De}$ | 0.48±0.02$^{Ce}$ | 0.55±0.05$^{Be}$ | 0.60±0.07$^{Aab}$ |
| | 10 | 0.20±0.05$^{EFef}$ | 0.22±0.03$^{Ee}$ | 0.35±0.03$^{De}$ | 0.44±0.13$^{Cf}$ | 0.52±0.05$^{Bf}$ | 0.61±0.05$^{Aab}$ |

Table 16 polydispersity index (PDI) obtained from the use of different pH (3, 4, 5, 6, 7, 8, 9, 10) and KGM concentration (continued)

| KGM | pH | Day |
|---|---|---|

| concentration (%) | | 0 | 2 | 4 | 6 | 8 | 10 |
|---|---|---|---|---|---|---|---|
| 0.06 | 3 | $0.26\pm0.21^{Ca}$ | $0.39\pm0.11^{Bbc}$ | $0.45\pm0.18^{Ae}$ | - | - | - |
| | 4 | $0.26\pm0.13^{Ca}$ | $0.38\pm0.12^{Bbc}$ | $0.45\pm0.12^{Ae}$ | - | - | - |
| | 5 (Native) | $0.27\pm0.10^{Ca}$ | $0.33\pm0.23^{Bbc}$ | $0.46\pm0.22^{Ae}$ | - | - | - |
| | 6 | $0.29\pm0.12^{Ba}$ | $0.34\pm0.24^{A}$ | $0.36\pm0.21^{Ae}$ | - | - | - |
| | 7 | $0.22\pm0.01^{CDcd}$ | $0.24\pm0.02^{Ce}$ | $0.54\pm0.03^{Bbc}$ | $0.62\pm0.05^{Aa}$ | - | - |
| | 8 | $0.22\pm0.01^{CDcd}$ | $0.24\pm0.05^{Ce}$ | $0.57\pm0.02^{Bab}$ | $0.62\pm0.12^{Aa}$ | - | - |
| | 9 | $0.24\pm0.02^{Dab}$ | $0.24\pm0.02^{De}$ | $0.43\pm0.09^{Cde}$ | $0.53\pm0.06^{Bd}$ | $0.68\pm0.07^{Aa}$ | - |
| 0.08 | 3 | $0.27\pm0.10^{Ca}$ | $0.34\pm0.12^{Bbc}$ | $0.43\pm0.18^{Ae}$ | - | - | - |
| | 4 | $0.29\pm0.12^{Ca}$ | $0.32\pm0.22^{Bbc}$ | $0.48\pm0.05^{Acd}$ | - | - | - |
| | 5 (Native) | $0.28\pm0.15^{Ca}$ | $0.34\pm0.15^{Bbc}$ | $0.33\pm0.12^{Ae}$ | - | - | - |
| | 6 | $0.27\pm0.03^{Ca}$ | $0.33\pm0.08^{Bbc}$ | $0.31\pm0.01^{Ae}$ | - | - | - |
| | 7 | $0.21\pm0.03^{Dde}$ | $0.25\pm0.08^{Ccd}$ | $0.54\pm0.05^{Bbc}$ | $0.62\pm0.06^{Aa}$ | - | - |
| | 8 | $0.22\pm0.03^{CDcd}$ | $0.25\pm0.01^{Ccd}$ | $0.56\pm0.03^{Bbc}$ | $0.62\pm0.12^{Aa}$ | - | - |
| | 9 | $0.20\pm0.09^{Def}$ | $0.24\pm0.07^{Ce}$ | $0.32\pm0.02^{Be}$ | $0.52\pm0.13^{Ad}$ | $0.52\pm0.09^{Af}$ | - |
| | 10 | $0.19\pm0.08^{CDf}$ | $0.22\pm0.04^{Ce}$ | $0.32\pm0.06^{Be}$ | $0.52\pm0.03^{Ad}$ | $0.52\pm0.03^{Af}$ | - |
| 0.1 | 3 | $0.24\pm0.09^{Bab}$ | $0.25\pm0.05^{Be}$ | $0.36\pm0.01^{Ae}$ | - | - | - |
| | 4 | $0.27\pm0.05^{Ca}$ | $0.35\pm0.06^{Bbc}$ | $0.44\pm0.12^{Ae}$ | - | - | - |
| | 5 (Native) | $0.22\pm0.09^{Ccd}$ | $0.34\pm0.13^{Bbc}$ | $0.43\pm0.11^{Ae}$ | - | - | - |
| | 6 | $0.25\pm0.08^{Ba}$ | $0.34\pm0.08^{Abc}$ | $0.35\pm0.17^{Ae}$ | - | - | - |
| | 7 | $0.21\pm0.01^{Cde}$ | $0.44\pm0.09^{Bbc}$ | $0.66\pm0.18^{Aa}$ | - | - | - |
| | 8 | $0.20\pm0.08^{Cef}$ | $0.42\pm0.08^{Bbc}$ | $0.65\pm0.06^{Aa}$ | - | - | - |
| | 9 | $0.22\pm0.03D^{Ecd}$ | $0.24\pm0.02^{De}$ | $0.40\pm0.02^{Ce}$ | $0.52\pm0.05^{Bd}$ | $0.62\pm0.02^{Abc}$ | - |
| | 10 | $0.19\pm0.03^{DEf}$ | $0.21\pm0.04^{De}$ | $0.49\pm0.04^{Ccd}$ | $0.52\pm0.06^{Bd}$ | $0.62\pm0.04^{Abc}$ | - |
| 0.2 | 3 | $0.26\pm0.04^{Ca}$ | $0.36\pm0.12^{Bbc}$ | $0.67\pm0.05^{Aa}$ | - | - | - |
| | 4 | $0.26\pm0.02^{Ca}$ | $0.40\pm0.15^{Bbc}$ | $0.62\pm0.03^{Aab}$ | - | - | - |
| | 5 (Native) | $0.25\pm0.06^{Ca}$ | $0.55\pm0.15^{Bab}$ | $0.68\pm0.16^{Aab}$ | - | - | - |
| | 6 | $0.23\pm0.09^{Cbc}$ | $0.59\pm0.13^{Bab}$ | $0.67\pm0.02^{Aa}$ | - | - | - |
| | 7 | $0.21\pm0.07^{Cde}$ | $0.43\pm0.07^{Bbc}$ | $0.66\pm0.03^{Aa}$ | - | - | - |
| | 8 | $0.20\pm0.06^{Cef}$ | $0.42\pm0.02^{Bbc}$ | $0.64\pm0.09^{Aa}$ | - | - | - |
| | 9 | $0.21\pm0.05^{Cde}$ | $0.42\pm0.01^{Bbc}$ | $0.61\pm0.06^{Aab}$ | - | - | - |
| | 10 | $0.21\pm0.01^{Cde}$ | $0.42\pm0.03^{Bbc}$ | $0.62\pm0.05^{Aab}$ | - | - | - |
| 0.3 | 3 | $0.23\pm0.03^{Bbc}$ | $0.55\pm0.03^{Aab}$ | - | - | - | - |
| | 4 | $0.28\pm0.03^{Ba}$ | $0.56\pm0.04^{Aab}$ | - | - | - | - |
| | 5 (Native) | $0.29\pm0.01^{Ba}$ | $0.55\pm0.12^{Aab}$ | - | - | - | - |
| | 6 | $0.23\pm0.02^{Bbc}$ | $0.56\pm0.15^{Aab}$ | - | - | - | - |
| | 7 | $0.22\pm0.03^{Bcd}$ | $0.62\pm0.09^{Aa}$ | - | - | - | - |
| | 8 | $0.19\pm0.08^{Bf}$ | $0.52\pm0.06^{Aab}$ | - | - | - | - |
| | 9 | $0.19\pm0.03^{Bf}$ | $0.52\pm0.07^{Aab}$ | - | - | - | - |
| | 10 | $0.24\pm0.06^{Bab}$ | $0.65\pm0.08^{Aa}$ | - | - | - | - |
| 0.4 | 3 | $0.20\pm0.07^{Bef}$ | $0.54\pm0.15^{Aab}$ | - | - | - | - |
| | 4 | $0.26\pm0.02^{Ba}$ | $0.57\pm0.13^{Aab}$ | - | - | - | - |
| | 5 (Native) | $0.27\pm0.07^{Ba}$ | $0.59\pm0.21^{Aab}$ | - | - | - | - |
| | 6 | $0.23\pm0.04^{Bbc}$ | $0.61\pm0.14^{Aa}$ | - | - | - | - |
| | 7 | $0.20\pm0.09^{Bef}$ | $0.52\pm0.17^{Aab}$ | - | - | - | - |
| | 8 | $0.22\pm0.01^{Bcd}$ | $0.62\pm0.05^{Aa}$ | - | - | - | - |
| | 9 | $0.24\pm0.06^{Bab}$ | $0.62\pm0.15^{Aa}$ | - | - | - | - |
| | 10 | $0.24\pm0.03^{Bab}$ | $0.62\pm0.06^{Aa}$ | - | - | - | - |

Table 16 polydispersity index (PDI) obtained from the use of different pH (3, 4, 5, 6, 7, 8, 9, 10) and KGM concentration (continued)

| KGM | pH | Day |
|---|---|---|

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| concentration (%) | | 0 | 2 | 4 | 6 | 8 | 10 |
|---|---|---|---|---|---|---|---|
| 0.5 | 3 | 0.20±0.07$^{Bef}$ | 0.54±0.15$^{Aab}$ | - | - | - | - |
| | 4 | 0.23±0.02$^{Bbc}$ | 0.57±0.23$^{Aab}$ | - | - | - | - |
| | 5 (Native) | 0.27±0.07$^{Ba}$ | 0.59±0.21$^{Aab}$ | - | - | - | - |
| | 6 | 0.23±0.04$^{Bbc}$ | 0.56±0.15$^{Aab}$ | - | - | - | - |
| | 7 | 0.23±0.07$^{Bbc}$ | 0.62±0.06$^{Aa}$ | - | - | - | - |
| | 8 | 0.22±0.04$^{Bcd}$ | 0.62±0.02$^{Aa}$ | - | - | - | - |
| | 9 | 0.20±0.03$^{Bef}$ | 0.57±0.08$^{Aab}$ | - | - | - | - |
| | 10 | 0.22±0.05$^{Bcd}$ | 0.55±0.08$^{Aab}$ | - | - | - | - |

The PDI of milk and oil (not added KGM) was 0.12±0.06 nm
All values are presented as the mean ± SD.
Mean values followed by different superscripted letters in the same row (A–D) or column (a–k) are significantly different, according to Duncan's multiple range test ($p < 0.05$).

It is possible to explain the increased stability of the emulsion when increased pH of KGM. When alkali is added to adjust the pH of KGM, the deacetylation of KGM occurred. As a result of this reaction, the decreasing water-solubility and the self-aggregation, so the expansion degree of the molecular chains became smaller. The negative charge density of the molecular chain was much larger than that in distilled water for the inductive effect of alkali. The alkali was seen as a kind of nucleophilic reagent, and it induced the electrons moving close to the oxygen atom of the hydroxyl group, even generated oxy anion, so the force of the electrostatic repulsion between the ionized molecular chain and hydroxide ion impeded the expansion of the molecular chain in solution. As a result, there was a competition relationship between the inductive effect of alkali and the hydration of water. The alkali damaged the hydrogen bonding of the hydration between the molecular chain and the water molecule, resulting in the expansion-suppression (Luo et al., 2013).

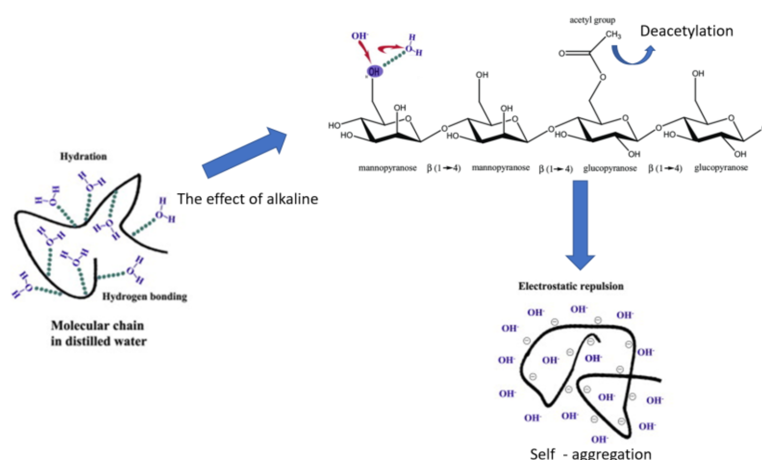


Figure 17 The diagram of the alkaline effects on the KGM molecular chain (Adapted from Luo et al. 2013).

When KGM is converted to anion, the addition of KGM to milk containing negatively charged casein makes no adsorption occurred between the milk proteins and KGM. As a result of deacetylation, KGM's acetyl group and hydrogen bonds are

weakened, making the hydrophobic interaction stronger. Thus, KGM are able to fill the gap between casein molecules and oil droplets in the emulsion, it may help to separate the oil droplets, which reducing the Brownian-motion of droplets (Lu et al., 2018).

