# Characterisation of Low Fat Instant Creamy Emulsions: Effects of Different Types and Concentrations of Hydrocolloids and Emulsifiers

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# Abstract

Instant creamy oil-in-water (O/W) emulsions prepared by a low shear (i.e. hand shaking) without using high shear forces (e.g. high-pressure homogeniser, microfluidizer, ultrasonicator, etc.) have not been well investigated. This research study was focused initially on the characterisation of instant creamy O/W low shear emulsions containing 40% oil prepared from a base formulation consisting of dry ingredients (starch, xanthan gum, citric acid, egg yolk powder), aqueous liquid (water, Tween 80) and oil (canola oil). The preparation of instant emulsions was conducted by a method called "instantaneous emulsification process" involving simply adding cold water to an oil mixture containing emulsifiers and thickening agents. More specifically, dry ingredients were mixed together (starch, xanthan gum, citric acid, egg yolk powder) and then added into canola oil to form an oil slurry by hand shaking. Then, cold water added with Tween 80 was added into the oil slurry and mixed with a moderate shear generated by hand shaking to form an instant creamy O/W emulsion. Different types and concentrations of starches (Ultra-Tex-4, Ultra-Sperse M, Purity HPC, Novation 5600, Quik-Tex 3331, Instant FTD 176, N-Creamer 46L), hydrocolloids as a cold-hydratable thickening agent (starch, xanthan gum, guar gum, carboxymethyl cellulose, carrageenan) and emulsifier ingredients (egg yolk powder, Tween 80, alpha-cyclodextrin and OSA modified starch) were used to determine their impact on the formation, properties and stability of instant emulsions. The instant emulsion samples prepared were analysed for their appearance, pH, mean particle size and particle size distribution, rheological and textural properties, microstructure (confocal microscope and light microscope images) and emulsion stability (creaming, phase separation, etc.).

The results revealed that starch plays an important role on the formation and properties of the instant creamy emulsions. All the physicochemical properties of the instant emulsions characterised by this study were found to be significantly influenced by the types and concentrations of starches that the emulsions were formed with. The most appropriate starch used in this application was found to be a cold water swelling waxy maize starch which was modified chemically by crosslinking and etherification (Ultra-Tex-4). The instant emulsion prepared with 4% w/w of this starch showed desirable viscosity and texture attributes with the pseudoplastic flow behaviour and a relatively small mean particle diameter of 50 μm. In addition, a non-starch hydrocolloid was incorporated into the formulation to enhance the properties of instant emulsions. Carboxymethyl cellulose (CMC)

was suitable to be used at a concentration of 0.2–0.4% w/w. The use of CMC aided the formation of instant emulsions with a smaller particle mean diameter of 45 μm and more uniform particle size distribution, and stabilised the instant emulsions by further increasing the viscosity of the emulsions, as well as enhancing the texture attributes and stability of the emulsions. The considerable potential of octenyl succinic anhydride (OSA) modified waxy maize starch (N-Creamer 46L) to emulsify and stabilise the instant emulsions was investigated in this project. It was found that the oil droplets with the smallest particle mean diameters at 45–50 μm could be formed and stabilised in the instant emulsions when prepared with OSA starch at a concentration above 0.1% w/w. However, the possible synergistic effects between the modified starch "Ultra-Tex-4", CMC and emulsifying OSA starch in instant emulsions at their appropriate concentrations were not investigated in this study, which is recommended for future research. This study provides an important insight into the preparation and characterisation of instant creamy emulsions for applications in food.

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# Chapter 1 Introduction

## 1.1 Background Information

Mayonnaise is one of the most widely used condiments in the worldwide. As an oil-in-water (O/W) emulsion, mayonnaise is traditionally prepared by mixing a mixture of oil, egg yolk, vinegar, and spices (Depree & Savage, 2001; Liu et al., 2007). Most commercial mayonnaise products typically have an oil content for 70-80%, which imparts the products a thick and creamy texture (Depree & Savage, 2001). However, nowadays, consumers become more aware of the negative effects of eating too much fat and saturated fat on their health. Mayonnaise-type emulsion products are regarded as a high-fat food and therefore there is an increasing demand for a low-fat version of mayonnaise or mayonnaise-type dressings (Daniells, 2010; Scott-Thomas, 2009). As a key ingredient in a mayonnaise-type emulsion product, fat contributes to the rheological properties and sensory characteristics of the final product, including its flavour, texture, appearance and shelf life (Amin et al., 2014; Puligundla et al., 2015). However, some non-fat ingredients with different functionalities, such as starches and gums, need to be incorporated into the formulation of a low-fat mayonnaise or mayonnaise-type dressings to replenish some of the fat functional attributes (Amin et al., 2014; Puligundla et al., 2015).

In addition, a concept of instant emulsions has been introduced and patented for the development of instant food emulsions (Ballard et al., 1986; Farrer et al., 2003; Foster et al., 2007; Torcello-Gómez & Foster, 2017). Unlike most food emulsion products in the market that are sold in a prepared format and packed in a tube or bottle, the instant emulsions are designed in the form of dry powders that contains a mixture of hydrocolloids and surface-active ingredients. The final emulsion is easily structured by consumers within a few minutes through simply adding cold water and oil into the instant powders and shaking it by hand (Ballard et al., 1986; Foster et al., 2007). Compared to the ready-to-use format, the approach of making instant emulsions from instant powders has several advantages (e.g. cost, storage, transport, convenience, diverse flavour and taste, etc.). There is less energy input required for the product manufacture. Instant emulsions can be prepared readily as small quantity as possible that could be all eaten without unfinished so no need to store the product, while most commercial mayonnaise and salad dressings must be kept in a fridge especially after opening the product package. In addition, instant creamy

emulsions can be prepared easily anywhere and anytime by adding water and oil into the dry ingredients and then shaking the mixture simply by hand.

In order to meet the increasing demand of low-fat dressings and the instant version of food emulsions, this research was carried out to develop a low-fat, cold-hydratable, mayonnaise-type instant creamy emulsion. By mixing the dry ingredients with oil and cold water, the final creamy, thick emulsion with a low-fat content could be created by a moderate shear generated via hand shaking within a few minutes. Since the emulsification process of the instant emulsion involves simply mixing of ingredients by hand shaking, the characteristics of the produced emulsions largely depend on the functional properties of the ingredients used to form the instant emulsions. Attempts have been made by some studies which used a starch-gum mixture for substituting fat to make it a reduced or low-fat product and also different types of emulsifiers for stabilising the mayonnaise-type emulsions (Lee et al., 2013; Puligundla et al., 2015; Riscardo et al., 2003; Wang et al., 2016). However, there is still some research areas to be further investigated. Thus, this project employed different types of hydrocolloids and surface active emulsifier ingredients at different concentrations for preparing the instant creamy emulsions containing 40% oil, aiming to explore the effects of types and concentrations of these ingredients (starch, non-starch hydrocolloids, emulsifiers) on the formation and physicochemical properties of the instant emulsions. The instant emulsions prepared were analysed for the determination of visual appearance, pH, rheological and textural properties, particle size and particle size distribution, microstructure and emulsion stability against creaming and phase separation.

The objective of this research project was to investigate the effects of types and concentrations of starches, non-starch hydrocolloids and emulsifiers on the formation and physicochemical properties of low-fat, cold-hydratable, instant creamy emulsions prepared by using spontaneous emulsification method.

## 1.2 Overview of Thesis

This thesis consists of seven chapters as follows:

Chapter 1 describes the background information and rationale of developing and characterising instant creamy emulsion systems including the main objective of the research project.

Chapter 2 is a literature review of this project in relation to the major ingredients, formation mechanism and properties of O/W emulsions as well as some analytical techniques being widely used to characterise the properties of emulsions. In addition, some previous studies on instant emulsions and low-fat mayonnaise-type emulsions are also reviewed.

Chapter 3 lists the information about the materials and methods used in this research work.

Chapter 4 describes the use of different types and concentrations of starches and their effects on the formation and properties of instant creamy emulsions prepared by hand shaking.

Chapter 5 describes the use of different types of non-starch hydrocolloids (xanthan gum, guar gum, CMC and carrageenan) at different concentrations on the properties of instant creamy emulsions.

Chapter 6 discusses the effects of types and concentrations of surface-active emulsifier ingredients (egg yolk powder, Tween 80, alpha-cyclodextrin and OSA- modified starch) on the properties of instant creamy emulsions.

Chapter 7 describes the main findings of this research project. Some recommendations for future work are also presented in Chapter 7.

# Chapter 2 Literature Review

## 2.1 Introduction of Emulsion

Emulsion is a type of dispersion system consisting of two immiscible liquids, in which one liquid is dispersed as small spherical droplets in the other (McClements, 2016). The most common immiscible liquids to form an emulsion are water and oil. According to the relative spatial distribution of oil and water phases, emulsions can be broadly divided into two types; oil-in-water (O/W) and water-in-oil (W/O) emulsions. O/W emulsions consist of oil droplets dispersed as small droplets in water that makes up the surrounding liquid known as the continuous phase. Examples include salad dressings, mayonnaise and sauces. On the other hand, W/O emulsions consist of water droplets being dispersed as small droplets in the surrounding oil phase. Examples of W/O emulsion are margarine and butter (Hasenhuettl & Hartel, 2008; McClements, 2016).