In addition to KGM affecting emulsion stability, casein protein also affects emulsion stability. The ability of casein proteins to stabilize emulsions is the most important criterion. The forces involved in stabilizing emulsions include van der Waals attractive forces, electrostatic interactions, and steric factors. As long as sufficient protein is present during homogenization to cover the oil droplets, emulsions stabilized by casein are generally very stable to coalescence over prolonged storage. However, these emulsions are susceptible to different types of flocculation, which in turn leads to enhanced creaming or serum separation. At low protein-to-oil ratios, there is insufficient protein to fully cover the oil-water interface during homogenization, and this results in bridging flocculation (Fellows & Doherty, 2005). Another consequence of protein insufficiency is the incorporation of droplets during or immediately after the emulsion. Bridging flocculation is commonly observed in emulsions formed with aggregated casein protein, in which the droplets are bridged by casein aggregates. Optimum stability can generally be attained at protein concentrations high enough to allow full saturation coverage at the oil-water interface. However, the presence of excess casein, un-adsorbed casein protein may lead to depletion flocculation in some emulsions. Both depletion flocculation and bridging flocculation cause an emulsion to cream more rapidly. The study of Srinivasan, Singh & MunroIn, (2001) which prepared sodium caseinate–3% soya oil emulsions, it was shown that, at a protein content of nearly 2.0%, the emulsion droplets were protected from flocculation by a thick steric-stabilizing layer of sodium caseinate. The emulsion was stable against flocculation, coalescence, and creaming for several weeks. However, when the protein content was increased to above 3.0 %, un-adsorbed protein gave rise to depletion flocculation. Because of this depletion flocculation, the effective diameter of the droplets increased, resulting in a marked an increase in the caseinate concentration from 3 to 5 % (Srinivasan, Singh, & Munro, 2001). The results indicated that casein protein correlate the flocculation of the emulsion.

4.1.2.2 Zeta potential analysis

Emulsion stability depends on the attraction and repulsion between the particles. The particle surface charge determines the attractive and repulsive forces and plays an important role in the stability of the emulsion. Zeta potential is the parameter which evaluates the surface charge to assess the stability of the emulsion. Emulsions with zeta potential higher than +30 mV or less than -30 mV are stable due to the repulses forced that maintain the particles during dispersion (Dai, Jiang, Shah, & Corke, 2017). In this study, the control emulsion (milk and fish oil without KGM added) had zeta potential less than -30 mV (-32.24 mV). However, separation between milk and fish oil occurred in the control emulsion within 4 h, although zeta potential

values showed that the emulsion was likely to be stable. But when adding KGM, the emulsion has increased stability. The effects of KGM concentration and pH values on the zeta potential are shown in table 17. The results showed that at the same pH, but different concentrations of KGM, the zeta sac values were quite similar. For example, at pH7, zeta potential of 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4 and 0.5% KGM were -34.51, -33.48, -34.28, -34.54, -33.88, -33.47, -34.42, -33.66 and -33.70 mV, respectively. But when the concentration of KGM was the same, but the pH was different, it was found that the zeta and zeta values were different. For example, at 0.04% KGM, pH 7, 8, 9 and 10 zeta potential were -33.48, -35.98, -36.94 -36.97 mV, respectively. The results of this study help confirm the deacetylation of KGM, which can help to stabilize the emulsion.

4.1.2.3 Precipitation percentage

Emulsion is classified as unstable thermodynamic systems. Aggregation and flocculation of oil droplets are among the factors that cause the emulsion instability. Precipitation percentage, refers to the volume percentage of the precipitation in emulsion from mixing to separation, which is an observed evidence of the stability of the emulsion (Ye & Harte, 2014). A value of 0% precipitation means emulsion was not separated. Table 18 shows the effect of KGM concentration and pH values on the precipitation percentage of samples. The precipitation percentage for 0.02-0.5 % KGM at any pH was 0% indicating a stable mixture after mixing up to 2 days. When the concentration of KGM in the emulsion increased, the separation occurred more rapidly and the precipitation percentage increased. For example, when KGM concentration was 0.02% (w/w) at pH 7, precipitation percentage was 0% until day 8 and increased to 7.39% at day 10 indicating that the emulsion was stable for 8 days before separation. Meanwhile, with 0.2% KGM at pH 7, the precipitation percentage was 0% until day 4 and increased to 6.85% on day 6 indicating that the emulsion was stable for 4 days before separation. In the present study, pH values were affecting the precipitation percentage. The increase in pH values, from 3-10 at the same concentration of KGM led to an increase of the stability of the emulsion. For example, at pH 7, 0.04% KGM concentration precipitation percentage was 7.05% on day 10, while at pH 10 at the same KGM concentration the precipitation percentage was 6.96% on day 12.

| 10 | - - - - - - -17.14±1.02 -18.13±1.54 | - - - - - - -18.02±1.61 -17.61±0.67 | - - - - - - - - | - - - - - - - - |
|---|---|---|---|---|

Table 17 The Zeta potential (mV) obtained from the use of different pH (3, 4, 5, 6, 7, 8, 9, 10) and KGM concentration.

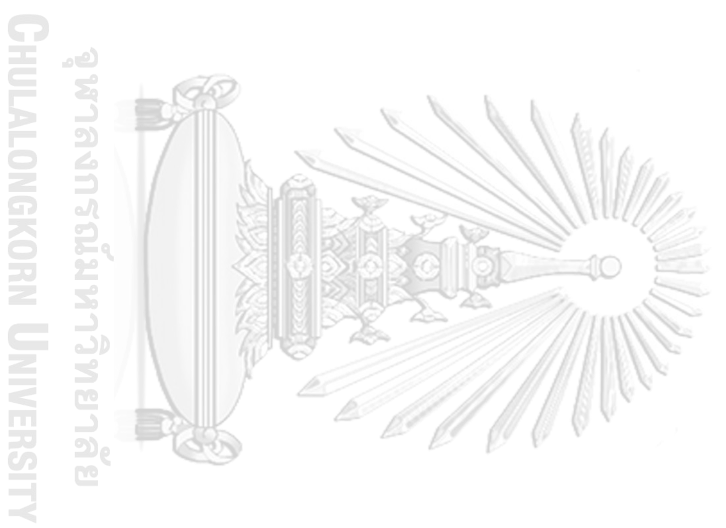
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Table 17 The Zeta potential (mV) obtained from the use of different pH (3, 4, 5, 6, 7, 8, 9, 10) and KGM concentration. (continued)

| KGM concentration (%) | pH | Day | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 |
| 0.1 | 3 | -32.40±0.48$^{Ca}$ | -23.50±1.48$^{Ba}$ | -19.59±1.38$^{Aa}$ | - | - | - |
| | 4 | -32.58±1.40$^{Ca}$ | -24.49±2.87$^{Bcd}$ | -20.52±0.33$^{Aa}$ | - | - | - |
| | 5 | -32.59±2.33$^{Ca}$ | -24.53±2.96$^{Bcde}$ | -19.33±0.13$^{Aa}$ | - | - | - |
| | 6 | -31.02±1.69$^{Ca}$ | -23.52±0.19$^{Ba}$ | -19.32±0.28$^{Aa}$ | - | - | - |
| | 7 | -33.88±0.23$^{Ca}$ | -23.30±0.08$^{Ba}$ | -19.98±0.08$^{Aab}$ | - | - | - |
| | 8 | -35.74±0.48$^{Cde}$ | -24.23±0.53$^{Babc}$ | -21.29±0.27$^{Adef}$ | - | - | - |
| | 9 | -37.63±0.74$^{Dij}$ | -24.59±0.46$^{Ccde}$ | -22.49±0.37$^{Bhi}$ | -21.02±1.03$^{Bbc}$ | -19.22±1.15$^{Acde}$ | - |
| | 10 | -37.66±0.34$^{Cj}$ | -24.09±1.18$^{Babc}$ | -24.13±1.16$^{Bk}$ | -21.66±0.56$^{Ac}$ | -19.73±0.65$^{Ade}$ | - |
| 0.2 | 3 | -30.49±1.49$^{Ca}$ | -24.59±1.97$^{Bcde}$ | -20.42±2.47$^{Aa}$ | - | - | - |
| | 4 | -31.30±1.35$^{Ca}$ | -25.30±1.43$^{Bijk}$ | -20.35±2.50$^{Aa}$ | - | - | - |
| | 5 | -30.25±2.42$^{Ca}$ | -23.42±1.60$^{Ba}$ | -19.39±0.39$^{Aa}$ | - | - | - |
| | 6 | -30.40±0.09$^{Cbc}$ | -24.30±1.76$^{Bab}$ | -19.37±0.02$^{Aa}$ | - | - | - |
| | 7 | -33.47±0.46$^{Ba}$ | -23.67±0.47$^{Babc}$ | -19.28±0.44$^{Aa}$ | - | - | - |
| | 8 | -35.81±0.74$^{Cdef}$ | -24.28±0.81$^{Babc}$ | -20.95±0.81$^{Ade}$ | - | - | - |
| | 9 | -36.45±1.94$^{Cghi}$ | -25.76±0.36$^{Bjk}$ | -22.78±0.94$^{Aij}$ | - | - | - |
| | 10 | -37.37±0.27$^{Bij}$ | -25.09±0.42$^{Aefg}$ | -24.28±1.03$^{Ak}$ | - | - | - |
| 0.3 | 3 | -32.42±1.68$^{Ba}$ | -24.25±1.65$^{Abc}$ | - | - | - | - |
| | 4 | -31.33±2.49$^{Ba}$ | -24.58±1.48$^{Aab}$ | - | - | - | - |
| | 5 | -31.90±1.56$^{Ba}$ | -23.67±0.44$^{Aa}$ | - | - | - | - |
| | 6 | -31.55±0.02$^{Ba}$ | -23.80±1.57$^{Aa}$ | - | - | - | - |
| | 7 | -34.42±0.19$^{Bbcd}$ | -23.85±0.49$^{Aabc}$ | - | - | - | - |
| | 8 | -34.32±0.89$^{Bab}$ | -24.78±0.77$^{Adef}$ | - | - | - | - |
| | 9 | -36.67±0.64$^{Bghi}$ | -24.54±0.48$^{Acde}$ | - | - | - | - |
| | 10 | -37.46±0.54$^{Bij}$ | -25.05±0.81$^{Aefg}$ | - | - | - | - |
| 0.4 | 3 | -31.49±1.40$^{Ba}$ | -24.58±0.24$^{Aab}$ | - | - | - | - |
| | 4 | -31.08±1.69$^{Ba}$ | -25.40±1.50$^{Aijk}$ | - | - | - | - |
| | 5 | -31.36±1.94$^{Ba}$ | -24.39±2.58$^{Abc}$ | - | - | - | - |
| | 6 | -30.30±0.39$^{Ba}$ | -24.32±1.50$^{Abc}$ | - | - | - | - |
| | 7 | -33.66±0.82$^{Ba}$ | -23.87±0.53$^{Aabc}$ | - | - | - | - |
| | 8 | -34.74±0.90$^{Bbcd}$ | -24.57±0.28$^{Aabc}$ | - | - | - | - |
| | 9 | -36.70±1.07$^{Bghi}$ | -24.52±0.31$^{Aabc}$ | - | - | - | - |
| | 10 | -37.17±0.55$^{Bhij}$ | -25.46±0.40$^{Aijk}$ | - | - | - | - |

Table 17 The Zeta potential (mV) obtained from the use of different pH (3, 4, 5, 6, 7, 8, 9, 10) and KGM concentration. (continued)

| KGM concentration (%) | pH | Day | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 |
| 0.5 | 3 | $-31.87 \pm 1.03^{Ba}$ | $-23.95 \pm 1.40^{Aa}$ | - | - | - | - |
| | 4 | $-31.47 \pm 2.03^{Ba}$ | $-23.05 \pm 2.31^{Aa}$ | - | - | - | - |
| | 5 | $-30.98 \pm 1.01^{Ba}$ | $-22.39 \pm 0.38^{Aa}$ | - | - | - | - |
| | 6 | $-30.23 \pm 2.11^{Ba}$ | $-22.12 \pm 1.55^{Aa}$ | - | - | - | - |
| | 7 | $-33.70 \pm 0.35^{Ba}$ | $-23.51 \pm 0.13^{Aab}$ | - | - | - | - |
| | 8 | $-35.63 \pm 0.33^{Bde}$ | $-24.68 \pm 0.61^{Adef}$ | - | - | - | - |
| | 9 | $-36.36 \pm 0.61^{Bghi}$ | $-24.49 \pm 0.34^{Abcd}$ | - | - | – | - |
| | 10 | $-37.40 \pm 0.31^{Bij}$ | $-25.33 \pm 0.36^{Aghi}$ | - | - | - | - |

The Zeta potential of milk and oil (not added KGM) was -32.24±0.64 mV
All values are presented as the mean ± SD.
Mean values followed by different superscripted letters in the same row (A–D) or column (a–k) are significantly different, according to Duncan's multiple range test ($p < 0.05$).

Table 18 The effects of KGM concentration and pH on the precipitation percentage of samples (0 = no separation and - = not analyzed)

| KGM concentration (%) | pH | Day | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | 10 | 12 |
| 0.02 | 3 | 0 | 0 | 8.30±0.10 | - | - | - |
| | 4 | 0 | 0 | 10.49±1.78 | - | - | - |
| | 5 | 0 | 0 | 0 | 7.10±0.57 | - | - |
| | 6 | 0 | 0 | 0 | 10.40±3.36 | - | - |
| | 7 | 0 | 0 | 0 | 0 | 7.39±0.10 | - |
| | 8 | 0 | 0 | 0 | 0 | 7.38±0.03 | - |
| | 9 | 0 | 0 | 0 | 0 | 0 | 9.10±0.56 |
| | 10 | 0 | 0 | 0 | 0 | 0 | 6.59 ±0.61 |
| 0.04 | 3 | 0 | 0 | 9.69±3.30 | - | - | - |
| | 4 | 0 | 0 | 8.38±0.21 | - | - | - |
| | 5 | 0 | 0 | 0 | 12.04±1.12 | - | - |
| | 6 | 0 | 0 | 0 | 11.30±1.09 | - | - |
| | 7 | 0 | 0 | 0 | 0 | 7.05±0.57 | - |
| | 8 | 0 | 0 | 0 | 0 | 8.25±0.14 | - |
| | 9 | 0 | 0 | 0 | 0 | 0 | 8.35±1.35 |
| | 10 | 0 | 0 | 0 | 0 | 0 | 6.96 ±0.58 |
| 0.06 | 3 | 0 | 0 | 8.04±1.30 | - | - | - |
| | 4 | 0 | 0 | 7.08±2.21 | - | - | - |
| | 5 | 0 | 0 | 10.40±3.30 | - | - | - |
| | 6 | 0 | 0 | 9.32±1.24 | - | - | - |
| | 7 | 0 | 0 | 0 | 7.61±0.32 | - | - |
| | 8 | 0 | 0 | 0 | 7.06±0.66 | - | - |
| | 9 | 0 | 0 | 0 | 0 | 7.48±0.34 | - |
| | 10 | 0 | 0 | 0 | 0 | 8.29±0.10 | - |
| 0.08 | 3 | 0 | 0 | 8.04±1.30 | - | - | - |
| | 4 | 0 | 0 | 7.08±2.21 | - | - | - |
| | 5 | 0 | 0 | 10.40±3.30 | - | - | - |
| | 6 | 0 | 0 | 9.32±1.24 | - | - | - |
| | 7 | 0 | 0 | 0 | 7.62±0.71 | - | - |
| | 8 | 0 | 0 | 0 | 7.56±0.27 | - | - |
| | 9 | 0 | 0 | 0 | 0 | 8.29±0.01 | - |
| | 10 | 0 | 0 | 0 | 0 | 8.39±0.10 | - |
| 0.1 | 3 | 0 | 0 | 9.40±3.25 | - | - | - |
| | 4 | 0 | 0 | 9.33±1.59 | - | - | - |
| | 5 | 0 | 0 | 10.55±2.40 | - | - | - |
| | 6 | 0 | 0 | 10.30±1.30 | - | - | - |
| | 7 | 0 | 0 | 6.70±0.52 | - | - | - |
| | 8 | 0 | 0 | 7.36±0.10 | - | - | - |
| | 9 | 0 | 0 | 0 | 0 | 8.61±1.22 | - |
| | 10 | 0 | 0 | 0 | 0 | 7.19±0.01 | - |
| 0.2 | 3 | 0 | 0 | 12.03±1.36 | - | - | - |
| | 4 | 0 | 0 | 13.40±1.40 | - | - | - |
| | 5 | 0 | 0 | 9.03± 2.33 | - | - | - |
| | 6 | 0 | 0 | 9.32±1.34 | - | - | - |
| | 7 | 0 | 0 | 6.85±0.55 | - | - | - |
| | 8 | 0 | 0 | 6.81±0.59 | - | - | - |
| | 9 | 0 | 0 | 7.09±0.69 | - | - | - |
| | 10 | 0 | 0 | 7.04±1.23 | - | - | - |

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Table 18 The effects of KGM concentration and pH on the precipitation percentage of samples (0 = no separation and - = not analyzed) (continued)

| KGM concentration (%) | pH | Day | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | 10 | 12 |
| 0.3 | 3 | 0 | 11.30±1.29 | - | - | - | - |
| | 4 | 0 | 10.22±1.30 | - | - | - | - |
| | 5 | 0 | 10.39± 0.37 | - | - | - | - |
| | 6 | 0 | 9.20±1.34 | - | - | - | - |
| | 7 | 0 | 5.23±0.67 | - | - | - | - |
| | 8 | 0 | 5.87±0.80 | - | - | - | - |
| | 9 | 0 | 6.40±0.58 | - | - | - | - |
| | 10 | 0 | 5.95±0.40 | - | - | - | - |
| 0.4 | 3 | 0 | 8.30±0.28 | - | - | - | - |
| | 4 | 0 | 7.42±1.30 | - | - | - | - |
| | 5 | 0 | 8.48±1.39 | - | - | - | - |
| | 6 | 0 | 9.39±1.23 | - | - | - | - |
| | 7 | 0 | 5.98±0.59 | - | - | - | - |
| | 8 | 0 | 6.53±0.38 | - | - | - | - |
| | 9 | 0 | 6.32±0.47 | - | - | - | - |
| | 10 | 0 | 6.10±0.45 | - | - | - | - |
| 0.5 | 3 | 0 | 9.53±0.35 | - | - | - | - |
| | 4 | 0 | 11.40±0.57 | - | - | - | - |
| | 5 | 0 | 7.24± 0.24 | - | - | - | - |
| | 6 | 0 | 9.39±1.30 | - | - | - | - |
| | 7 | 0 | 5.32±0.03 | - | - | - | - |
| | 8 | 0 | 6.43±0.74 | - | - | - | - |
| | 9 | 0 | 6.34±0.23 | - | - | - | - |
| | 10 | 0 | 6.40±0.10 | - | - | - | - |

All values are presented as the mean ± SD.

4.1.2.4 Microscopic structure

Nile Red and FITC were used to stain fat globules and protein in a milk or dairy products, respectively (Gaygadzhiev, Hill, & Corredig, 2009). However, FITC was used to label polysaccharide (Hans Tromp, van de Velde, van Riel, & Paques, 2001). The orange or red in the picture represented milk protein and the green one represented KGM rich area. The results are consistent with the phenomenon of micellar casein-konjac mixture (1.7% fat and 77.4% protein) that the Konjac-FITC conjugate (green channel) shows homogeneity in solution and the mixtures separated in protein (red channel) and konjac (green phase) domains (Abhyankar, Mulvihill, Chaurin, & Auty, 2011). Emulsion stability can be analyzed by shape, size and connection of the aggregate structures of phase separation dynamics theory (Bates, 1991). The nucleation structure and growth are that tiny droplet of the minor phase, it is developed by diffusion of the component from the supersaturated continuum. This happens in the metastable area of the phase diagram in a stable mixture (Abhyankar et al., 2011).

As mentioned above, different KGM concentration and pH values are a factor of stability of emulsion. The present study, the microscopic image of emulsion prepared at pH 7, 8, 9 and 10 and 0.04% KGM concentration are chosen and shown in Figure 17. All emulsions were stables with homogeneous CLSM image after mixing (Day 0). During storage time, phase separation of emulsion could be observed.

Emulsion stability can be determined by shape, size and connection of the aggregate structure by phase separation (Abraham, 2012). At day 10, the microstructure of 0.04% KGM stabilized emulsion was stable due to the influence of pH, it was found that lowering the pH resulted in an increase in the size of the aggregate and the shape. of aggregated changed from isolated shape to interconnected shape, which is shown in emulsion at pH 7 compared to emulsion at pH 10.

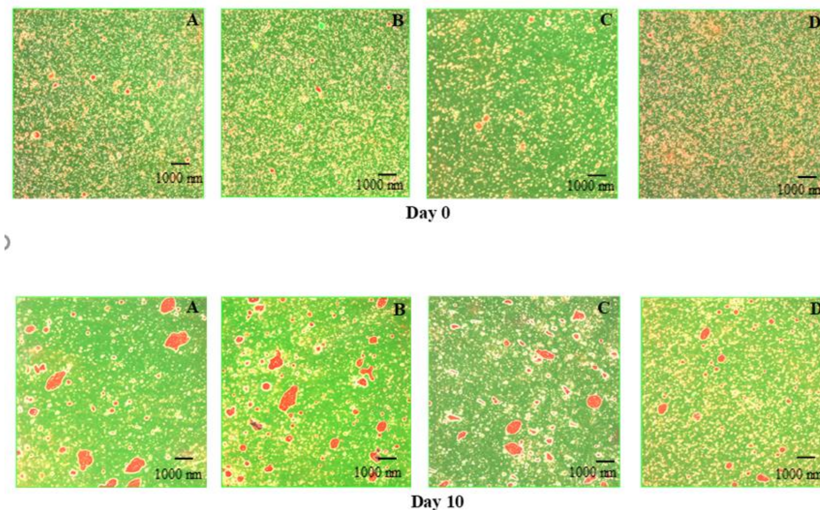


Figure 18 Microscopic image of emulsion at 0.04% KGM concentration as observed by confocal laser scanning microscopy (CLSM). A, B, C and D represent the pH emulsion at pH 7, 8, 9 and 10, respectively.

4.1.2.5 Viscosity

Table 19 shows the apparent viscosity and pH of freshly prepared emulsions measured at 31.10 $^{s-1}$ using viscometer. Apparent viscosity and pH of whole goat milk were 6.77 and 3.35 mP.s, respectively.

Increasing KGM concentration obviously increased the viscosity of the emulsions. For example, at pH 5, 0.04%,0.06% and 0.08%KGM, apparent viscosities of emulsion were 6.10, 9.75, 10.70 mPa.s, respectively. In this case, viscosity increased due to the viscous nature of KGM caused an increase in the emulsion continuous phase viscosity (Chivero et al., 2015). Moreover, increasing pH of KGM at the same concentration increased the viscosity of the emulsions. For example, at pH 8,9,10 at 0.04% KGM, apparent viscosities of emulsion were 16.85, 19.50, 21.40 mPa.s, respectively. In this case, viscosity increased due to deacetylation of KGM which play an important role in the gelation behavior of KGM (Du, Li, Chen, & Li, 2012).

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Table 19 The apparent viscosity and pH of freshly prepared emulsions measured at $31.10^{s-1}$ using viscometer.

| KGM concentration (%) | pH | Apparent viscosity (mPa.s) | pH of emulsion |
|---|---|---|---|
| 0.02 | 3 | 3.20±0.00 | 6.60 |
| | 4 | 3.45±0.21 | 6.66 |
| | 5 (native) | 3.85±0.07 | 6.60 |
| | 6 | 10.30±0.14 | 6.77 |
| | 7 | 14.20±0.14 | 6.77 |
| | 8 | 15.20±0.28 | 6.77 |
| | 9 | 15.55±0.35 | 6.78 |
| | 10 | 17.30±0.42 | 6.78 |
| 0.04 | 3 | 6.50±0.57 | 6.67 |
| | 4 | 6.50±0.14 | 6.67 |
| | 5 (Native) | 6.10±0.14 | 6.68 |
| | 6 | 10.70±0.14 | 6.77 |
| | 7 | 14.50±0.57 | 6.77 |
| | 8 | 16.85±0.07 | 6.7 |
| | 9 | 19.50±0.14 | 6.78 |
| | 10 | 21.40±0.57 | 6.79 |
| 0.06 | 3 | 8.45±0.18 | 6.60 |
| | 4 | 8.85±0.04 | 6.67 |
| | 5 (Native) | 9.75±0.04 | 6.71 |
| | 6 | 23.70±0.14 | 6.77 |
| | 7 | 26.45±0.04 | 6.77 |
| | 8 | 28.30±0.64 | 6.78 |
| | 9 | 28.55±0.04 | 6.78 |
| | 10 | 30.55±0.11 | 6.78 |
| 0.08 | 3 | 10.95±0.64 | 6.73 |
| | 4 | 11.45±0.21 | 6.74 |
| | 5 (Native) | 10.70±0.85 | 6.73 |
| | 6 | 24.55±0.35 | 6.77 |
| | 7 | 26.85±0.07 | 6.78 |
| | 8 | 28.70±0.28 | 6.78 |
| | 9 | 30.25±0.21 | 6.79 |
| | 10 | 32.35±0.35 | 6.80 |
| 0.1 | 3 | 10.45±0.49 | 6.73 |
| | 4 | 10.75±0.21 | 6.73 |
| | 5 (Native) | 10.10±1.13 | 6.74 |
| | 6 | 26.55±0.35 | 6.77 |
| | 7 | 30.40±0.28 | 6.78 |
| | 8 | 32.65±0.35 | 6.79 |
| | 9 | 35.35±0.39 | 6.80 |
| | 10 | 37.25±0.35 | 6.80 |
| 0.2 | 3 | 13.65±0.35 | 6.72 |
| | 4 | 13.50±0.14 | 6.73 |
| | 5 (Native) | 13.85±0.07 | 6.74 |
| | 6 | 32.20±0.85 | 6.77 |
| | 7 | 34.20±0.00 | 6.79 |
| | 8 | 37.45±0.07 | 6.81 |
| | 9 | 39.70±0.28 | 6.82 |
| | 10 | 43.45±0.07 | 6.83 |

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Table19 The apparent viscosity and pH of freshly prepared emulsions measured at $31.10^{s-1}$ using viscometer. (Continued)

| KGM concentration (%) | pH | Apparent viscosity (mPa.s) | pH of emulsion |
|---|---|---|---|
| 0.4 | 3 | 17.70±0.14 | 6.69 |
| | 4 | 16.70±0.71 | 6.72 |
| | 5 (Native) | 17.25±0.07 | 6.74 |
| | 6 | 37.55±0.21 | 6.78 |
| | 7 | 40.70±0.28 | 6.80 |
| | 8 | 44.20±0.00 | 6.82 |
| | 9 | 48.30±0.14 | 6.83 |
| | 10 | 51.40±0.14 | 6.84 |
| 0.5 | 3 | 19.25±0.21 | 6.69 |
| | 4 | 19.00±0.14 | 6.72 |
| | 5 (Native) | 18.95±0.78 | 6.75 |
| | 6 | 40.35±0.07 | 6.79 |
| | 7 | 43.10±0.14 | 6.80 |
| | 8 | 49.30±0.14 | 6.82 |
| | 9 | 50.55±0.14 | 6.83 |
| | 10 | 53.40±0.14 | 6.84 |

All values are presented as the mean ± SD.

The results from particle size, zeta potential and viscosity indicated that the emulsion was stable due to the effect casein and KGM. Casein protein may cover the oil droplets during homogenization. Deacetyled KGM was create network to fill gap between casein molecules and oil droplets in the emulsion, it may help to separate the oil droplets, which reducing the Brownian-motion of droplets. However, casein is not enough to cover the oil droplet. And the network building of the deacetyled KGM is thermally enhanced, which was not used in this study. Thus, the highest emulsions in this study were stability for 10 days.