Emulsions can also be classified based on their droplet sizes. If all the droplets in an emulsion have the same dimension, the emulsion is then referred to as a monodisperse emulsion. The droplet size of a monodisperse emulsion is therefore completely described by using one single dimension such as a droplet diameter (McClements, 2016). However, in fact, a monodisperse emulsion is too difficult to produce and almost all the real emulsion-based food products consist of a range of droplets with different sizes. Then, the emulsion is referred to as a polydisperse emulsion. The full particle size distribution should be taken into consideration when analysing the size of droplets within a polydisperse emulsion (McClements, 2016; Tadros, 2013*)*. Furthermore, according to the mean emulsion droplet diameter, emulsion can also be divided into micro- (10-100 nm), mini- (100-1000 nm) and macro-emulsions (0.5-100 μm) (Windhab et al., 2005). This literature review focus on the O/W emulsions unless stated otherwise.

## 2.2 Emulsion Ingredients

Emulsion is a complex mixture of different ingredients including oil, water, emulsifiers, polysaccharides, salt and others. Each of these ingredients has different functionalities, which contributes to the bulk properties of emulsions. The role of major ingredients is discussed and reviewed in the following section.

### 2.2.1 Aqueous Phase

Water, the continuous phase in an O/W emulsion, plays a vital role in determining the bulk physicochemical properties of food emulsions. As a solvent, it comprises a variety of water-soluble materials such as emulsifier, protein, polysaccharide, and acid. These components being presented as solutes and their intermolecular interactions with water have a significantly impact on the overall characteristics of the emulsion. For example, thickening agents, such as starch and gums, are dissolved in water to increase the viscosity of the aqueous phase of the emulsion system. Such change in the rheological properties of aqueous phase can aid the emulsion formation and stability, as well as affect the texture and appearance of emulsion (McClements, 2016).

### 2.2.2 Oil Phase

Oil is an important component for making up the body of the O/W food emulsions, such as salad dressings and mayonnaise. Traditionally, salad dressings and mayonnaise have been made by using vegetable oil that remains in the liquid form at refrigerator and room temperatures. Canola oil is considered as a healthy oil due to its fatty acid composition, which contains approximately 60% of oleic acid, 20% of linoleic acid and 10% of alpha linolenic acid (ALA) (Barthet, 2016). Hence, canola oil was used in this research project involved in making an instant creamy O/W emulsion which has the similar appearance to mayonnaise.

Oil plays multiple roles that affect the nutritional, sensory and physicochemical properties of food emulsions. Firstly, oil is the major energy component of salad dressing and mayonnaise. As reported by the USDA Food Composition Databases (2018), every 100 grams of canola oil contribute about 884 calories. Traditionally, dressings and mayonnaise contain up to 80% of oil, which makes them to be known as “high-calories” foods (Depree & Savage, 2001). The high fat content also imparts to the dressings and mayonnaise a unique flavour, creamy texture and thicken mouthfeel. Moreover, the rheological properties of emulsion are influenced by the fat content as well, as reported by Wendin et al. (1997) that the viscosity and elasticity of mayonnaise increase with increasing fat content.

However, nowadays, with the increased nutritional consciousness of consumers and aging population, there is a growing demand for the low and reduced fat dressings and mayonnaise (Yang & Lai, 2003b). As defined by Food Standards Australia New Zealand

(2016), the claim of "reduced-" or "light-" can be made when the food contains at least 25% less fat than a standard food product, and a "low-fat" claim can be made when a solid food containing no more than 3 g fat per 100 g product. However, in some cases, a decrease in the fat content can have a marked impact on the flavour release and textural properties of the food products. Thus, other ingredients, such as starches, gums and proteins, are often incorporated into the reduced fat products for replenishing some of the fat functional attributes (Puligundla et al., 2015).

### 2.2.3 Emulsifiers

Emulsifiers are the surface-active molecules which are capable of forming an adsorbed layer at the oil-water interface to lower the interfacial tension. This aids the process of emulsification and provides the stability of emulsions by protecting emulsion droplets from flocculation and coalescence (Robins & Wilde, 2003). The emulsifying property of emulsifiers is determined by their molecular characteristics. Generally, surface-active substances often called surfactants consists of a polar hydrophilic head group, which has a high affinity for water, attached to a non-polar lipophilic tail group that has a high affinity for oil. Thus, when the surfactants adsorb to an oil-water interface, the direct unfavourable contact between oil and water is replaced by the more thermodynamically favourable interaction between the polar head and water and between the non-polar tail and oil (McClements, 2016).

Surfactants can be classified by hydrophilic-lipophilic balance (HLB), which describes the ratio of hydrophilic groups to lipophilic groups in their molecules. A surfactant with a high HLB number is considered as predominantly hydrophilic, which shows high solubility in water and forms an O/W emulsion. A surfactant with a low HLB number is relatively hydrophobic, which is soluble in oil and used to form and stabilise a W/O emulsion (McClements, 2016). The commonly used emulsifiers in the food industry can be divided into two types; 1) low-molecular-weight surfactants, such as mono-and diglycerides, lecithin, polysorbates and sugar esters, and 2) biopolymers, such as protein and polysaccharides (McClements, 2016; Robins & Wilde, 2003). Depending on the application, emulsifiers can be used individually or in combinations for obtaining desirable functional properties (McClements, 2016; Riscardo et al., 2003).

### 2.2.4 Stabilisers

Some hydrocolloids (e.g. starches, xanthan gum, guar gum and carrageenan) are often used as stabilisers to enhance the stability of O/W emulsion products by enhancing the viscosity of the aqueous phase of the emulsion system (McClements, 2016; Saha & Bhattacharya, 2010). The viscous continuous phase traps the dispersed oil droplets, thus effectively inhibiting their movement and retarding the rate of emulsion creaming or sedimentation. Some stabilisers can also gel to form a three-dimensional network that immobilises a large volume of water, giving the emulsion a solid-like structure that is resistant to flow (McClements, 2016; Robins & Wilde, 2003; Saha & Bhattacharya, 2010). However, it should be mentioned that when the amount of a polymer added as stabiliser is low in its concentration, it may promote droplet flocculation, thus causing the instability and destabilisation of emulsion. This is due to a depletion mechanism which causes an increase in the droplet size of emulsion (McClements, 2000; Robins & Wilde, 2003).

## 2.3 Emulsion Formation

The physicochemical and sensory properties of a food emulsion is not only affected by the type and concentration of ingredients used but also the emulsification process used to fabricate it. To produce an O/W emulsion, oil phase must be disrupted into small droplets and dispersed into the water phase. The breakdown of oil phase into smaller oil droplets in usually achieved by using a mechanical shear force (e.g. high-pressure homogeniser) referred to as a homogenisation process. This results in a massive increase in the interfacial area between oil and water, which is strongly thermodynamically unfavourable and the systems shows very high tendency to separate into two layers (McClements, 2016). However, the oil-water interfacial area is adsorbed and stabilised by surface-active emulsifiers that can rapidly adsorb and accumulate at the interfaces, reducing the interfacial tension between oil and water phases. Thus, the faster the absorption time and the greater the absorption efficiency, the emulsification will be more effective (McClements, 2016; Robins & Wilde, 2003).

## 2.4 Characteristics and Properties of Emulsions

### 2.4.1 Droplet Characterisation

An O/W food emulsion, including salad dressing and mayonnaise, is a mixture of oil and water in which the oil is presented as small droplets and distributed throughout the water phase. It is believed that many of the most important properties of such a particulate system, such as the stability, shelf life, appearance and texture, are often dominated by the size of the constituent oil droplets (McClements, 2016). Thus, it becomes important to measure, report and control the particle size in emulsions. In the following section, factors influencing the droplet size, methods of measuring and reporting particle size are reviewed.

#### 2.4.1.1 Droplet Size

Oil is dispersed in water in the spherical form, which enables the oil droplets to be described by a single dimension such as a diameter. There are two mean diameters that could be used: D[3,2] and D[4,3]. The term D[3,2] is the surface-weighted mean diameter whereas the term D[4,3] is referred to as the volume-weighted mean diameter. The equations for the calculations of these two values are as the equations shown below where $d_i$ is expressed as the diameter of the particles in *i*th size class and $n_i$ represents the number of particles in the *i*th size class per unit volume of emulsion (McClements, 2016).

$$\mathrm{D}[3,2] = {\Sigma n_i d_i^3}\Big/{\Sigma n_i d_i^2}$$

$$\mathrm{D}[4,3] = {\Sigma n_i d_i^4}\Big/{\Sigma n_i d_i^3}$$

The surface mean diameter and the volume mean diameter are the same for a monodisperse emulsion. However, as mentioned before, almost all the food emulsions are polydisperse emulsions containing a range of different particle sizes. In this case, the surface mean diameter and the volume mean diameter are considerably different. Generally, D[3,2] is more sensitive to the presence of fine particulates in the size distribution while D[4,3] is more sensitive to the presence of large particulates. This makes D[4,3] to be more useful for detecting flocculation or coalescence in an emulsion, and D[3,2] is more appropriate for monitoring the proportion of fines present (Malvern Instruments Limited, 2015; McClements, 2016). When comparing the mean values of particle sizes, it should be

remembered that the larger the difference between D[3,2] and D[4,3], the greater the polydispersity of the emulsions (McClements, 2016).

#### 2.4.1.2 Laser Diffraction Particle Sizing

There are various techniques that can be used to characterise the particle size of emulsions. In this study, laser diffraction particle characterisation technique was used. Laser diffraction technique is suitable for detecting particles in a wide dynamic range, sizing from approximate 10 nm to about 10 mm, coupled with high repeatability and rapid measurements (Malvern Instruments Limited, 2015). The principle of laser diffraction particle sizing is based on the measurement of angular variation in the intensity of light scattered by the dispersed particles. More specifically, the incident laser light is scattered by the interaction with the droplet surface. However, particles with varying sizes would produce different angles of light scattering. This produces a pattern of light intensity that can be captured by detectors around the particles, then the collected data are analysed to calculate the size of the particle responsible for producing the scattering pattern (Malvern Instruments Limited, 2015; Mingard et al., 2009).

#### 2.4.1.3 Particle Size Distribution

By using the laser diffraction particle size analyser, a volume weighted particle size distribution can be obtained. This model clearly shows the contribution of each particles in the distribution related to the volume of that particles (Malvern Instruments Limited, 2015). When analysing the volume weighted particle size distribution, there are three useful values being measured and reported, such as d(0.1), d(0.5) and d(0.9). These values indicate that 10%, 50% and 90% of the particles are smaller than the values of d(0.1), d(0.5) and d(0.9) in size, respectively. Through monitoring these three parameters, the changes in the main and the extreme particle size distribution could be detected, which might be caused by the presence of fine or oversize particles (Malvern Instruments Limited, 2015; Mingard et al., 2009).

#### 2.4.1.4 Factors Influencing the Droplet Size

Oil droplets are disrupted into small droplets during the emulsion formation process. However, for an emulsion to remain high stability for a significant period, the oil droplets must be small enough to slow down the emulsion breakdown process such as creaming. As

suggested by McClement (2016), the formation of droplets is affected by the properties of component ingredients as well as the processing conditions. In details, there are four major factors influencing the size of oil droplets during the emulsion formation, such as the type and concentration of emulsifier, energy input, temperature and the properties of component phases.

**Emulsifier type and concentration**

There are many types of emulsifier that can be used in food industry, and different types of emulsifier exhibit different characteristics in term of the adsorption rates, the maximum reduction in the interfacial tension, and the effectiveness of emulsifier coating layer to protect droplets from coalescence (McClements, 2016). In order to obtain smaller droplets, the emulsifiers must be able to be adsorbed to the surfaces of the newly formed droplets rapidly during the emulsion formation. The faster and more efficient the adsorption, the greater the interfacial tension reduction, and therefore the smaller are the droplets produced (McClements, 2016; Robins & Wilde, 2003).

In addition to the types of emulsifier, the concentration of emulsifier also influences the size of droplets produced. It is believed that with the increasing content of emulsifiers in an emulsion, a marked reduction in mean droplet size will be obtained at a given high pressure homogenisation process (Chung et al., 2017; McClements, 2016). When the concentration of emulsifier is low, there are insufficient emulsifiers to cover all the droplet surface areas created during the emulsion formation. Thus, the produced small droplets tend to coalesce with each other and result in an increase of droplet size. Under this condition, the produced droplet size is primarily dominated by the emulsifier concentration rather than the energy input applied to breakdown oil phase into smaller droplets. However, when the emulsifier concentration is high, there are sufficient emulsifiers present to completely cover the droplet surface and protect the droplets from aggregating each other. In this case, the droplet size becomes relatively independent to the emulsifier concentration and primarily governed by the energy input during the emulsion formation (McClements, 2016).

**Energy input**

During emulsion formation, a free energy is required for the system to deform and disrupt the bulk oil into small droplets. This energy input can be achieved by a given

homogenisation device (e.g. high shear mixer, high pressure homogeniser). Generally, when sufficient emulsifiers are present in the system, the droplet size can be effectively reduced by increasing the energy input. This can be achieved through increasing the intensity or duration of disruptive energy supplied. However, when using certain types of emulsifier, the increase in energy input may induce an increase in droplet sizes, which is called as overprocessing (McClements, 2016). For example, when processing a protein-stabilised emulsion with high pressure homogeniser, the exceeded pressure can denaturalise the adsorbed protein and cause a loss of emulsifying functional property of emulsifiers. As a result, the droplets will merge together due to flocculation or coalescence and lead to an increase in droplet size (Rampon et al., 2003).

**Properties of component phases**

The composition and physicochemical properties of both the oil and water phases can influence the droplets size produced during emulsion formation (McClements, 2016). Firstly, different types of oil have different molecular structures, which result in the different viscosities. As reported by some previous studies, with the increasing viscosity of dispersed phase, a larger mean particle size is formed because it gets harder for droplets to breakup during the formation of emulsion (Jafari et al., 2008; Qian & McClements, 2011). In addition, different oils may have different interfacial tensions when placed in contact with water as they contain varying amount of surface-active impurities, such as free fatty acid and monoacyglycerols. These impurities tend to accumulate at the oil-water interface and lower the interfacial tension. Thus, different amount of energy input is required depending on different types of oil (McClements, 2016). Secondly, continuous phase contains a wide variety of components, such as minerals, acid, bases, biopolymers, or gas bubbles. These components can alter the physicochemical properties of continuous phase and therefore further influence the size of droplets produced (McClements, 2016). For example, the presence of salt in aqueous phase affects the solubility of functional ingredients such as proteins, which then affects the adsorption of the ingredients and influences the droplet size (Depree & Savage, 2001).

**Temperature**

Temperature can influence the droplet size through influencing the properties of component molecules. Firstly, temperature can alter the rheological properties of oil and water phases.

It is known that the viscosity of both oil and water phases depends on temperature. Therefore, the particle size produced can be controlled via altering the viscosity ratio of oil and aqueous phase. In addition, high temperature may affect the functionality of certain types of emulsifiers, such as the protein-based emulsifiers. The loss of emulsifying property will induce droplet aggregation and result in an increase of droplet size (McClements, 2016).

### 2.4.2 Microstructure

In this study, the microstructure of emulsion samples was characterised at different magnification levels by using optical light microscopy and confocal laser scanning microscopy. Thus, these two techniques are reviewed in the following section.

#### 2.4.2.1 Optical Light Microscopy

Optical light microscopy is a well-developed and widely used technique for studying the microstructure of a food system. The principle of optical microscopy is to render a two-dimension magnified view through lenses, by which the different transmitted light through the specimen due to the different absorbance by the microstructural phases is observed.

A number of researches have utilised this technique to observe and investigate the microstructure of O/W emulsions (Chivero et al., 2016; Ghazaei et al., 2015; Perrechil et al., 2010). However, there are several limitations of using optical microscopy for visualisation of microstructure. In order to obtain good quality and high-resolution images, samples must be sufficiently transparent. Thus, the thick samples like mayonnaise must be diluted, or a substantial shear or compressive forces may have applied to samples when preparing the observation slides, which may promote the migration and disruption of emulsion droplets (Cardona et al., 2013).

#### 2.4.2.2 Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy (CLSM) is an advanced imaging technique and can be used to provide some valuable information about the microstructure of food emulsions (Auty, 2013). Compared to conventional optical light microscopy, CLSM can provide higher resolution and contrast images without the need of sample dilution (Langton et al., 1999; Verboven et al., 2018). Also, CLSM has the advantage of generating the three-dimensional images of structures without sectioning the samples (McClements, 2016).

By using CLSM, specific components in the system of emulsions can be labelled selectively by using fluorescent dyes. For example, protein and lipid can be stained by Fast Green and Nile Red to the colour of green and red, respectively (Kasprzak et al., 2018). By doing so, the structure of food emulsions, including the droplet size, aggregation state, and location of droplets and other components can be better determined through the use of CLSM (McClements, 2016).

## 2.5 Emulsion Rheology

Mayonnaise and salad dressings are a compositionally and structurally semi-solid that exhibits non-Newtonian and pseudoplastic flow behaviours. This implies a decrease in the viscosity of emulsions with increasing shear rate, thus also being referred to as shear thinning (Alvarez et al., 2006; Maruyama et al., 2007). It is important to understand the rheological properties of food emulsions because they have a significant impact on the creaminess, smoothness, thickness and flowability of emulsion systems. In addition, the stability and shelf life of mayonnaise and salad dressings are strongly dependent on their rheological characteristics (Maruyama et al., 2007; McClements, 2016).

### 2.5.1 Measurement of Rheological Property

The rheological properties of mayonnaise and salad dressings can be characterised by investigating the relationship between the apparent viscosity and the shear rate. This can be carried out by utilising a dynamic shear rheometer that applies a controlled shear rate to the sample under a temperature-controlled condition. The resulting shear stress and apparent viscosity are measured and analysed by the instrument, and the flow behaviour of the sample is then determined. This method has been widely used by many researchers for studying the flow properties of mayonnaise and salad dressings (Alvarez et al., 2006; Bortnowska & Tokarczyk, 2009; De Cássia da Fonseca et al., 2009; Wu et al., 2009).