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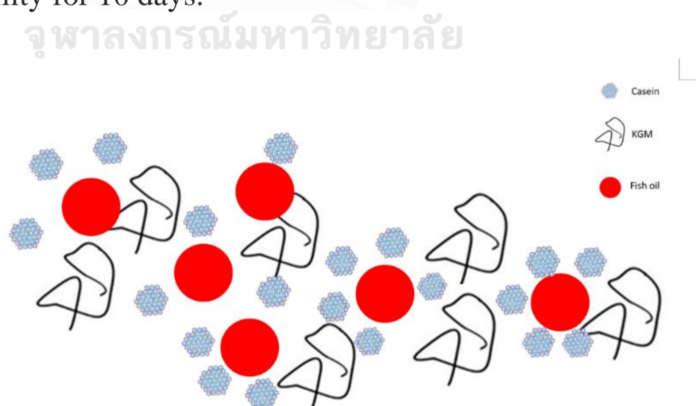



Figure 19 Schematic illustration of the structure of 5% fish oil KGM-milk emulsion

The result of this section would present the proper concentration of KGM and pH, which had the highest KGM – fish oil milk emulsion stability. The results showed that, 0.04% KGM (W/W) at pH 9 and 10 provided the highest stability of the prepared emulsion. Therefore, this condition was chosen for the next section.

### 4.2 Effect of high pressure on stability and microbiology of the KGM- fish oil milk emulsion

The emulsion from previous section was treated using a high-pressure unit at pressures of 400, 500 and 600 MPa for 3 min and cooled immediately. Physical properties (included particle size and zeta potential) were analyzed. In addition, oxidative stability (PV and TBAR) and microbiological was analyzed too. All analyzes were performed until separation of emulsion occurs. It was found that, separation of emulsion under all conditions studied occurred after day 14.

4.2.1 Particle size analysis

HPP is often used to produce food emulsions, with fine texture and stable emulsion (Desrumaux & Marcand, 2002). As the processed, emulsion flows through a high-pressure valve, combination of intense shear, cavitation, and turbulent result in breakage of droplets. Emulsion stability can be improved by the reduction of droplet particle size (McClements, 2004).

The effect of high-pressure processing (400, 500 and 600 MPa for 3 min) on the particle size and PDI was determined for 5 wt% fish oil-milk emulsions stabilized by 0.04 wt% KGM at pH9 and 10 (Table 20). A decrease in particle size with increasing pressure was observed, which is agreement with previous studies (Qian & McClements, 2011; Tan & Nakajima, 2005). There was a decrease in the particle size when the coarse emulsions were passed through HPP at all pressures studied. In addition, when compared to non-HPP at the same KGM concentration and pH, the particle size of emulsion was reduced. For example, on Day 0, the particle size of all emulsion was approximately 930 nm before HPP to approximately 922, 906 and 901 nm after HPP at 400, 500 and 600 MPa, respectively. The particle size after pass through the HPP is important because it determines the stability of the emulsion (McClements, Decker, & Weiss, 2007). Emulsions with smaller particle sizes are generally found to be more stable than emulsions with larger particle sizes. This is because the relatively small particle size means that the Brownian motion effect dominates gravitational forces (McClements, 2004). The better stability to resist coalescence and flocculation is due to the range of the attractive forces acting between droplets decreases as the particle size decreases whereas the range of steric repulsion is less dependent on particle size (Tadros, Izquierdo, Esquena, & Solans, 2004). However, from this study it was found that the particle size was increasing over time. This increase occurs with both untreated and treated HPP emulsions. For example, at pH 9 emulsions which was not HPP treated, had a 31% increase in particle size prior to separations while the increase of particle size was about 30, 31 and 31% when HPP

treated at 400, 500 and 600 MPa, respectively. Therefore, the stability of the emulsion, which increased from 10 days to 14 days, was due to the small initial particle size after the HPP. The PDI values of all controllers and emulsions ranged from 0.12-0.66, indicating that the emulsion possesses monodisperse properties. However, when the emulsion was kept, the PDI value tended to increase in all emulsion conditions (Table 15).

Table 20 Effect of pressures on particle size (average diameter, nm) of 5% fish oil emulsion made with 0.04 % KGM at pH9 and 10

| Pressure (MPa) | pH | Storage period (Day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 | 12 | 14 |
| 0 | 9 | $938±30^{Ea}$ | $1006±11^{Da}$ | $1215±81^{Ca}$ | $1296±6^{Ba}$ | $1306±5^{Ba}$ | $1439±15^{Aa}$ | - | - |
| | 10 | $931±47^{Ea}$ | $1011±7^{Da}$ | $1201±3^{Ca}$ | $1143±48^{Ba}$ | $1208±14^{Ba}$ | $1419±35^{Aa}$ | - | - |
| 400 | 9 | $919±17^{Dc}$ | $920±3^{Dd}$ | $995±5^{Cc}$ | $1000±0^{Cd}$ | $1011±10^{Cc}$ | $1156±54^{Bb}$ | $1241±58^{Aa}$ | $1303±6^{Aa}$ |
| | 10 | $925±33^{Fb}$ | $926±11^{Fb}$ | $987±1^{EFd}$ | $1002±4^{DEb}$ | $1010±1^{CDd}$ | $1106±5^{Cc}$ | $1204±7^{Bb}$ | $1302±4^{Ab}$ |
| 500 | 9 | $901±1^{Gd}$ | $910±2^{Fc}$ | $996±6^{Eb}$ | $1000±1^{Ed}$ | $1014±6^{Db}$ | $1146±71^{Cd}$ | $1238±19^{Bc}$ | $1303±6^{Ab}$ |
| | 10 | $911±16^{Ed}$ | $924±2^{Ec}$ | $996±5^{Db}$ | $998±3^{De}$ | $1007±6^{Db}$ | $1105±3^{Ce}$ | $1209±95^{Bd}$ | $1301±3^{Ac}$ |
| 600 | 9 | $902±4^{He}$ | $904±2^{Gd}$ | $995±6^{Fc}$ | $1001±2^{Ec}$ | $1010±2^{Dc}$ | $1111±9^{Cf}$ | $1213±2^{Be}$ | $1301±5^{Ac}$ |
| | 10 | $901±3^{Gf}$ | $903±1^{Gg}$ | $996±3^{Fb}$ | $1000±1^{Ed}$ | $1009±0^{Dd}$ | $1109±1^{Cg}$ | $1208±4^{Bf}$ | $1300±0^{Ad}$ |

- represents separated mixture

All values are presented as the mean ± SD.

Mean values followed by different superscripted letters in the same column (a–g) or row (A–H) are significantly different, according to Duncan's multiple range test ($p < 0.05$).

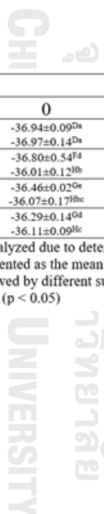
### 4.2.2 Zeta potential analysis

The results of the zeta potential analysis are presented in Table 21. Zeta potential could give information about the droplets' electrostatic repulsion, and this could be related to the stability of the emulsions. In fact, the emulsion with low absolute zeta potentials were less electrically stable with tend to coagulate or flocculate than high absolute zeta potentials (Cho et al., 2014). As shown in Table 16, absolute zeta-potential were about 36.94 and 36.97 mV in the untreated emulsion with KGM at pH 9 and 10, respectively. After HPP at 400, 500 and 600 MPa, the absolute zeta-potential of emulsion with KGM at pH 9 and 10 were about 36.80, 36.46, 36.29, 36.01, 36.07 and 36.11 mV, respectively. These results were agreement with the results of Yuan et al. (2013) on the HPP of whey protein isolate and chitosan mixtures (Yuan et al., 2013)

Table 22 Effect of pressures on Zeta potential (mV) of 5% fish oil emulsion made with 0.04 % KGM at pH9 and 10

| Pressure (MPa) | pH | Storage period (Day) 0 | 2 | 4 | 6 | 8 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 9 | $-36.94\pm0.09^{Da}$ | $-25.27\pm0.62^{Ca}$ | $-21.37\pm0.10^{Ba}$ | $-20.21\pm0.58^{Ba}$ | $-20.18\pm0.11^{Ba}$ | $-18.02\pm1.61^{Aa}$ | - | - |
| | 10 | $-36.97\pm0.14^{Da}$ | $-25.23\pm0.69^{Ca}$ | $-23.70\pm0.24^{Cb}$ | $-21.75\pm1.07^{Bb}$ | $-20.67\pm1.53^{Ba}$ | $-17.61\pm0.67^{Ab}$ | - | - |
| 400 | 9 | $-36.80\pm0.54^{Fd}$ | $-26.55\pm0.11^{Ec}$ | $-24.61\pm0.38^{Dc}$ | $-22.15\pm0.31^{Cc}$ | $-20.62\pm0.23^{Bb}$ | $-20.04\pm0.08^{Bc}$ | $-18.55\pm0.15^{Aa}$ | $-18.35\pm0.02^{Aa}$ |
| | 10 | $-36.01\pm0.12^{Hb}$ | $-27.06\pm0.11^{Gd}$ | $-26.28\pm0.47^{Fd}$ | $-23.48\pm0.32^{Ed}$ | $-21.45\pm0.42^{Dd}$ | $-20.51\pm0.27^{Cd}$ | $-18.84\pm0.14^{Ba}$ | $-18.15\pm0.17^{Aa}$ |
| 500 | 9 | $-36.46\pm0.02^{Ge}$ | $-26.25\pm0.44^{Fbc}$ | $-24.94\pm0.07^{Ec}$ | $-22.06\pm0.10^{Dc}$ | $-20.93\pm0.09^{Cc}$ | $-20.27\pm0.08^{Bc}$ | $-18.42\pm0.11^{Aa}$ | $-18.71\pm0.13^{Aa}$ |
| | 10 | $-36.07\pm0.17^{Hbc}$ | $-27.25\pm0.28^{Gd}$ | $-26.27\pm0.51^{Fd}$ | $-23.69\pm0.34^{Ee}$ | $-21.49\pm0.33^{Dd}$ | $-20.76\pm0.28^{Ce}$ | $-19.06\pm0.22^{Ba}$ | $-18.07\pm0.69^{Aa}$ |
| 600 | 9 | $-36.29\pm0.14^{Gd}$ | $-26.01\pm0.08^{Fb}$ | $-24.99\pm0.03^{Ec}$ | $-22.39\pm0.40^{Dc}$ | $-20.90\pm0.20^{Cc}$ | $-20.46\pm0.18^{Bd}$ | $-18.81\pm0.13^{Aa}$ | $-18.68\pm0.16^{Aa}$ |
| | 10 | $-36.11\pm0.09^{Hc}$ | $-27.33\pm1.01^{Gd}$ | $-26.31\pm0.54^{Fd}$ | $-23.57\pm0.40^{Ede}$ | $-21.50\pm0.06^{Dd}$ | $-20.71\pm0.16^{Ce}$ | $-19.13\pm0.30^{Ba}$ | $-18.29\pm0.32^{Aa}$ |

- represents not analyzed due to deterioration
All values are presented as the mean ± SD.
Mean values followed by different superscripted letters in the same column (a–g) or row (A–H) are significantly different, according to Duncan's multiple range test ($p < 0.05$)



Overall, the results showed that the applied pressure have great influence on the stability of the 5% fish oil emulsion made with 0.04 % KGM at pH 9 and 10. However, the stability of the emulsion is likely due to the reduction in particle size due to HPP, but not due to the effects of electrostatic reactions. This was because there was no change in the zeta potential of the emulsion before and after HPP.

4.2.3 Viscosity

The results of the viscosity are presented in Table 22. The apparent viscosity of the samples at a constant shear rate of 31.10 $^{s-1}$ increased with the increase in the pressure applied.

Table 23 Effect of pressures on viscosity (mP.s) of 5% fish oil emulsion made with 0.04 % KGM at pH9 and 10

| pH | Before HPP | After HPP | | |
|---|---|---|---|---|
| | | 400 MPa | 500 MPa | 600 MPa |
| pH 9 | 19.50±0.11 | 48.30±0.28 | 49.80±0.14 | 57.60±0.42 |
| pH 10 | 21.40±0.22 | 51.40±0.00 | 59.30±0.42 | 60.20±0.28 |

All values are presented as the mean ± SD.

Application of HPP treatment was reported to enhance the functionality of polysaccharides and protein macromolecules and consequently, it improves the emulsification and stabilization of food grade-emulsion systems (Mohamad et al., 2018). The structural, morphological and physicochemical modifications of HPP treated polysaccharides and protein macromolecules, such as starch gelatinization, protein denaturation, and increase in interactions between food constituents, can significantly improve the viscosity and stability of food-grade emulsions (Gharibzahedi et al., 2019). The partial denaturation via structural unfolding leads to the exposure of a high number of hydrophobic groups and more absorption of protein moieties at the interface of oil and water and reduction of emulsion droplets size (Zhu, Lin, Ramaswamy, Yu, & Zhang, 2017).

4.2.4 Oxidative stability (Hydroperoxides values and thiobarbituric acid values) analysis

HPP is another technology used in the past decades to pasteurize or sterilize food matrices. HPP has the advantage of reducing food spoilage while preserving the organoleptic characteristics of products that would otherwise be destroyed in heat treatments. However, high pressure changes the thermodynamic equilibrium of chemical reactions. For example, lipid oxidation in which kinetics are accelerated at high hydrostatic pressures (Medina-Meza, Barnaba, & Barbosa-Cánovas, 2014).