Furthermore, the flow curves of food emulsions can be evaluated by more complex models. It has been shown that the Power Law model is the most often used rheological model for describing the rheological properties of foods emulsions showing pseudoplastic flow behaviour, including mayonnaise and salad dressings (Alvarez et al., 2006; Nikzade et al., 2012; Perrechil et al., 2010; Santana et al., 2015). The model is described by the equation shown below, where $\delta$ is the shear stress in Pa, $\gamma$ is the shear rate in $s^{-1}$, $n$ is the flow behaviour index and $k$ is the consistency coefficient in $Pa \cdot s^n$. By this law, the sample can

be classified as a dilatant fluid if $n > 1$, showing the viscosity of the emulsion increases as the shear rate is increased; when $n = 1$, it is a Newtonian fluid that the viscosity of the sample keeps constant regardless of changing shear rate; when $n < 1$, it is a pseudoplastic fluid that the viscosity of the sample decreases with the increasing shear rate (Santana et al., 2015).

$$\delta = k \cdot \gamma^n$$

### 2.5.2 Factors Influencing Emulsion Rheology

As a compositional and structural food product, the rheological property of mayonnaise and salad dressings directly depends on their composition of ingredients used and the processing and storage conditions applied. Usually, the rheology of food systems like mayonnaise and salad dressings is dominated by the rheological property of continuous phase. Thus, the rheology of these food emulsions could be efficiently modified by altering the viscosity of the continuous phase. As thus, gelling or thickening agents are often incorporated into the aqueous phase of O/W emulsion-based food products to obtain desirable rheological properties.

The rheological properties of emulsions are also controlled by the dispersed phase in terms of the concentration and size of dispersed droplets (Robins & Wilde, 2003). In an emulsion with low particle concentration, droplets are far apart from each other. There is none or weak interactions between droplets and consequently the emulsion shows a relatively low viscosity. However, as the particle concentration increases, more droplets are packed closely in the emulsion and the particles start to interact with neighbouring particles very strongly. As a result, the viscosity of the emulsion system increases and the emulsion changes its flow behaviour from a liquid-like towards a solid-like behaviour (McClements, 2016). Furthermore, the size of particles also has an impact on the rheology of emulsions. As the droplet size decreases, there are more droplets crowded in per unit volume of the emulsion. Thus, these particles are packed more closely together and cannot easily move. Therefore, the emulsion has stronger resistance to flow (Perrechil et al., 2010). Also, the decrease in particle size gives a larger interface area, which may introduce more interactions between oil droplets and consequently increase viscosity of the emulsion (Maruyama et al., 2007).

## 2.6 Emulsion Stability

McClements (2016) defined the term of "emulsion stability" as the ability of an emulsion system to resist to changes in its properties. The more stable the emulsion, the slower its changes occur. Generally, emulsions are thermodynamically unstable systems and undergo destabilisation leading to phase separation into two layers of water and oil (Friberg, 1997; McClements, 2016). Under the action of different forces existing in the emulsions including gravitational forces, interparticle repulsive and attractive forces, flow forces and molecular forces, the emulsions tend to break down during storage after the emulsion preparation. This results in a loss of some of their desirable properties such as appearance and texture. These breakdowns may be physical or chemical processes and these processes may take place simultaneously (Chiralt, 2009; McClements, 2016; Tadros, 2013). Figure 2.1 illustrates the possible physical breakdown processes that can occur in emulsions including creaming, sedimentation, flocculation, coalescence, phase inversion and Ostwald ripening. In the following section, some of the common breakdown processes are further discussed.

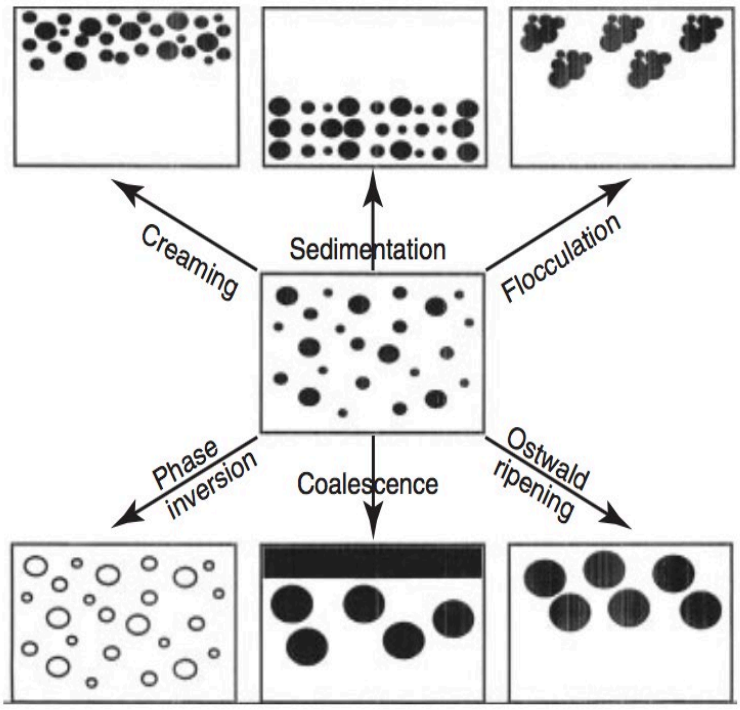



**Figure 2.1 Schematic representation of different types of breakdown processes in emulsions (Tadros, 2013).**

### 2.6.1 Creaming and Sedimentation

Normally, dispersed phase and the continuous phases in emulsions have different densities. Therefore, under the gravitational or centrifugal force, the emulsion droplets may move upwards (droplets with lower density) or downwards (droplets with larger density) to cause gravitational phase separation. This is referred as an emulsion creaming or sedimentation correspondingly. Normally, in an O/W emulsion, the density of oil phase is lower than that of water phase, and there is a tendency for foaming a layer of emulsion oil droplets on top of the aqueous phase, which is called the "creaming" of the emulsion (McClements, 2016). Theoretically, the rate of this gravitational instability of emulsion can be determined by the Stokes' Law which is shown in the equation below (Chiralt, 2009; McClements, 2016), where $v$ is the creaming rate, $g$ is the acceleration of gravity, $r$ is the radius of the droplets, $\rho$ is the density, $\eta$ is the viscosity, and *1* and *2* refer to the continuous and dispersed phases, respectively. Thus, based on the Stokes' Law, it is possible to control the rate of creaming through a number of ways, including reducing the droplet sizes, increasing the viscosity of aqueous phase and minimising the density difference between continuous and dispersed phases.

$$v = \frac{2gr^2\ (\rho_2 - \ \rho_1)}{9\eta_1}$$

### 2.6.2 Flocculation

Flocculation is a process whereby emulsion droplets aggregate but remain their individual integrity. This is caused when there is not sufficient repulsion to keep the droplets apart to distances where the van der Waals attraction is weak (McClements, 2016; Tadros, 2013). Flocculation may have advantageous or adverse effect on the emulsion properties. On one hand, flocculation in diluted O/W emulsions accelerates the rate of creaming due to the increase of mean particle size. This is undesirable because the shelf-life of the product is reduced. On another hand, in concentrated emulsions where there is sufficiently high droplet concentration, creaming is usually retarded or prevented when flocculation occurs. This is because the formation of a three-dimensional network of aggregated droplets prevents droplets from moving (McClements, 2016). The possible methods for controlling

flocculation include increasing the viscosity of continuous phase and regulating the colloidal interactions between the droplets (McClements, 2016).

### 2.6.3 Coalescence

Coalescence is a type of droplet aggregation similarly to flocculation but coalescence refers to the process whereby emulsion droplets merge together into a single larger one (Tadros, 2013). Coalescence results in an increase in droplet sizes and a decrease in the interfacial area between oil and water phases. As a result, coalescence can accelerate the creaming rate of emulsion. Coalescence can be prevented by keeping oil droplets apart from each other using an emulsifier that can provide a strong repulsive force between the oil droplets (McClements, 2016).

### 2.6.4 Ostwald Ripening

Ostwald ripening occurs when the solubility of a dispersed droplet increases with decreasing droplet sizes. Thus, the smaller droplets tend to shrink and disappear with time. Their solute molecules present in the disperse phase move from the small droplets to the larger droplets due to diffusion through the continuous phase, leading to a growth in size of large droplets. This process results in an overall net increase in the mean droplet size (McClements, 2016; Tadros, 2013). However, in food emulsions containing long chain triacylglycerols as the oil phase (e.g. canola oil), Ostwald ripening can be negligible as the mutual solubility between these triacylglycerols and water are very low and the mass transport does not occur. In an emulsion containing more water-soluble oil phase, such as short chain triacylglycerols, Ostwald ripening is important to take into consideration (McClements, 2016).

### 2.6.5 Phase Inversion

Phase inversion refers to the process whereby there is an inversion between the dispersed and continuous phases. For example, an O/W emulsion is converted to a W/O emulsion (Tadros, 2013). Phase inversion is undesirable for many food products, such as mayonnaise and salad dressings, because it will cause an adverse effect on their appearance, texture, stability and taste so that phase inversion should be avoided. In order to prevent phase inversion, the volume fraction between the dispersed and continuous phases should be well

controlled, and the appropriate type and concentration of emulsifiers must be used (McClements, 2016).

## 2.7 Instant Creamy Emulsions

### 2.7.1 Dressings and Mayonnaise

Yang and Lai (2003b) defined "salad dressings" as the liquid or semisolid products being used to flavour, moisturise and enrich salads, as well as being used in sandwich or other hot and cold recipes. Salad dressings can be divided into four categories based on their formulation and stability. One major type of salad dressing is emulsified dressings and the typical example of an emulsified dressing is mayonnaise (Yang & Lai, 2003b). Mayonnaise is a condiment sauce made from vegetable oil, egg yolks, vinegar or other acidifying agents, and additional seasonings (e.g. mustard, salt, sweeteners) (Yang & Lai, 2003b). In many countries, the manufacture of commercial mayonnaise has a minimum level of oil content which is required by legal standards (e.g. at least 65% by weight in USA, 70% by weight in Italy and UK) (Food and Agriculture Organization of the United Nations, 2000; U.S. Food and Drug Administration, 2019). In fact, the oil level in most commercial mayonnaises is in the range of 70-80% by weight for providing a thicker and creamier texture that has been shown to be more acceptable to consumers (International Food Information Service, 2009; U.S. Food and Drug Administration, 2019).

Commercial dressings and mayonnaise are mostly sold in a prepared format which are packed in a tube or bottle. However, the stability of such products is limited by various factors, such as shaking during transport, and storage at non-refrigerated temperature after opening the package. Consequently, the products may break down easily with phase separations (Farrer et al., 2003; Yang & Lai, 2003a). Other than purchasing commercially packed products, mayonnaise and dressings can also be made at home by following a recipe and using an electrical blender or mixer. However, homemade dressings normally have a very limited shelf stability and cannot be stored for a long period of time or shipped over long distances (Farrer et al., 2003). Furthermore, it has been shown that the homemade mayonnaise prepared with raw eggs may cause serious foodborne illness related to *Salmonella* (Keerthirathne et al., 2016). As a solution to all these issues, the development of more convenient, functional, end-user friendly products, such as an instant emulsion, has drawn an increasing interest (Torcello-Gómez & Foster, 2017).

### 2.7.2 Studies on Instant Emulsions

In recent years, there have been some studies conducted on the development of an instant mayonnaise-type emulsion powder which involves the dehydration of an initial emulsion through spray drying (Christensen et al., 2001; Lu et al., 2019; Plati et al., 2019). The powdered foods have the advantages that meet the consumers' demand for convenience and easy-to-use. They have also gained economic importance for the food industry because of the powder format that lowers the transportation and storage costs along with enhanced stability and shelf life (Lu et al., 2019). However, the production of the re-dispersible dry emulsion is relatively more costly than the liquid dressings, because a high level of "wall" ingredients, such as maltodextrin, is required to be incorporated into the formulations for making the instant salad dressing powders (Lu et al., 2019). In addition, the production of emulsion powder is more sophisticated as more steps are required, such as drying, which may have a negative impact on the quality of products (Torcello-Gómez & Foster, 2017).

It has also been shown that instant creamy emulsions (e.g. instant mayonnaise) can also be formed using an instantaneous emulsification process which involves simply adding cold water to an oil mixture containing emulsifiers and thickening agents followed by mixing the mixture of oil and water with a moderate shear generated by hand shaking (Foster et al., 2007; Torcello-Gómez & Foster, 2017). It is important to understand that this product concept is different from the aforementioned instant mayonnaise-type emulsion powder which are prepared by spray drying and require the rehydration (reconstitution) of the spray-dries emulsions in water. Some patent literatures have described several approaches to making the instant creamy emulsions, even the reduced-fat or fat-free mayonnaise-type emulsions can be prepared from an instant dry mix (Bakal et al., 1992; Eckhardt et al., 2005; Farrer et al., 2003; Тарасова et al., 1998). For example, Ballard et al. (1986) have patented the preparation of a low-fat salad dressing by simple hand shaking. The oil content of this dressing was reduced to 10-30% by volume. To obtain the desirable texture and mouthfeel, a pre-cooked, cold-swelling and acid stable starch in the conjunction of xanthan gum and guar gum was employed in the formulation. These dry ingredients were blended and packed, and then mixed with a certain amount of vinegar, oil and water when a dressing was needed to be made. Coote et al. (2002) also describes a formulation composition containing emulsifiers, fat blend and a cold water hydratable viscosifying agent, which can be mixed with water by stirring with a spoon or by shaking to form a stable O/W emulsion. As shown in Table 2.1, some studies on instant emulsions, in terms of the formulations and methods

used, are summarised. These studies showed the feasibility of applying different types of hydrocolloid (e.g. starch, xanthan gum, guar gum) and emulsifiers (e.g. Tween 20, lecithin) in preparing low-fat instant emulsions by hand shaking.

### 2.7.3 Major Ingredients for Instant Emulsions

As discussed in the previous section, the formation of instant emulsions involves an emulsification process taking place instantly under a moderate shear as can be produced by hand shaking. Thus, the resulting emulsion properties are predominately governed by the constituent ingredients, such as the thickener (stabiliser) concentration, oil phase volume and emulsifier types (Foster et al., 2007). In the following section, some representative examples of emulsifiers and thickening agents (e.g. hydrocolloids) are discussed, including their molecular structure, characteristics and functional properties, which enable them to be suitable for use in the instant emulsion application.

#### 2.7.3.1 Emulsifiers

**Egg Yolk**

Among all the ingredients, egg yolk is the key to the salad dressings, especially mayonnaise, due to its outstanding emulsifying capacity for forming an O/W emulsion as well as its organoleptic properties (e.g. flavour, texture) (Chivero et al., 2016; Depree & Savage, 2001). Egg yolk is a complex system consisting of non-soluble protein aggregates suspended in a clear yellow fluid. The protein aggregates are referred as the “granules” and consisted of 70% high-density lipoproteins (HDL), 16% phosvitin and 12% low-density lipoproteins (LDL). The yellow fluid is referred as the “plasma” containing 85% LDL and 15% water-soluble protein livetins (Anton, 2013).

It is believed that LDL is the major contributor to the emulsifying properties of egg yolk (de Souza & Rojas, 2012; Magnusson & Nilsson, 2011). The LDL particles consist of a core full of triacylglycerides and cholesterol, along with an outer layer of phospholipids and proteins. When the LDL particles approach the emulsion droplets, LDL breaks down to release its inner lipids, phospholipids and proteins to spread at the interface, which can lower the interfacial tension between oil and water phases and create a protective layer around the droplets (Anton, 2013; de Souza & Rojas, 2012; Magnusson & Nilsson, 2011).

**Table 2.1 Formulations and methods for preparing the instant emulsions in literatures.**

| Product name | Oil Content | Ingredients | Emulsification Method | References |
|---|---|---|---|---|
| Low-fat salad dressing | 10-30% v/v | To produce 1 litre of salad dressing:<br>• Instant waxy maize starch: 10 – 11 g<br>• Xanthan gum: 1 - 2.25 g<br>• Guar gum:0.25 – 2 g<br>• Flavouring agents (e.g. sugar, salt, garlic, spices): 0.1 – 5 g<br>• Vinegar: 200 – 300 ml<br>• Water: 500 – 600 ml | The mixture of dry ingredients was added into the aqueous phase which contains water and vinegar and the mixture was shaken thoroughly by hand for 10 seconds. Then the oil was added to the mixture of aqueous phase and shaken thoroughly for 10 seconds. Then the resultant dressing was left to stand at room temperature for 10 minutes. | (Ballard et al., 1986) |
| Instant emulsion | 47.39% w/w | • Starch (Ultra-tex 4): 2.37%<br>• Guar gum: 0.95%<br>• Tween 20: 0.28%<br>• Trisodium citrate: 0.28%<br>• Citric acid: 0.12%<br>• Potassium sorbate: 0.09%<br>• Sodium chloride: 1.13%<br>• Water: 47.39% | A paste was prepared by adding all the ingredients except for water into oil at room temperature and shaking the mixture in a sealed container to disperse the powder in the oil. Then the water was added to the oil phase. The resulting product was then prepared by shaking the oil and aqueous phases for 2 minutes. | (Farrer et al., 2003) |
| Instant spreadable emulsion | 43.15% w/w | • Starch (Ultra-tex-4): 3.45%<br>• Sodium caseinate: 0.85%<br>• Lecithin: 0.85%<br>• Sodium chloride: 1.05%<br>• Buffer: 0.55%<br>• Potassium sorbate: 0.1%<br>• Milk: 50% | Firstly, the lecithin was mixed with oil at room temperature, followed by the addition of other ingredients. Then the mixture was shaken in a sealed container to disperse the powders into oil phase. Lastly, the milk was added to the oil phase and the container was closed and shaken for 2 minutes. | (Eckhardt et al., 2005) |

Egg yolk used for mayonnaise may be fresh, frozen or dried. Since the liquid egg yolk can only be stored for a limited period of time, frozen and dried egg yolk are often used in the production of mayonnaise (Depree & Savage, 2001). In a study by Yang and Lal (2003b), the mayonnaise made from frozen salted egg yolk was reported to be thick and creamy, while the mayonnaise made from dried egg yolk was even thicker than that obtained from frozen egg as the dried egg yolk can disperse rapidly in the water phase of mayonnaise. However, the process of drying egg yolk may disrupt the structure of egg yolk and interfere with its emulsifying properties. The mayonnaise made from the dried egg yolk has larger oil droplets and higher tendency to coalesce (Depree & Savage, 2001).