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Table 24 Effect of pressures on hydroperoxides values (PV) of 5% fish oil emulsion made with 0.04 % KGM at pH9 and 10.

| Pressure (MPa) | pH | Hydroperoxides values (mM kg oil–1) | | |
|---|---|---|---|---|
| | | Day 0 | Day 14 | Difference (Day 14-Day0) |
| no KGM, 0 | | $0.34 \pm 0.13$ | - | - |
| 0 | pH9 | $0.57 \pm 0.10^{c}$ | - | - |
| 0 | pH10 | $0.55 \pm 0.06^{c}$ | - | - |
| 400 | pH9 | $0.58 \pm 0.03^{c}$ | $1.52 \pm 0.06^{c}$ | $0.94 \pm 0.04^{b}$ |
| | pH10 | $0.57 \pm 0.02^{c}$ | $1.31 \pm 0.06^{e}$ | $0.74 \pm 0.04^{d}$ |
| 500 | pH9 | $0.62 \pm 0.07^{b}$ | $1.60 \pm 0.12^{b}$ | $0.98 \pm 0.95^{a}$ |
| | pH10 | $0.62 \pm 0.02^{b}$ | $1.43 \pm 0.08^{d}$ | $0.81 \pm 0.50^{c}$ |
| 600 | pH9 | $0.65 \pm 0.02^{a}$ | $1.64 \pm 0.19^{a}$ | $0.99 \pm 0.11^{a}$ |
| | pH10 | $0.64 \pm 0.03^{a}$ | $1.44 \pm 0.08^{d}$ | $0.80 \pm 0.05^{c}$ |

- represents not analyzed due to deterioration
All values are presented as the mean ± SD.
Mean values followed by different superscripted letters in the same column (a–e) are significantly different, according to Duncan's multiple range test ($p < 0.05$).

Table 25 Effect of pressures on thiobarbituric acid values (TBARS) of 5% fish oil emulsion made with 0.04 % KGM at pH9 and 10.

| Pressure (MPa) | pH | thiobarbituric acid values (mM kg oil–1) | | |
|---|---|---|---|---|
| | | Day 0 | Day 14 | Difference (Day 14-Day0) |
| no KGM, 0 | | $0.44 \pm 0.13$ | - | - |
| 0 | pH9 | $0.53 \pm 0.22^{g}$ | - | - |
| | pH10 | $0.56 \pm 0.14^{g}$ | - | - |
| 400 | pH9 | $0.69 \pm 0.12^{f}$ | $1.66 \pm 0.26^{e}$ | $0.93 \pm 0.19^{e}$ |
| | pH10 | $0.78 \pm 0.11^{d}$ | $1.57 \pm 0.15^{f}$ | $0.79 \pm 0.13^{f}$ |
| 500 | pH9 | $0.71 \pm 0.34^{e}$ | $1.74 \pm 0.53^{d}$ | $1.03 \pm 0.43^{c}$ |
| | pH10 | $0.84 \pm 0.57^{c}$ | $1.83 \pm 0.62^{c}$ | $0.99 \pm 0.60^{d}$ |
| 600 | pH9 | $0.88 \pm 0.46^{b}$ | $2.20 \pm 0.46^{a}$ | $1.32 \pm 0.66^{a}$ |
| | pH10 | $0.90 \pm 0.75^{a}$ | $2.11 \pm 0.35^{b}$ | $1.21 \pm 0.55^{b}$ |

- represents not analyzed due to deterioration
All values are presented as the mean ± SD.
Mean values followed by different superscripted letters in the same column (a–e) are significantly different, according to Duncan's multiple range test ($p < 0.05$).

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Table 23 and 24 shows the PVs and TBARS of the emulsions treated at different pressures at day 0 and day 14. At KGM pH 9 and 10, the PVs and TBARS of all emulsions increased with increasing pressure. Similar results were reported by Zhu et al. (2014) which study on fish oil-in-water emulsions stabilized by 0.5 wt% whey protein isolate or sodium caseinate at 300, 400, 500, 600 and 700 MPa (Xiangqian et al., 2014).

HPP led to increases in the PV and TBAR values of 5% fish oil emulsion made with 0.04 % KGM at pH9 and 10, indicating that oxidation of the fish oil-in-water emulsions was promoted in the high-pressure conditions. Overall, HPP enhances the propagation step and slows down the termination step for the radicals. This resulted in increased in the PVs and TBA values in fish oil-in-water emulsions (Xiangqian et al., 2014). Previous studies demonstrate that HPP can accelerate both the formation and decomposition of hydroperoxide. (Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010). Hydroperoxides, which are considered primary products

of lipid oxidation, can further decompose into secondary oxidation products such as TBARS (Frankel, 2014). From this experiment, it was found that the change of TBAR values was higher than PV values change (0.79-1.32 and 0.74-0.99, respectively). As a result, it can say that, the rate of decomposition of hydroperoxides to form secondary oxidation products may be higher than the rate of formation of hydroperoxides, which was shown by the considerable increase in the TBA values, which indicate the amount of secondary oxidation products.

4.2.3 Microbiological analysis

HPP can decrease the total microbial count of yeasts, molds, and bacteria in store emulsions. This protective role was related to the pressure applied (Gharibzahedi et al., 2019). Pressure treatment induces many changes in the bacterial cell, such as key enzymes inhibition, protein synthesis inhibition, change in cell morphology and the cell membrane, which affecting on survival and reproduction (Considine, Kelly, Fitzgerald, Hill, & Sleator, 2008).

The effect of HPP (400, 500 and 600 for 3 min) on a population of microbes in emulsion sample was evaluated during refrigerated storage (14 days at 4 C). The obtained results are show in table 25, 26 and 27

Table 26 Total plate count, TPC (log CFU/ml) of 5% fish oil-in-water emulsions made with 0.04 % KGM at pH9, pH10 and control under refrigeration conditions

| condition | Storage period (Day) | | | |
|---|---|---|---|---|
| | 0 | 4 | 9 | 14 |
| no KGM, untreated with HPP | 3.8±0.3[a] | - | - | - |
| KGM pH9, untreated with HPP | 3.7±0.2[a] | - | - | - |
| KGM pH10, untreated with HPP | 3.7±0.3[a] | - | - | - |
| no KGM, treated with HPP at 400 MPa | 0.6±0.1[Dc] | 2.9±0.2[Ca] | 5.0±0.8[Ba] | 5.5±0.9[Aa] |
| KGM pH9, treated with HPP at 400 MPa | 0.9±0.2[Db] | 3.0±0.2[Ca] | 4.9±0.5[Bb] | 5.4±0.7[Aa] |
| KGM pH10, treated with HPP at 400 MPa | 0.7±0.3[Db] | 2.9±0.1[Ca] | 4.6±0.6[Bc] | 5.6±0.4[Aa] |
| no KGM, treated with HPP at 500 MPa | 0.2±0.0[Bd] | 1.8±0.2[Bb] | 3.0±0.2[Ad] | 3.1±0.3[Ab] |
| KGM pH9, treated with HPP at 500 MPa | 0.1±0.0[Be] | 1.6±0.2[Bb] | 3.0±0.3[Ad] | 3.1±0.4[Ab] |
| KGM pH10, treated with HPP at 500 MPa | 0.2±0.2[Dd] | 1.8±0.4[Cb] | 2.8±0.4[Bd] | 3.1±0.4[Ab] |
| no KGM, treated with HPP at 600 MPa | 0.1±0.0[Ce] | 0.3±0.1[Bc] | 1.8±0.2[Ac] | 1.9±0.2[Ac] |
| KGM pH9, treated with HPP at 600 MPa | 0.1±0.0[Ae] | 0.5±0.1[Ac] | 1.8±0.3[Ac] | 1.9±0.3[Ac] |
| KGM pH10, treated with HPP at 600 MPa | 0.1±0.0[Ce] | 0.5±0.2[Bc] | 1.8±0.2[Ac] | 2.0±0.1[Ac] |

- represents not analyzed due to deterioration

All values are presented as the mean ± SD.

Mean values followed by different superscripted letters in the same column (a–e) are significantly different, according to Duncan's multiple range test ($p < 0.05$).

Table 27 Coliform bacteria (log CFU/ml) of 5% fish oil-in-water emulsions made with 0.04 % KGM at pH9, pH10 and control under refrigeration conditions.

| condition | Storage period (Day) | | | |
|---|---|---|---|---|
| | 0 | 4 | 9 | 14 |
| no KGM, untreated with HPP | 2.5±0.2$^{a}$ | - | - | - |
| KGM pH9, untreated with HPP | 2.3±0.4$^{a}$ | - | - | - |
| KGM pH10, untreated with HPP | 2.2±0.2$^{a}$ | - | - | - |
| no KGM, treated with HPP at 400 MPa | 0.1±0.0$^{Cb}$ | 0.1±0.0$^{Ca}$ | 1.9±0.4$^{Ba}$ | 2.5±0.5$^{Aa}$ |
| KGM pH9, treated with HPP at 400 MPa | 0.1±0.0$^{Cb}$ | 0.1±0.0$^{Ca}$ | 1.9±0.3$^{Ba}$ | 2.4±0.3$^{Aa}$ |
| KGM pH10, treated with HPP at 400 MPa | 0.1±0.0$^{Cb}$ | 0.1±0.0$^{Ca}$ | 1.7±0.4$^{Bb}$ | 2.3±0.3$^{Aa}$ |
| no KGM, treated with HPP at 500 MPa | 0.1±0.0$^{Bb}$ | 0.1±0.0$^{Ba}$ | 0.1±0.0$^{Bc}$ | 2.3±0.4$^{Ab}$ |
| KGM pH9, treated with HPP at 500 MPa | 0.1±0.0$^{Bb}$ | 0.1±0.0$^{Ba}$ | 0.1±0.0$^{Bc}$ | 2.3±0.4$^{Ab}$ |
| KGM pH10, treated with HPP at 500 MPa | 0.1±0.0$^{Bb}$ | 0.1±0.0$^{Ba}$ | 0.1±0.0$^{Bc}$ | 2.1±0.3$^{Ab}$ |
| no KGM, treated with HPP at 600 MPa | 0.1±0.0$^{Ab}$ | 0.1±0.0$^{Aa}$ | 0.1±0.0$^{Ac}$ | 0.1±0.0$^{Ac}$ |
| KGM pH9, treated with HPP at 600 MPa | 0.1±0.0$^{Ab}$ | 0.1±0.0$^{Aa}$ | 0.1±0.0$^{Ac}$ | 0.1±0.0$^{Ac}$ |
| KGM pH10, treated with HPP at 600 MPa | 0.1±0.0$^{Ab}$ | 0.1±0.0$^{Aa}$ | 0.1±0.0$^{Ac}$ | 0.1±0.0$^{Ac}$ |

- represents not analyzed due to deterioration

All values are presented as the mean ± SD.

Mean values followed by different superscripted letters in the same column (a–e) are significantly different, according to Duncan's multiple range test ($p < 0.05$).

Table 28 *S. aureus* (log CFU/ml) of 5% fish oil-in-water emulsions made with 0.04 % KGM at pH9, pH10 and control under refrigeration conditions.

| condition | Storage period (Day) | | | |
|---|---|---|---|---|
| | 0 | 4 | 9 | 14 |
| no KGM, untreated with HPP | 3.6±0.3$^{a}$ | - | - | - |
| KGM pH9, untreated with HPP | 3.4±0.2$^{a}$ | - | - | - |
| KGM pH10, untreated with HPP | 3.4±0.3$^{a}$ | - | - | - |
| no KGM, treated with HPP at 400 MPa | 1.3±0.3$^{Db}$ | 1.9±0.4$^{Ca}$ | 2.8±0.7$^{Ba}$ | 3.0±0.3$^{Aa}$ |
| KGM pH9, treated with HPP at 400 MPa | 1.1±0.5$^{Dc}$ | 2.0±0.4$^{Ca}$ | 2.8±0.3$^{Ba}$ | 3.0±0.3$^{Aa}$ |
| KGM pH10, treated with HPP at 400 MPa | 1.2±0.2$^{Dbc}$ | 1.9±0.3$^{Ca}$ | 2.8±0.2$^{Ba}$ | 3.0±0.3$^{Aa}$ |
| no KGM, treated with HPP at 500 MPa | 0.1±0.0$^{Dd}$ | 1.1±0.2$^{Cbc}$ | 1.6±0.6$^{Bc}$ | 1.9±0.2$^{Ab}$ |
| KGM pH9, treated with HPP at 500 MPa | 0.1±0.0$^{Dd}$ | 1.0±0.6$^{Cc}$ | 1.7±0.4$^{Bc}$ | 1.9±0.3$^{Ab}$ |
| KGM pH10, treated with HPP at 500 MPa | 0.1±0.0$^{Dd}$ | 1.2±0.4$^{Cb}$ | 1.9±0.3$^{Bb}$ | 2.0±0.2$^{Ab}$ |
| no KGM, treated with HPP at 600 MPa | ND | ND | ND | ND |
| KGM pH9, treated with HPP at 600 MPa | ND | ND | ND | ND |
| KGM pH9, treated with HPP at 600 MPa | ND | ND | ND | ND |

- represents not analyzed due to deterioration

All values are presented as the mean ± SD.

Mean values followed by different superscripted letters in the same column (a–e) are significantly different, according to Duncan's multiple range test ($p < 0.05$).

ND (not detected)

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As shown in table 25, 26 and 27 a significant decrease was observed in the population of TPC, coliform and *S. aureus* during HPP treated at 400, 500 and 600 MPa at KGM pH 9 and 10. The reduction of microorganisms occurred maximally at a pressure of 600 MPa (approximately 3 log for TPC and *S. aureus*, approximately 2 log for coliform bacteria. Furthermore, the pH of KGM at 9 and 10 had no effect on the change in the population of TPC, coliform and *S. aureus*. However, it was found that the amount of microorganisms increased throughout the shelf life, but found that pressure more than 500 MPa the microorganisms still did not exceed the acceptance (≤4 log for TPC, ≤2 log for coliform and *S. aureus*) ("Notification of the ministry of public health (No.352) ", 2013; "Notification of the ministry of public health (No.416)," 2020).

HPP can help increase the stability of the emulsion. It was found that HPP resulted in a decrease in particle size with increasing pressure. The better stability to resist emulsion separation is due to the range of the attractive forces acting between

droplets decreases as the particle size decreases whereas the range of steric repulsion is less dependent on particle size. However, HPP does not affect zeta potential values.

HPP stimulates lipid oxidation by enhances the propagation step and slows down the termination step for the radicals. For the analysis of microorganisms, it was found that, HPP was more effective in reducing microorganisms due to induces many changes in the microbial cell, such as alterations in cell morphology and the cell membrane responsible for survival and reproduction.