In terms of health aspect, the use of egg yolk in making mayonnaise can raise consumers' concern about its high cholesterol content, and it is also relatively costly to use compared to synthetic small molecule surfactants (Ghazaei et al., 2015; Riscardo et al., 2003). Therefore, many studies have been conducted in recent years, which focus on the utilisation of other emulsifiers to substitute egg yolk totally or partially in the low-fat mayonnaise-type emulsions (Bortnowska & Tokarczyk, 2009; Ghazaei et al., 2015; Nikzade et al., 2012; Riscardo et al., 2003).

**Tween 80**

Tweens are widely used as emulsifiers in the food industry since their hydrophilic character can be selectively enhanced by substituting different numbers of polyoxyethylene chains of different length in hydroxyl groups, which results in several kinds of Tweens, such as Tween 20, Tween 60 and Tween 80 (Riscardo et al., 2003). In the research project reported in this thesis, Tween 80 has been used and it is therefore focused on and reviewed in more detail as follows.

Tween 80, also known as polysorbate 80 or polyoxyethylene (20) sorbitan monooleate, is a low molecular weight, non-ionic surfactant with an HLB value of 15 (Cottrell & Peij, 2015; Espert et al., 2019). Tween 80 is an oily liquid at ambient temperature and exhibits good solubility in hot and cold water as it can act as both hydrogen bond donors and hydrogen bond acceptors (Cottrell & Peij, 2015; EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2015; European Food Emulsifier Manufacturers Association, 2019). As shown in Figure 2.2, Tween 80 has a long unsaturated hydrocarbon chain attached to the ethylene oxide groups, which provides the hydrophobic and

hydrophilic characters to the Tween 80 molecule, respectively (Nayak et al., 2012). The structural features of Tween 80 enable it to stabilise O/W emulsions through the steric stabilisation mechanism, in which a large number of hydrophilic groups are adsorbed to the interface and extended into the aqueous phase. Hence, when the groups come close to each other, repulsion will occur to keep the droplets apart (Cottrell & Peij, 2015; Magnusson & Nilsson, 2011).

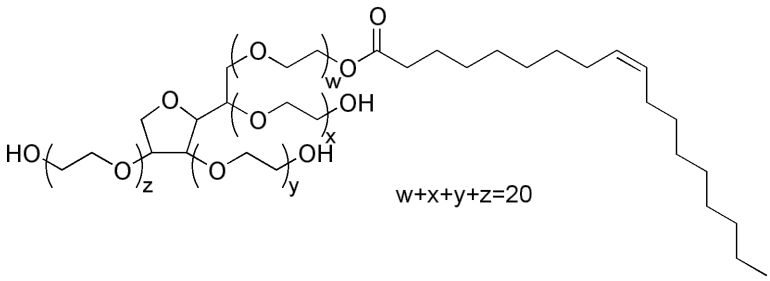



**Figure 2.2 Chemical structure of Tween 80 (Edgar181, 2008).**

Tween 80 is approved by Food Standards Australia New Zealand to be used as emulsifiers in food applications with an E number of E433 (Ministry for Primary Industries, 2017). It is a powerful emulsifier as a small amount of addition to water can result in a dramatic reduction in the interfacial tension between oil and water phases (Cottrell & Peij, 2015). However, due to the increased awareness of health and wellness, the use of Tween 80 in food products may raise some concerns as it is chemically synthesised, thus being related to the label of "chemical", even though there is no indication of health issues corresponding to the consumption of Tween 80 with a group Acceptable Daily Intake (ADI) of 25mg/kg body weight (bw) per day (Campbell, 2019; EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2015). The use of Tween 80 in low-fat and fat-free products may also have another issue of bitter flavour as there is no sufficient fat to mask the flavour of Tween 80 (Cottrell & Peij, 2015).

**Alpha-cyclodextrin**

Alpha-cyclodextrin is a member of the cyclic oligosaccharides which contains six 1,4-linked D-glucose residues (van der Veen et al., 2000). It is a starch derivative produced via an enzymatic conversion process (Li et al., 2014). As shown in Figure 2.3, α-cyclodextrin has a three-dimensional torus shape due to its unique chain arrangement, which gives the

molecule a hydrophobic cavity on the inside with a hydrophilic cover on the outside. Hence the exterior can dissolve in water and the cavity can attract and encapsulate the lipophilic guest molecules to form inclusion complexes (Li et al., 2014; van der Veen et al., 2000).

The structure of α-cyclodextrin gives it a surface-active property, which enable it to be used to stabilise the food emulsions (da Silva, 2009; Del Valle, 2004; Mathapa & Paunov, 2013). When α-cyclodextrin is added into an O/W emulsion, the hydrophobic cavity can attract and encapsulate the fatty acid tail of triglycerides while the hydrophilic exterior can dissolve in the aqueous phase. As a result, a layer of α-cyclodextrin forms in the surface of oil droplets, leading to a reduction of interfacial tension and protect the oil droplets from aggregation (Figure 2.3C). In New Zealand, α-cyclodextrin is classified by FSANZ as a novel food ingredient with no specific limit of use (Food Standards Australia New Zealand, 2004).

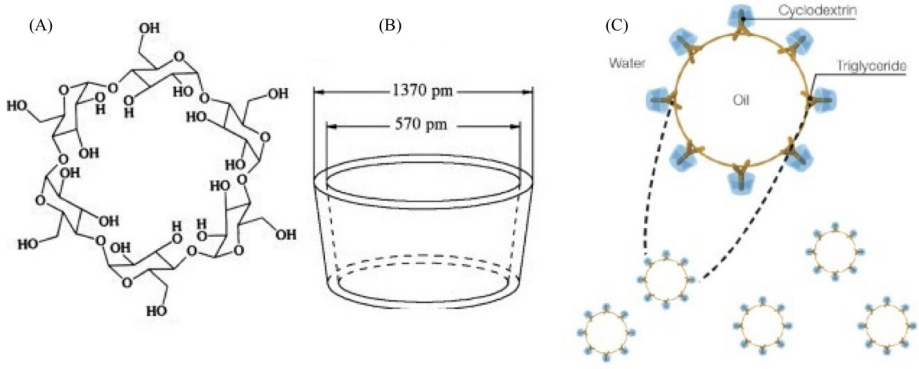



**Figure 2.3 Chemical structure of (A) α-cyclodextrin and (B) its three-dimensional form; (C) the emulsion-stabilizing properties of α-cyclodextrin (Astray et al., 2009; Wacker Chemie AG, n.d.).**

### 2.7.3.2 Starch

Starch is a polysaccharide consisting of a large number of glucose units, which was joined together by glyosidic bonds. Starch mainly comprises of two macromolecular components, namely amylose and amylopectin. As shown in Figure 2.4, the unit structures of amylose and amylopectin are illustrated.

Typically, normal starch granules consist of 15-25% amylose and 75-85% amylopectin and the ratio might vary in starches from different botanical sources (Tako et al., 2014). Amylose is a linear molecule consisting of repeating α-(1,4)-glucan units linked together by α-(1,4)-linkages. On the other hand, in amylopectin, glucose residues are first joined together by α-(1,4)-linkages to form short- and long-unit chains, which are then combined with α-(1,6)-bonds to form a very large, highly-branched structure (Bertoft, 2015; Hanashiro, 2015). The different structures of amylose and amylopectin give them different properties. For example, amylose is unstable and has a strong tendency to re-associate (retrograde) unlike amylopectin, which can lead to the formation of gels and films. On the other hand, amylopectin is more stable in aqueous dispersion and less sensitive to gelation (Jane, 2009; Zia-ud-Din et al., 2017). Waxy starch does not contain amylose but is almost exclusively made of amylopectin. Compared to normal starch, waxy starch is solubilised faster in water as the high content of amylose in normal starch inhibits the extensive starch granule swelling. Also, with the absence of amylose, waxy starch produces a viscoelastic gel with better clarity, lower gel hardness, and less tendency to retrograde (Schirmer et al., 2013; Tako et al., 2014).