# CHAPTER 5
# CONCLUSION

Konjac glucomannan (KGM) could be an interesting material for stabilized fish oil – milk emulsion as well as a stabilizing agent.

KGM solution from different concentration (0.02%-0.5%) and different pH (3-10) were mixed with 5% fish oil and skim milk, skim milk without casein and whole milk sample. The mixtures were homogenized using high pressure homogenizer at 40 MPa. After that stability of emulsion including particle size, polydispersity index (PDI), zeta-potential, viscosity, precipitation percentage and microscopic structure were determined. The results showed that KGM at a concentration of 0.04% pH 9 and 10 provided the most stable milk-fat emulsion without separation for 10 days. It suggests that, deacetylation of KGM was occur at alkaline condition, resulting in KGM's acetyl group and hydrogen bonds are weakened, making the hydrophobic interaction stronger. Thus, KGM are able to fill the gap between casein molecules and oil droplets in the emulsion, it may help to separate the oil droplets, which reducing the Brownian-motion of droplets. These results present the benefit of KGM as stabilizing agent, and 0.04% of KGM at pH 9 and 10 were selected for study the effect of pressure on stability of fish oil- milk emulsion via high pressure processing machine (HPP).

0.04% KGM solution at pH 9-10 were mixed and homogenized follow the previous method. After that, mixtures were subjected to HPP at 400, 500 and 600 MPa. Next, stability of emulsion including particle size, PDI, zeta-potential, oxidative stability and microbial population count were determined. The results in this section were found that, increased pressure resulting in decreased particle size and PDI. However, pressure had no effect on zeta potential values. HPP improved emulsion stability compared to previous studies from 10 days to 14 days.

As for the oxidative stability, which was reported as hydroperoxides values (PV) and thiobarbituric acid values (TBARS), it was found that HPP led to increases in the PV and TBAR values of 5% fish oil emulsion made with 0.04 % KGM at pH9 and 10, indicating that oxidation of the emulsions was promoted in the high-pressure conditions. As well as the amount of microorganisms, which the results showed that the pressure reduced the microbial load. The reduction of microorganisms occurred maximally at a pressure of 600 MPa (approximately 3 log for Total plate count and *S. aureus*, approximately 2 log for coliform bacteria).

In conclusion, KGM has potential to stabilize fish oil-milk emulsion. The use of HPP helps to stabilize the emulsion, however, KGM does not reduce lipid oxidation. The last, it was found that appropriate pressure for microbial shelf assessment was 500 MPa with the microorganisms still did not exceed the acceptance (≤4 log for TPC, ≤2 log for coliform and *S. aureus*). This study provides information that may facilitate the design of emulsion-based delivery systems for polyunsaturated

lipids. Further studies on the effects of other components in milk, such as calcium will provide further information on the stability of the emulsion.

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# REFERENCES


Abhyankar, A. R., Mulvihill, D. M., Chaurin, V., & Auty, M. A. E. (2011). Techniques for localisation of konjac glucomannan in model milk protein–polysaccharide mixed systems: Physicochemical and microscopic investigations. *Food Chemistry, 129*(4), 1362-1368. doi:https://doi.org/10.1016/j.foodchem.2011.05.052

Abraham, F. F. (2012). *Homogeneous Nucleation Theory: The Pretransition Theory of Vapor Condensation*: Academic Press.

Ackman, R. G., & Lamothe, F. (1989). Marine mammals. *Marine biogenic lipids, fats and oils, 2*, 179-381.

Adkins, Y., & Kelley, D. S. (2010). Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. *J Nutr Biochem, 21*(9), 781-792. doi:10.1016/j.jnutbio.2009.12.004

Akbari, S. (2018). Emulsion types, stability mechanisms and rheology: A review.

Albert, B. B., Cameron-Smith, D., Hofman, P. L., & Cutfield, W. S. (2013). Oxidation of marine omega-3 supplements and human health. *BioMed research international, 2013*, 464921-464921. doi:10.1155/2013/464921

Amaral, A. B., Silva, M. V. d., & Lannes, S. C. d. S. (2018). Lipid oxidation in meat: mechanisms and protective factors   a review. *Food Science and Technology, 38*, 1-15. Retrieved from http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0101-20612018000500001&nrm=iso

Amr, B., Shabbar, A., Barkat, A., Hamid, M., Mohamed, A., Ahmed, M., & Li, L. (2015). Microencapsulation of Oils: A Comprehensive Review of Benefits, Techniques, and Applications. *Comprehensive Reviews in Food Science and Food Safety, 15*, 143-182. doi:10.1111/1541-4337.12179

Anal, A. K., Shrestha, S., & Sadiq, M. B. (2019). Biopolymeric-based emulsions and their effects during processing, digestibility and bioaccessibility of bioactive compounds in food systems. *Food Hydrocolloids, 87*, 691-702. doi:10.1016/j.foodhyd.2018.09.008

Arab-Tehrany, E., Jacquot, M., Gaiani, C., Imran, M., Desobry, S., & Linder, M. (2012). Beneficial effects and oxidative stability of omega-3 long-chain polyunsaturated fatty acids. *Trends in Food Science & Technology, 25*(1), 24-33. doi:https://doi.org/10.1016/j.tifs.2011.12.002

Augustin, M. A., Sanguansri, L., & Bode, O. (2006). Maillard reaction products as encapsulants for fish oil powders. *Journal of food science, 71*(2), E25-E32.

Bakry, A. M., Abbas, S., Ali, B., Majeed, H., Abouelwafa, M. Y., Mousa, A., & Liang, L. (2016). Microencapsulation of Oils: A Comprehensive Review of Benefits, Techniques, and Applications. *Comprehensive Reviews in Food Science and Food Safety, 15*(1), 143-182. doi:https://doi.org/10.1111/1541-4337.12179

Ballabio, C., Chessa, S., Rignanese, D., Gigliotti, C., Pagnacco, G., Terracciano, L., . . . Caroli, A. M. (2011). Goat milk allergenicity as a function of αS1-casein genetic polymorphism. *Journal of Dairy Science, 94*(2), 998-1004. doi:https://doi.org/10.3168/jds.2010-3545

Balny, C., & Masson, P. (1993). Effects of high pressure on proteins. *Food Reviews International, 9*(4), 611-628.

Barba, F. J., Terefe, N. S., Buckow, R., Knorr, D., & Orlien, V. (2015). New opportunities and perspectives of high pressure treatment to improve health and safety attributes of foods. A review. *Food Research International, 77*, 725-742. doi:10.1016/j.foodres.2015.05.015

Barrow, C. J., Nolan, C., & Holub, B. J. (2009). Bioequivalence of encapsulated and microencapsulated fish-oil supplementation. *Journal of Functional Foods, 1*(1), 38-43. doi:10.1016/j.jff.2008.09.006

Bates, F. S. (1991). Polymer-Polymer Phase Behavior. *Science, 251*(4996), 898. doi:10.1126/science.251.4996.898

Belitz, H.-D., Grosch, W., & Schieberle, P. (2009). Milk and dairy products. *Food Chemistry*, 498-545.

Bermúdez-Aguirre, D., & Barbosa-Cánovas Gustavo V, G. V. (2011). Quality of selected cheeses fortified with vegetable and animal sources of omega-3. *LWT - Food Science and Technology, 44*(7), 1577-1584. doi:10.1016/j.lwt.2011.01.023

Bhattacharjee, S., Elimelech, M., & Borkovec, M. (1998). DLVO interaction between colloidal particles: Beyond Derjaguin's approximation. *Croatica Chemica Acta, 71*(4), 883-903.

Bimbo, A. P. (2013). 2 - Sources of omega-3 fatty acids. In C. Jacobsen, N. S. Nielsen, A. F. Horn, & A.-D. M. Sørensen (Eds.), *Food Enrichment with Omega-3 Fatty Acids* (pp. 27-107): Woodhead Publishing.

Bos, M. A., & Van Vliet, T. (2001). Interfacial rheological properties of adsorbed protein layers and surfactants: A review. *Advances in Colloid and Interface Science, 91*(3), 437-471. doi:10.1016/S0001-8686(00)00077-4

Bouzid, H., Rabiller-Baudry, M., Paugam, L., Rousseau, F., Derriche, Z., & Bettahar, N. E. (2008). Impact of zeta potential and size of caseins as precursors of fouling deposit on limiting and critical fluxes in spiral ultrafiltration of modified skim milks. *Journal of Membrane Science, 314*(1), 67-75. doi:https://doi.org/10.1016/j.memsci.2008.01.028

Cai, Y., Deng, X., Liu, T., Zhao, M., Zhao, Q., & Chen, S. (2018). Effect of xanthan gum on walnut protein/xanthan gum mixtures, interfacial adsorption, and emulsion properties. *Food Hydrocolloids, 79*, 391-398. doi:10.1016/j.foodhyd.2018.01.006

Calvo, P., Castaño, Á. L., Hernández, M. T., & González-Gómez, D. (2011). Effects of microcapsule constitution on the quality of microencapsulated walnut oil. *European Journal of Lipid Science and Technology, 113*(10), 1273-1280. doi:https://doi.org/10.1002/ejlt.201100039

Ceballos, L. S., Morales, E. R., de la Torre Adarve, G., Castro, J. D., Martínez, L. P., & Sampelayo, M. R. S. (2009). Composition of goat and cow milk produced under similar conditions and analyzed by identical methodology. *Journal of Food Composition and Analysis, 22*(4), 322-329. doi:https://doi.org/10.1016/j.jfca.2008.10.020

Chawla, R., Patil, G. R., & Singh, A. K. (2011). High hydrostatic pressure technology in dairy processing: a review. *Journal of food science and technology, 48*(3), 260-268. doi:10.1007/s13197-010-0180-4

Chivero, P., Gohtani, S., Yoshii, H., & Nakamura, A. (2015). Effect of xanthan and guar gums on the formation and stability of soy soluble polysaccharide oil-in-water emulsions. *Food Research International, 70*, 7-14.

Chulalongkorn University

doi:https://doi.org/10.1016/j.foodres.2015.01.025

Cho, H. T., Salvia-Trujillo, L., Kim, J., Park, Y., Xiao, H., & McClements, D. J. (2014). Droplet size and composition of nutraceutical nanoemulsions influences bioavailability of long chain fatty acids and Coenzyme Q10. *Food Chemistry, 156*, 117-122. doi:10.1016/j.foodchem.2014.01.084

Choe, E., & Min, D. B. (2006). Mechanisms and Factors for Edible Oil Oxidation. *Comprehensive Reviews in Food Science and Food Safety, 5*(4), 169-186. doi:https://doi.org/10.1111/j.1541-4337.2006.00009.x

Chua, M., Baldwin, T. C., Hocking, T. J., & Chan, K. (2010). Traditional uses and potential health benefits of Amorphophallus konjac K. Koch ex N.E.Br. *Journal of Ethnopharmacology, 128*(2), 268-278. doi:https://doi.org/10.1016/j.jep.2010.01.021

Considine, K. M., Kelly, A. L., Fitzgerald, G. F., Hill, C., & Sleator, R. D. (2008). High-pressure processing – effects on microbial food safety and food quality. *FEMS Microbiology Letters, 281*(1), 1-9. doi:10.1111/j.1574-6968.2008.01084.x

Dacaranhe, C. D., & Terao, J. (2001). Effect of Phosphatidic Acid and Phosphatidylserine on Lipid Oxidation in Beef Homogenate During Storage and in Emulsified Sardine Oil. *Journal of food science, 66*(3), 422-427. doi:https://doi.org/10.1111/j.1365-2621.2001.tb16121.x

Dai, S., Jiang, F., Shah, N. P., & Corke, H. (2017). Stability and phase behavior of konjac glucomannan-milk systems. *Food Hydrocolloids, 73*, 30-40. doi:https://doi.org/10.1016/j.foodhyd.2017.06.025

Dalgleish, D. G. (1996). Conformations and structures of milk proteins adsorbed to oil-water interfaces. *Food Research International, 29*(5-6), 541-547.

Delahaije, R. J. B. M., Hilgers, R. J., Wierenga, P. A., & Gruppen, H. (2017). Relative contributions of charge and surface coverage on pH-induced flocculation of protein-stabilized emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 521*, 153-160. doi:10.1016/j.colsurfa.2016.10.043

Delgado, J., Franco, M., Sánchez Casas, J., Miguel, C., Ramírez, R., & Martín-Vertedor, D. (2013). Comparative effect of high pressure processing and traditional thermal treatment on the physicochemical, microbiology, and sensory analysis of olive jam. *Grasas y Aceites, 64*, 432-441. doi:10.3989/gya.023613

Desai, K. G. H., & Jin Park, H. (2005). Recent Developments in Microencapsulation of Food Ingredients. *Drying Technology, 23*(7), 1361-1394. doi:10.1081/DRT-200063478

Desrumaux, A., & Marcand, J. (2002). Formation of sunflower oil emulsions stabilized by whey proteins with high-pressure homogenization (up to 350 MPa): Effect of pressure on emulsion characteristics. *International Journal of Food Science and Technology, 37*(3), 263-269. doi:10.1046/j.1365-2621.2002.00565.x

Dickinson, E. (1998). Proteins at interfaces and in emulsions stability, rheology and interactions. *Journal of the Chemical Society, Faraday Transactions, 94*(12), 1657-1669.

Dickinson, E. (2008). Interfacial structure and stability of food emulsions as affected by protein–polysaccharide interactions. *Soft Matter, 4*, 932-942. doi: 10.1039/B718319D

Dobarganes, C., & Márquez-Ruiz, G. (2003). Oxidized fats in foods. *Current Opinion in Clinical Nutrition & Metabolic Care, 6*(2), 157-163.

Doyle, J. P., Lyons, G., & Morris, E. R. (2009). New proposals on "hyperentanglement" of galactomannans: Solution viscosity of fenugreek gum under neutral and alkaline conditions. *Food Hydrocolloids, 23*(6), 1501-1510.
Du, X., Li, J., Chen, J., & Li, B. (2012). Effect of degree of deacetylation on physicochemical and gelation properties of konjac glucomannan. *Food Research International, 46*(1), 270-278. doi:https://doi.org/10.1016/j.foodres.2011.12.015
Dziezak, J. D. (1988). Microencapsulation and encapsulated ingredients. *Food technology (Chicago), 42*(4), 136-153.
Earnshaw, R. (1996). High pressure food processing. *Nutrition & Food Science*.
Fan, Z. (2018). Modifications of konjac glucomannan for diverse applications. *Food Chemistry, 256*, 419-426. doi:10.1016/j.foodchem.2018.02.151
Fang, Z., & Bhandari, B. (2010). Encapsulation of polyphenols – a review. *Trends in Food Science & Technology, 21*(10), 510-523. doi:https://doi.org/10.1016/j.tifs.2010.08.003
Fank, W., & Wu, P. (2004). Variations of Konjac glucomannan (KGM) from Amorphophallus konjac and its refined powder in China. *Food Hydrocolloids, 18*(1), 167-170. doi:https://doi.org/10.1016/S0268-005X(03)00044-4
Farkas, D. F., & Hoover, D. G. (2000). High pressure processing. *Journal of food science, 65*, 47-64.
Fellows, C. M., & Doherty, W. O. (2005). *Insights into bridging flocculation.* Paper presented at the Macromolecular symposia.
Frankel, E. N. (2014). *Lipid oxidation*: Elsevier.
Friberg, S., Larsson, K., & Sjoblom, J. (2003). *Food emulsions*: CRC Press.
Fuentes, V., Ventanas, J., Morcuende, D., Estévez, M., & Ventanas, S. (2010). Lipid and protein oxidation and sensory properties of vacuum-packaged dry-cured ham subjected to high hydrostatic pressure. *Meat science, 85*(3), 506-514.
Gaygadzhiev, Z., Hill, A., & Corredig, M. (2009). Influence of the emulsion droplet type on the rheological characteristics and microstructure of rennet gels from reconstituted milk. *Journal of Dairy Research, 76*(3), 349-355. doi:10.1017/S002202990900418X
Gharibzahedi, S. M. T., Hernández-Ortega, C., Welti-Chanes, J., Putnik, P., Barba, F. J., Mallikarjunan, K., . . . Roohinejad, S. (2019). High pressure processing of food-grade emulsion systems: Antimicrobial activity, and effect on the physicochemical properties. *Food Hydrocolloids, 87*, 307-320. doi:https://doi.org/10.1016/j.foodhyd.2018.08.012
Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International, 40*(9), 1107-1121. doi:https://doi.org/10.1016/j.foodres.2007.07.004
Gibbs, B. F., Kermasha, S., Alli, I., & Mulligan, C. N. (1999). Encapsulation in the food industry: A review. *International Journal of Food Sciences and Nutrition, 50*(3), 213-224. doi:10.1080/096374899101256
Goldstein, A., & Seetharaman, K. (2011). Effect of a novel monoglyceride stabilized oil in water emulsion shortening on cookie properties. *Food Research International, 44*(5), 1476-1481. doi:10.1016/j.foodres.2011.03.029
Hans Tromp, R., van de Velde, F., van Riel, J., & Paques, M. (2001). Confocal scanning light microscopy (CSLM) on mixtures of gelatine and polysaccharides. *Food*

*Research International, 34*(10), 931-938. doi:https://doi.org/10.1016/S0963-9969(01)00117-X

Haug, A., Høstmark, A. T., & Harstad, O. M. (2007). Bovine milk in human nutrition–a review. *Lipids in health and disease, 6*(1), 1-16.

Hege, J., Palberg, T., & Vilgis, T. A. (2020). Interactions of different hydrocolloids with milk proteins. *Journal of Physics: Materials, 3*(4), 044003. doi:10.1088/2515-7639/aba2b7

Holzmüller, W., Gmach, O., Griebel, A., & Kulozik, U. (2016). Casein precipitation by acid and rennet coagulation of buttermilk: Impact of pH and temperature on the isolation of milk fat globule membrane proteins. *International Dairy Journal, 63*, 115-123. doi:https://doi.org/10.1016/j.idairyj.2016.08.006

Hu, Y.-T., Ting, Y., Hu, J.-Y., & Hsieh, S.-C. (2017). Techniques and methods to study functional characteristics of emulsion systems. *Journal of Food and Drug Analysis, 25*(1), 16-26. doi:https://doi.org/10.1016/j.jfda.2016.10.021

Huang, C. Y., Zhang, M. Y., Peng, S. S., Hong, J. R., Wang, X., Jiang, H. J., . . . Yu, Y. R. (1990). Effect of Konjac food on blood glucose level in patients with diabetes. *Biomedical and environmental sciences : BES, 3*(2), 123-131. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-0025447594&partnerID=40&md5=342beaf9334098f2cb506cfef5c41faf

Jacobsen, C. (2010). Challenges when developing omega-3 enriched foods. *Oléagineux, Corps gras, Lipides, 17*, 251-258. doi:10.1051/ocl.2010.0327

Jacobsen, C., Hartvigsen, K., Lund, P., Thomsen, M. K., Skibsted, L. H., Hølmer, G., . . . Meyer, A. S. (2001). Oxidation in fish oil-enriched mayonnaise: 4. Effect of tocopherol concentration on oxidative deterioration. *European Food Research and Technology, 212*(3), 308-318.

Jafari, S. M., Assadpoor, E., Bhandari, B., & He, Y. (2008). Nano-particle encapsulation of fish oil by spray drying. *Food Research International, 41*(2), 172-183. doi:https://doi.org/10.1016/j.foodres.2007.11.002

Janssen, P. H., Noik, C., & Dalmazzone, C. (2001). *Emulsion Formation in a Model Choke-valve.* Paper presented at the SPE Annual Technical Conference and Exhibition.

Jian, W., Siu, K.-C., & Wu, J.-Y. (2015). Effects of pH and temperature on colloidal properties and molecular characteristics of Konjac glucomannan. *Carbohydrate Polymers, 134*, 285-292. doi:https://doi.org/10.1016/j.carbpol.2015.07.050

Kabalnov, A. S., & Shchukin, E. D. (1992). Ostwald ripening theory: applications to fluorocarbon emulsion stability. *Advances in Colloid and Interface Science, 38*(C), 69-97. doi:10.1016/0001-8686(92)80043-W

Kashiwagi, S., & Huang, P. (2012). Dietary Supplements and Cardiovascular Disease: What is the Evidence and What Should We Recommend? In.

Katsuraya, K., Okuyama, K., Hatanaka, K., Oshima, R., Sato, T., & Matsuzaki, K. (2003). Constitution of konjac glucomannan: Chemical analysis and 13C NMR spectroscopy. *Carbohydrate Polymers, 53*(2), 183-189. doi:10.1016/S0144-8617(03)00039-0

Kolanowski, W., Jaworska, D., & Weißbrodt, J. (2007). Importance of instrumental and sensory analysis in the assessment of oxidative deterioration of omega-3 long-chain polyunsaturated fatty acid-rich foods. *Journal of the Science of Food and Agriculture, 87*(2), 181-191. doi:https://doi.org/10.1002/jsfa.2733

Chulalongkorn University

Kolanowski, W., Jaworska, D., Weißbrodt, J., & Kunz, B. (2007). Sensory Assessment of Microencapsulated Fish Oil Powder. *Journal of the American Oil Chemists' Society, 84*(1), 37-45. doi:https://doi.org/10.1007/s11746-006-1000-x

Kris-Etherton, P. M., Harris, W. S., & Appel, L. J. (2002). Fish Consumption, Fish Oil, Omega-3 Fatty Acids, and Cardiovascular Disease. *Circulation, 106*(21), 2747-2757. doi:doi:10.1161/01.CIR.0000038493.65177.94

Lagarde, M. (2010). Oxygenated metabolites of polyunsaturated fatty acids: Formation and function in blood and vascular cells. *European Journal of Lipid Science and Technology, 112*(9), 941-947. doi:https://doi.org/10.1002/ejlt.201000045

Larrieu, S., Letenneur, L., Helmer, C., Dartigues, J. F., & Barberger-Gateau, P. (2004). Nutritional factors and risk of incident dementia in the PAQUID longitudinal cohort. *Journal of Nutrition, Health and Aging, 8*(3), 150-154. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-2442641693&partnerID=40&md5=f94783b5e657aa2fcb2e8e68428974d4

Ledward, D. A. (1995). *High pressure processing of foods*: Nottingham University Press.

Lembke, P., & Schubert, A. (2014). Chapter 37 - Introduction to Fish Oil Oxidation, Oxidation Prevention, and Oxidation Correction. In R. R. Watson & F. De Meester (Eds.), *Omega-3 Fatty Acids in Brain and Neurological Health* (pp. 455-460). Boston: Academic Press.

Li, S., & Shah, N. P. (2015). Effects of Pleurotus eryngii polysaccharides on bacterial growth, texture properties, proteolytic capacity, and angiotensin-I-converting enzyme–inhibitory activities of fermented milk. *Journal of Dairy Science, 98*(5), 2949-2961. doi:https://doi.org/10.3168/jds.2014-9116

Liu, G., Carøe, C., Qin, Z., Munk, D. M. E., Crafack, M., Petersen, M. A., & Ahrné, L. (2020). Comparative study on quality of whole milk processed by high hydrostatic pressure or thermal pasteurization treatment. *LWT, 127*, 109370. doi:https://doi.org/10.1016/j.lwt.2020.109370

Lorente-Cebrián, S., Costa, A. G., Navas-Carretero, S., Zabala, M., Martínez, J. A., & Moreno-Aliaga, M. J. (2013). Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: a review of the evidence. *J Physiol Biochem, 69*(3), 633-651. doi:10.1007/s13105-013-0265-4

Lu, W., Zheng, B., & Miao, S. (2018). Improved emulsion stability and modified nutrient release by structuring O/W emulsions using konjac glucomannan. *Food Hydrocolloids, 81*, 120-128. doi:https://doi.org/10.1016/j.foodhyd.2018.02.034

Luo, X., He, P., & Lin, X. (2013). The mechanism of sodium hydroxide solution promoting the gelation of Konjac glucomannan (KGM). *Food Hydrocolloids, 30*(1), 92-99. doi:https://doi.org/10.1016/j.foodhyd.2012.05.012

M. Danaei, M. D., S. Ataei, F. Hasanzadeh Davarani, R. Javanmard, A. Dokhani,, & Mozafari, S. K. a. M. R. (2018). Impact of Particle Size and Polydispersity Index on

the Clinical Applications of Lipidic

Nanocarrier Systems. *Pharmaceutics 10*, 17.

Marry Ann Augustin, Abeywardena, M. Y., Patten, G., Head, R., Lockett, T., De Luca, A., & Sanguansri, L. (2011). Effects of microencapsulation on the gastrointestinal transit and tissue distribution of a bioactive mixture of fish oil,

tributyrin and resveratrol. *Journal of Functional Foods, 3*(1), 25-37. doi:https://doi.org/10.1016/j.jff.2011.01.003

McClements, D. J. (2004). *Food emulsions: principles, practices, and techniques*: CRC press.

McClements, D. J. (2011). Edible nanoemulsions: Fabrication, properties, and functional performance. *Soft Matter, 7*(6), 2297-2316. doi:10.1039/c0sm00549e

McClements, D. J., Bai, L., & Chung, C. (2017). Recent Advances in the Utilization of Natural Emulsifiers to Form and Stabilize Emulsions. *Annual Review of Food Science and Technology, 8*(1), 205-236. doi:10.1146/annurev-food-030216-030154

McClements, D. J., Decker, E. A., & Weiss, J. (2007). Emulsion-based delivery systems for lipophilic bioactive components. *Journal of food science, 72*(8), R109-R124. doi:10.1111/j.1750-3841.2007.00507.x

Medina-Meza, I. G., Barnaba, C., & Barbosa-Cánovas, G. V. (2014). Effects of high pressure processing on lipid oxidation: A review. *Innovative Food Science & Emerging Technologies, 22*, 1-10. doi:https://doi.org/10.1016/j.ifset.2013.10.012

Mertens, B., & Gould, G. (1995). New methods of food preservation. *New York: Blackie Academic and Professional*, 135.

Miyagawa, Y., & Adachi, S. (2017). Dispersion and oxidative stability of O/W emulsions and oxidation of microencapsulated oil. *Bioscience, Biotechnology, and Biochemistry, 81*(4), 625-633. doi:10.1080/09168451.2016.1270742

Mohamad, Z., Nur Syahida, N. N., Othman, N., Baharulrazi, N., Man, S. H. C., Jusoh, M., & Majid, R. A. (2018). Properties enhancement of polylactic acid biocomposite. *Chemical Engineering Transactions, 63*, 361-366. doi:10.3303/CET1863061

Moreno-Villares, J. M., & Germán-Díaz, M. (2019). Chapter 29 - Human Milk as a Bioactive Food. In R. R. Watson & V. R. Preedy (Eds.), *Bioactive Food as Dietary Interventions for Diabetes (Second Edition)* (pp. 425-445): Academic Press.

Mozafari, M. R., Khosravi-Darani, K., Borazan, G. G., Cui, J., Pardakhty, A., & Yurdugul, S. (2008). Encapsulation of food ingredients using nanoliposome technology. *International Journal of Food Properties, 11*(4), 833-844. doi:10.1080/10942910701648115

Mudalige, T., Qu, H., Van Haute, D., Ansar, S. M., Paredes, A., & Ingle, T. (2019). Chapter 11 - Characterization of Nanomaterials: Tools and Challenges. In A. López Rubio, M. J. Fabra Rovira, M. martínez Sanz, & L. G. Gómez-Mascaraque (Eds.), *Nanomaterials for Food Applications* (pp. 313-353): Elsevier.