**Figure 2.4 Unit structure of starch molecules: (A) amylose molecule and (B) amylopectin molecule (Pérez et al., 2009).**

Starch is rarely used in the food processing industry in its native form due to its shear and thermal instability as well as its strong tendency to decompose and retrograde during food processing (Alcázar-Alay & Meireles, 2015). Therefore, modification of starch has been adopted to improve its properties and expand its application. In general, starch could be modified by three different methods (physical, chemical and enzymatic). Physical

modification involves the use of heat, moisture or shear, resulting in changes in the physical properties of starches (McDonagh, 2012; Zia-ud-Din et al., 2017). For example, pregelatinised starches, which are precooked and dried, are instantly soluble in cold water and produce a moderate stable viscous suspension without heating (Zia-ud-Din et al., 2017). The chemical modification of starch is accomplished by introducing the functional groups into the starch polymer molecules. This type of modification brings a variety of physicochemical benefits in starch behaviour, gelatinisation capacity, retrogradation and paste properties (Alcázar-Alay & Meireles, 2015). Starches may also undergo a controlled degradation by using hydrolysing enzymes in its enzymatic modifications (Kaur et al., 2012). It is worth to notice that commercially available modified starches are often prepared by not only one single modification method, but also through more complex modifications involving two or more processes (BeMiller, 2003). The resulting modified starches are permitted to use in the food industry as food additives with their applicable E numbers.

In this research project reported in this thesis, three different types of modified starches (E1422, E1442, E1450) were involved in the preparation of instant emulsions as thickeners and stabilisers. Thus, these starches are reviewed in terms of their modification processes and chemical and physical properties in the following section.

**Acetylated distarch adipate (E1422)**

Acetylated distarch adipate is a modified starch obtained by cross-linking and esterification with adipic acid and acetic anhydride (Tian et al., 2018). The chemical structure of the modified starch is shown in Figure 2.5. The cross-linking adds intra- and inter-connection between the hydroxyl groups within the molecules of starch granule. As a result, a three-dimensional network forms and it increases the degree of polymerisation and molecular mass of starch (Alcázar-Alay & Meireles, 2015; Zia-ud-Din et al., 2017).

By cross-linking, starch becomes less sensitive to the processing conditions and shows higher resistance to heat, pH and shear. The starch pastes of cross-linked starch are more viscous with heavier-bodied, shorter-textured and are less likely to break down during cooking and severe agitation (BeMiller, 2003). In addition, the acetylation with acetic anhydride results in replacement of the hydroxyl groups in starch polymers by acetyl groups. This process not only increases the swelling solubility and swelling capacity of starch but also reduces the retrogradation tendency and gelatinisation temperature compared to native

starch (Masina et al., 2017). Overall, acetylated distarch adipate has the characteristics developed both from cross-linking and acetylation, enabling the starch to be used as adhesion, thickening, texturising, stabilising and binding agent in numerous food products including sauces and salad dressings (Tian et al., 2018; Zia-ud-Din et al., 2017).

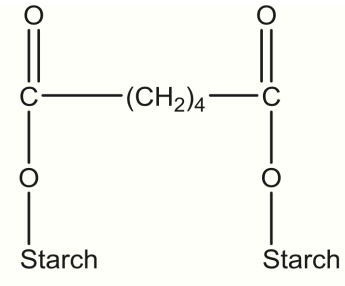



**Figure 2.5 Structural representation of distarch adipate; the acetyl groups present in acetylated distarch adipate are not shown (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) et al., 2017).**

**Hydroxypropyl distarch phosphate (E1442)**

Hydroxypropyl distarch phosphate is a type of modified starch produced by the combination of substitution and cross-linking. By the etherification with propylene oxide, the hydroxyl groups in starch polymers are substituted with 2-hydroxylpropyl ether. Then the etherified starch is cross-linked under the presence of sodium trimetaphosphate or phosphorus oxychloride. Thereby, the phosphate groups between the neighbouring glucose are crosswise linked, resulting in the hydroxypropyl distarch phosphate (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) et al., 2017; Kishida et al., 2001). The structure of the phosphate cross-link was shown in Figure 2.6.

Through hydroxypropylation, the retrogradation property of starch is reduced and the starch has the improved swellability and viscosity (Masina et al., 2017). Similar to the acetylated distarch adipate, hydroxypropyl distarch phosphate is more shear and acid resistant with improved paste stability (BeMiller, 2003).

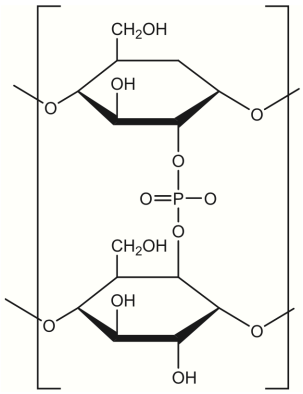



**Figure 2.6 Structural representation of a phosphate cross-link between two glucopyranose units in distarch phosphate; the substitution of the hydroxyl groups in starch is not shown (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) et al., 2017).**

### Starch sodium octenyl succinate (E1450)

Starch sodium octenyl succinate is a modified starch produced by starch esterification with ocetenylsuccinic anhydride (OSA) and neutralisation with sodium hydroxide (Tian et al., 2018). Through the modification, the hydrophilic starch polymer is introduced with the hydrophobic octenyl groups, resulting in whole molecules with an amphiphilic character and being used as a safe and reliable emulsifier thickener (Sweedman et al., 2013; Tian et al., 2018). With the presence of octenyl groups, the interconnections between starch molecules are weakened, which allows the granules to swell more rapidly at lower temperature and produce more viscous paste than native starches (Sweedman et al., 2013). The structure of the starch sodium octenyl succinate is shown in Figure 2.7.

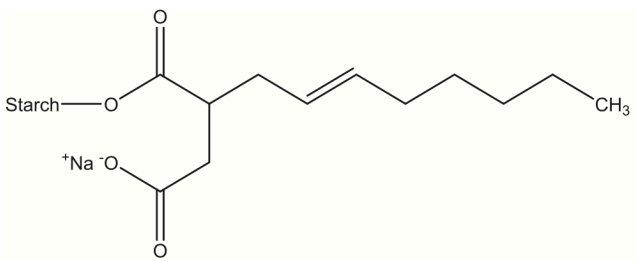



**Figure 2.7 Structural representation of starch sodium octenyl succinate (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) et al., 2017).**

### 2.7.3.3 Non-Starch Hydrocolloids

The combination of starch with other gums (e.g. xanthan gum, guar gum, carboxymethyl cellulose) has been employed in commercial formulations of mayonnaise and dressings to improve the rheological properties of the food products (Dolz et al., 2004; Lee et al., 2013). In this project, a variety of different non-starch hydrocolloids (xanthan gum, guar gum, carboxymethyl cellulose and λ-carrageenan) were involved in the preparation of instant emulsions as thickeners and stabilisers. Thus, the molecular structure, characteristic and functional properties of these hydrocolloids are discussed in the following section.

**Xanthan Gum**

Xanthan gum is a heteropolysaccharide, which is produced by the fermentation of glucose and sucrose using the bacterium *Xanthomonas campestris* (Urlacher & Noble, 1999). Figure 2.8 shows the primary chemical structure of xanthan gum consisting of a linear 1,4-linked β-D-glucose backbone (like cellulose) with side chains containing one glucuronic acid and two mannose units (Sworn, 2009; Urlacher & Noble, 1999). Generally, the side chains of xanthan gum overlap, thus protecting the cellulose backbone from degradation against acids and alkalis, enzymes and prolonged mix (Sworn, 2009; Urlacher & Noble, 1999). Besides, owing to the side chains, xanthan gum completely hydrates and is readily soluble even in cold water (Urlacher & Noble, 1999).

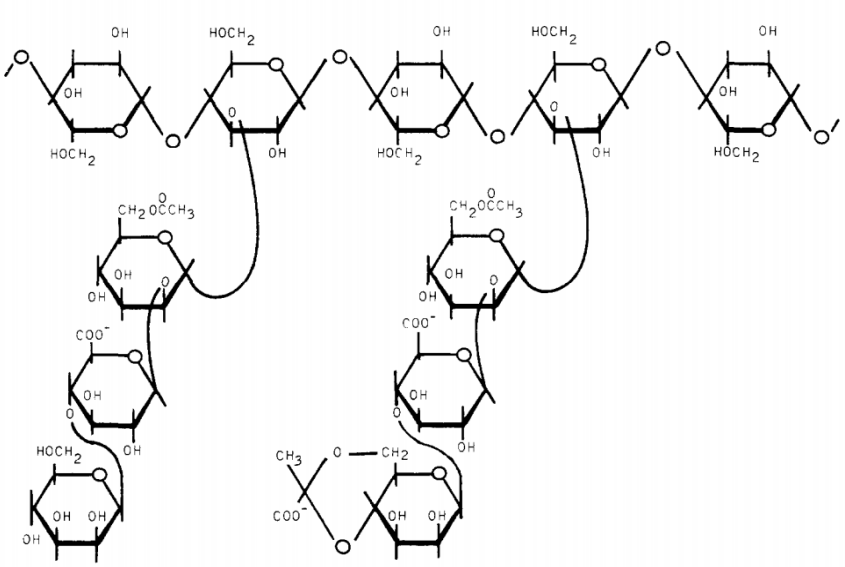


**Figure 2.8 Primary structure of xanthan gum (Holzwarth, 1976).**

The most important feature of xanthan gum is its rheological properties. Xanthan gum forms highly viscous solution even at low concentration. This solution exhibits highly pseudoplastic floe behaviour, which means the viscosity of xanthan solution is decreased progressively when the shear applied is increased. But when the shear is removed, the initial viscosity of xanthan gum solution can be recovered almost instantaneously (Urlacher & Noble, 1999). This feature makes products containing xanthan gum become easier to pour, mix or pump when a shear is applied but remain good stability during transportation and storage. Theoretically, the rheological property of xanthan gum is given by its molecular structure. In solution, xanthan gum molecule forms complex intermolecular aggregates through weak intermolecular forces, including hydrogen bonds and polymer entanglements. At low shear rate, this highly ordered network contributes to the high viscosity. When shear increases, these aggregates are gradually disrupted and results in a highly pseudoplastic flow behaviour (Sworn, 2009).