Mújica Paz, H., Valdez-Fragoso, A., Samson, C., Welti-Chanes, J., & Torres, J. A. (2011). High-Pressure Processing Technologies for the Pasteurization and Sterilization of Foods. *Food and Bioprocess Technology, 4*, 969-985. doi:10.1007/s11947-011-0543-5

O'Kennedy, B. T. (2011). 2 - Caseins. In G. O. Phillips & P. A. Williams (Eds.), *Handbook of Food Proteins* (pp. 13-29): Woodhead Publishing.

Oh, Y. A., Oh, Y. J., Song, A. Y., Won, J. S., Song, K. B., & Min, S. C. (2017). Comparison of effectiveness of edible coatings using emulsions containing lemongrass oil of different size droplets on grape berry safety and preservation.

*LWT, 75*, 742-750. doi:10.1016/j.lwt.2016.10.033

Ohlsson, T., & Bengtsson, N. (2002). *Minimal processing technologies in the food industries*: Elsevier.

Olsson, S. (1995). High pressure processing of foods. In: Nottingham University Press: Nottingham, UK.

Otsuka, M., & Ueki, A. (2001). The study of eicosapentaenoic aci EPA efficacy for dementia patients. *Dementia Jpn, 15*, 21-22.

Palacios, L. E., & Wang, T. (2005). Egg-yolk lipid fractionation and lecithin characterization. *JAOCS, Journal of the American Oil Chemists' Society, 82*(8), 571-578. doi:10.1007/s11746-005-1111-4

Papanikolaou, Y., Brooks, J., Reider, C., & Fulgoni, V. L., 3rd. (2014). U.S. adults are not meeting recommended levels for fish and omega-3 fatty acid intake: results of an analysis using observational data from NHANES 2003-2008. *Nutr J, 13*, 31. doi:10.1186/1475-2891-13-31

Patrick KE, Abbas S, Lv Y, Ntsama ISB, & X, Z. (2013). Microencapsulation by complex coacervation of fish oil using gelatin/SDS/NaCMC. *The Pakistan Journal of Food Sciences, 23*, 17-25.

Pestana, J., Gennari, A., Monteiro, B., Lehn, D., & Souza, C. (2015). Effects of Pasteurization and Ultra-High Temperature Processes on Proximate Composition and Fatty Acid Profile in Bovine Milk. *American Journal of Food Technology, 10*, 265-272. doi:10.3923/ajft.2015.265.272

Qian, C., & McClements, D. J. (2011). Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. *Food Hydrocolloids, 25*(5), 1000-1008. doi:https://doi.org/10.1016/j.foodhyd.2010.09.017

Qin, Z., Guo, X., Lin, Y., Chen, J., Liao, X., Hu, X., & Wu, J. (2013). Effects of high hydrostatic pressure on physicochemical and functional properties of walnut (juglans regia l.) protein isolate. *Journal of the Science of Food and Agriculture, 93*(5), 1105-1111. doi:10.1002/jsfa.5857

Rasti, B., Erfanian, A., & Selamat, J. (2017). Novel nanoliposomal encapsulated omega-3 fatty acids and their applications in food. *Food Chemistry, 230*, 690-696. doi:10.1016/j.foodchem.2017.03.089

Richter, C., Skulas-Ray, A., & Kris-Etherton, P. M. (2016). Recommended Intake of Fish and Fish Oils Worldwide. In (pp. 27-48).

Romeu-Nadal, M., Chávez-Servín, J. L., Castellote, A. I., Rivero, M., & López-Sabater, M. C. (2007). Oxidation stability of the lipid fraction in milk powder formulas. *Food Chemistry, 100*(2), 756-763. doi:10.1016/j.foodchem.2005.10.037

Ryan, A. S., Astwood, J. D., Gautier, S., Kuratko, C. N., Nelson, E. B., & Salem, N. (2010). Effects of long-chain polyunsaturated fatty acid supplementation on neurodevelopment in childhood: A review of human studies. *Prostaglandins Leukotrienes and Essential Fatty Acids, 82*(4-6), 305-314. doi:10.1016/j.plefa.2010.02.007

Sahu, J., & Mallikarjunan, P. (2012). Modeling of particle size distribution of heat assisted high-pressure treated reconstituted cow milk: Effect of high pressure, pressurization time and heat treatment temperature. *LWT - Food Science and Technology, 48*, 255–260. doi:10.1016/j.lwt.2012.03.023

Schram, L. B., Nielsen, C. J., Porsgaard, T., Nielsen, N. S., Holm, R., & Mu, H. (2007).

Food matrices affect the bioavailability of (n - 3) polyunsaturated fatty acids in a single meal study in humans. *Food Research International, 40*(8), 1062-1068. doi:10.1016/j.foodres.2007.06.005

Schrooyen, P. M. M., Van der Meer, R., & De Kruif, C. G. (2001). Microencapsulation: Its application in nutrition. *Proceedings of the Nutrition Society, 60*(4), 475-479. doi:10.1079/PNS2001112

Shao, P., Feng, J., Sun, P., Xiang, N., Lu, B., & Qiu, D. (2020). Recent advances in improving stability of food emulsion by plant polysaccharides. *Food Research International, 137*, 109376. doi:https://doi.org/10.1016/j.foodres.2020.109376

Singh, H., Zhu, X.-Q., & Ye, A. (2015). United state of america Patent No. US 8,993,019 B2.

Sliwinski, E. L., Roubos, P. J., Zoet, F. D., Van Boekel, M. A. J. S., & Wouters, J. T. M. (2003). Effects of heat on physicochemical properties of whey protein-stabilised emulsions. *Colloids and surfaces B: Biointerfaces, 31*(1-4), 231-242. doi:10.1016/S0927-7765(03)00143-7

Smelt, J. P. P. M. (1998). Recent advances in the microbiology of high pressure processing. *Trends in Food Science and Technology, 9*(4), 152-158. doi:10.1016/S0924-2244(98)00030-2

Srinivasan, M., Singh, H., & Munro, P. (2001). Creaming Stability of Oil-in-Water Emulsions Formed with Sodium and Calcium Caseinates. *Journal of food science, 66*(3), 441-446.

Su, J., Flanagan, J., Hemar, Y., & Singh, H. (2006). Synergistic effects of polyglycerol ester of polyricinoleic acid and sodium caseinate on the stabilisation of water-oil-water emulsions. *Food Hydrocolloids, 20*(2-3 SPEC. ISS.), 261-268. doi:10.1016/j.foodhyd.2004.03.010

Suriyarak, S., & Weiss, J. (2014). Cutoff Ostwald ripening stability of alkane-in-water emulsion loaded with eugenol. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 446*, 71-79. doi:10.1016/j.colsurfa.2014.01.009

Tadros, T. (2015). Viscoelastic properties of sterically stabilised emulsions and their stability. *Advances in Colloid and Interface Science, 222*, 692-708. doi:10.1016/j.cis.2015.03.001

Tadros, T., Izquierdo, P., Esquena, J., & Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science, 108-109*, 303-318. doi:10.1016/j.cis.2003.10.023

Tan, C. P., & Nakajima, M. (2005). β-Carotene nanodispersions: preparation, characterization and stability evaluation. *Food Chemistry, 92*(4), 661-671. doi:https://doi.org/10.1016/j.foodchem.2004.08.044

Tual, A., Bourles, E., Barey, P., Houdoux, A., Desprairies, M., & Courthaudon, J. L. (2006). Effect of surfactant sucrose ester on physical properties of dairy whipped emulsions in relation to those of O/W interfacial layers. *Journal of Colloid and Interface Science, 295*(2), 495-503. doi:10.1016/j.jcis.2005.09.010

Türkmen, N. (2017). The Nutritional Value and Health Benefits of Goat Milk Components. In (pp. 441-449).

Valero, E., Villamiel, M., Miralles, B., Sanz, J., & Martínez-Castro, I. (2001). Changes in flavour and volatile components during storage of whole and skimmed UHT milk. *Food Chemistry, 72*(1), 51-58. doi:10.1016/S0308-8146(00)00203-X

Van Oss, C. J. (2007). Development and applications of the interfacial tension between

water and organic or biological surfaces. *Colloids and surfaces B: Biointerfaces, 54*(1), 2-9.

Vazquez-Landaverde, P. A., Torres, J. A., & Qian, M. C. (2006). Effect of High-Pressure−Moderate-Temperature Processing on the Volatile Profile of Milk. *Journal of Agricultural and Food Chemistry, 54*(24), 9184-9192. doi:10.1021/jf061497k

Vichi, S., Pizzale, L., Conte, L. S., Buxaderas, S., & López-Tamames, E. (2003). Solid-Phase Microextraction in the Analysis of Virgin Olive Oil Volatile Fraction: Modifications Induced by Oxidation and Suitable Markers of Oxidative Status. *Journal of Agricultural and Food Chemistry, 51*(22), 6564-6571. doi:10.1021/jf030268k

Vilgis, T. A. (2020). Unser Essen wird industrialisiert. In *Biophysik der Ernährung* (pp. 115-189): Springer.

Wan, Y., Bankston Jr, J. D., Bechtel, P. J., & Sathivel, S. (2011). Microencapsulation of menhaden fish oil containing soluble rice bran fiber using spray drying technology. *Journal of food science, 76*(4), E348-E356.

Wei, X., Yongzhao, X., Zhifan, L., Denglin, L., Zhao, W., Yuqing, S., & Ramin, S. B. (2020). Stability, microstructural and rheological properties of complex prebiotic emulsion stabilized by sodium caseinate with inulin and konjac glucomannan. *Food Hydrocolloids, 105*, 105772. doi:https://doi.org/10.1016/j.foodhyd.2020.105772

Weitz, D., Weintraub, H., Fisher, E., & Schwartzbard, A. Z. (2010). Fish Oil for the Treatment of Cardiovascular Disease. *Cardiology in Review, 18*(5), 258-263. doi:10.1097/CRD.0b013e3181ea0de0

Weixun, F., & Wu, P. (2004). Variations of Konjac glucomannan (KGM) from Amorphophallus konjac and its refined powder in China. *Food Hydrocolloids, 18*(1), 167-170. doi:https://doi.org/10.1016/S0268-005X(03)00044-4

Williams, M. A., Foster, T. J., Martin, D. R., Norton, I. T., Yoshimura, M., & Nishinari, K. (2000). A molecular description of the gelation mechanism of konjac mannan. *Biomacromolecules, 1*(3), 440-450.

Xiangqian, Z., Aiqian, Y., Jiahan, T. H., Jeanne, L. S., & Harjinder, S. (2014). Oxidative stability of fish oil-in-water emulsions under high-pressure treatment. *International Journal of Food Science & Technology, 49*(6), 1441-1448. doi:10.1111/ijfs.12462

Xu, G., Wang, C., & Yao, P. (2017). Stable emulsion produced from casein and soy polysaccharide compacted complex for protection and oral delivery of curcumin. *Food Hydrocolloids, 71*, 108-117. doi:10.1016/j.foodhyd.2017.05.010

Ye, R., & Harte, F. (2014). High pressure homogenization to improve the stability of casein–hydroxypropyl cellulose aqueous systems. *Food Hydrocolloids, 35*, 670-677. doi:https://doi.org/10.1016/j.foodhyd.2013.08.022

Ying, Z., Xie, B.-J., & Gan, X. (2005). Advance in the applications of konjac glucomannan and its derivatives. *Carbohydrate polymers., 60*(1), 27-31. doi:10.1016/j.carbpol.2004.11.003

Yingqing, Z., Xie, B.-j., & Gan, X. (2005). Advance in the applications of konjac glucomannan and its derivatives. *Carbohydrate Polymers, 60*, 27-31. doi:10.1016/j.carbpol.2004.11.003

Yoshimura, M., & Nishinari, K. (1999). Dynamic viscoelastic study on the gelation of

konjac glucomannan with different molecular weights. *Food Hydrocolloids, 13*(3), 227-233. doi:https://doi.org/10.1016/S0268-005X(99)00003-X

Yuan, F., Xu, D., Qi, X., Zhao, J., & Gao, Y. (2013). Impact of High Hydrostatic Pressure on the Emulsifying Properties of Whey Protein Isolate-Chitosan Mixtures. *Food and Bioprocess Technology, 6*(4), 1024-1031. doi:10.1007/s11947-011-0745-x

Zhang, Chen, J.-d., & Yang, F.-q. (2014). Konjac glucomannan, a promising polysaccharide for OCDDS. *Carbohydrate Polymers, 104*, 175-181. doi:https://doi.org/10.1016/j.carbpol.2013.12.081

Zhang, F., Cai, X., Ding, L., & Wang, S. (2021). Effect of pH, ionic strength, chitosan deacetylation on the stability and rheological properties of O/W emulsions formulated with chitosan/casein complexes. *Food Hydrocolloids, 111*, 106211. doi:https://doi.org/10.1016/j.foodhyd.2020.106211

Zhao, Q., Long, Z., Kong, J., Liu, T., Sun-Waterhouse, D., & Zhao, M. (2015). Sodium caseinate/flaxseed gum interactions at oil–water interface: Effect on protein adsorption and functions in oil-in-water emulsion. *Food Hydrocolloids, 43*, 137-145. doi:https://doi.org/10.1016/j.foodhyd.2014.05.009

Zhou, Y., Cao, H., Hou, M., Nirasawa, S., Tatsumi, E., Foster, T. J., & Cheng, Y. (2013). Effect of konjac glucomannan on physical and sensory properties of noodles made from low-protein wheat flour. *Food Research International, 51*(2), 879-885. doi:10.1016/j.foodres.2013.02.002

Zhu, S. M., Lin, S. L., Ramaswamy, H. S., Yu, Y., & Zhang, Q. T. (2017). Enhancement of Functional Properties of Rice Bran Proteins by High Pressure Treatment and Their Correlation with Surface Hydrophobicity. *Food and Bioprocess Technology, 10*(2), 317-327. doi:10.1007/s11947-016-1818-7

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