**Galactomannans**

Guar, locust bean (carob) and tara gums are chemically known as galactomannans and are derived from the seed of leguminous plants, such as Cyamopsis tetragonoloba, Ceratonia siliqua and Caesalpinia spinosa, respectively. Galactomannans are a long linear polysaccharide composed of D-mannose residues connected by 1,4-β-linkages to which α-D-galactose is linked as side chains (branches) through a 1,6-glycosidic bond (Figure 2.9). However, the number of galactose branches varies depending on the source of galactomannans. In locust bean gum (LBG), the ratio of mannose to galactose is 4:1 whereas it approximately 2:1 ratio in guar gum and 3:1 ratio in tara gum (Fox, 1999; Wielinga, 2009).

It is believed that the mannose-galactose ratios have a significant impact on the gums' water solubility. With the high number of galactose branches, the cohesion between the mannose backbones of different neighbouring galactomannans is prohibited. As a result, gum becomes more hydrated and dissolved in water as water can easily penetrate between the molecules even at room temperature (Wielinga, 2009). Thus, commercial gums which have a high ratio of galactose substitution, such as guar gum, can be fully dissolved even in cold water, whereas gums with low ratio of galactose side chains, such as LBG, are less water soluble and hydrate completely only in hot water (Pegg, 2012). Tara gum is partially dissolved in cold water bridging the gap between guar gum and LBG (Wu et al., 2018).

Guar gum, LBG and tara gum are well known mainly for their ability to change the rheological properties of aqueous systems: galactomannans can product the high low-shear viscosity at relatively low concentrations and exhibit the high shear thinning rheological properties (Saha & Bhattacharya, 2010). Among those three gums described above, guar gum has the highest thickening ability, which is followed by tara gum. LBG generates the least viscosity among the three seed gums (Lopes da Silva & Gonçalves, 1990; Wu et al., 2018).

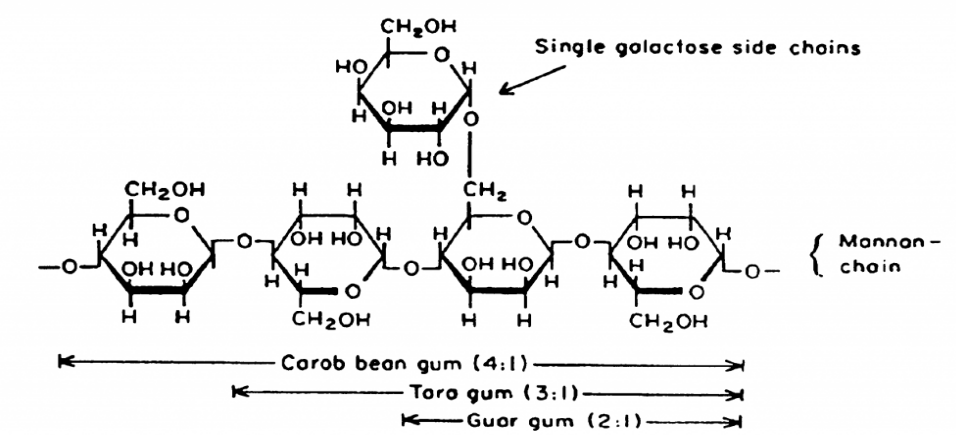



**Figure 2.9 Structure of a galactomannan chain.**

**Cellulose Gum**

Cellulose is one of the most abundant materials available in nature. It is a long-chain polysaccharide made up of glucose monomeric units joined by 1,4-β-linkages without side chains. Due to its chemical structure, cellulose molecules are bound together to form crystalline structure, which makes it insoluble in water and in most organic solvents (Morana et al., 2011). In the food industry, modified cellulose is used as food additives. For example, sodium carboxymethyl cellulose, which is called simply carboxymethyl cellulose (CMC), is widely used as thickening agent and stabiliser.

CMC is derived from raw cellulose by substituting the anhydroglucose monomers of the cellulose chain with methyl carboxylic group. Theoretically, each anhydroglucose unit contains three hydroxyl groups that can be substituted. In fact, the degree of substitution (DS) required to produce desirable properties is much lower than the theoretical maximum. Typically, CMC used in food application has a DS within the range of 0.65 to 0.95 (Murray,

2009; Zecher & Gerrish, 1999). Figure 2.10 shows the structures of cellulose and modified cellulose (CMC with a DS 1).

**Figure 2.10: Structural features of (A) cellulose and (B) CMC with a DS 1 (Murray, 2009).**

CMC is a linear anionic polysaccharide, which is soluble either in hot or cold water. It can build up viscosity rapidly to produce a clear and colourless solutions with neutral flavour. The rheology of CMC solution is significantly affected by the DS together with the uniformity of substitution. As the DS increases, the hydration rate of the polymer increases, and the CMC solution changes from thixotropic to more pseudoplastic (Murray, 2009; Zecher & Gerrish, 1999). Similarly, uniformly substituted CMC tends to exhibit pseudoplasticity and results in a smoother mouthfeel compared to the randomly substituted CMC (Murray, 2009; Zecher & Gerrish, 1999).

CMC has an E-number of 466 and is used as a thickener and stabiliser in a wide range of food applications (Pegg, 2012). It can be used in sauces and dressings due to its property of rapid viscosity production, along with its water-binding ability and good tolerance to pH (Murray, 2009). Generally, there is no limitation in the usage level of cellulose gum. However, excessive use of CMC can raise the costs and result in an unpleasant ‘gummy’ mouthfeel (Murray, 2009).

## Carrageenan

Carrageenan is a naturally occurring polysaccharide extracted from red seaweeds. It has been extensively utilised in a wide range of food systems due to its strong thickening and stabilising properties. Carrageenan is a large molecular weight linear polysaccharide which exists in three different types: κ-carrageenan (kappa), ι-carrageenan (iota) and λ-carrageenan (lambda). All of these have a galactose backbone joined together by alternating α-(1,3) and β-(1,4) glycosidic bonds, but differ in the proportion and location of ester sulphate groups and the proportion of 3,6-anhydrogalactose (3,6-AG) (Figure 2.11) (Imeson, 2009; Thomas, 1999).

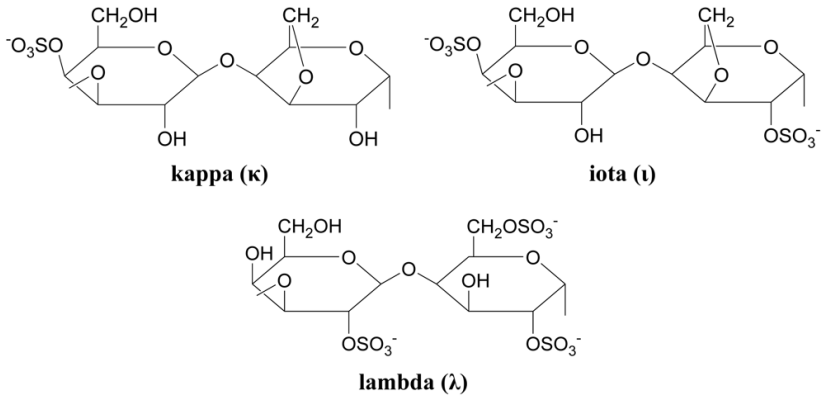



**Figure 2.11 Basic structural unit of carrageenan molecules (O'Sullivan et al., 2010).**

Lambda carrageenan contains approximately 35% of ester sulphate with little or no 3,6-AG, while the proportion of ester sulphate group and 3,6-AG are around 25% and 34%, respectively, for kappa carrageenan and 32% and 30%, respectively, for iota carrageenan (Thomas, 1999). The solubility of carrageenan is influenced by the overall balance of hydrophilicity as provided by the hydrophilic sulphate and hydroxyl groups and the hydrophobic 3,6-AG residues. Thus, although all three types are soluble in hot water, lambda type is the only one that can produce viscosity in cold water (Thomas, 1999). Thus, only lambda carrageenan was taken into consideration to use in this research project. Unlike kappa and iota carrageenan can form gel, lambda does not form gel but it exhibits random distribution of polymer chains in solution can confers high viscosity to the solution (Thomas, 1999). This viscous solution exhibits shear-thinning when a shear is applied (Imeson, 2009).

Carrageenan is classified as E 407 as food additive. Carrageenan has a very wide variety of uses. As recommended by Thomas (1999), carrageenan can be utilised in the dry mix and cold process salad dressings to promote the emulsion stabilisation, with a usage level of 0.6-1.0% and 0.2-0.5%, respectively.

## 2.8 Conclusions

The purpose of this review is to understand the formation and characterisation of O/W food emulsions as well as the chemical structures and properties of some food additives (starches, gums and emulsifiers) used in the preparation of O/W food emulsions. Thus, based on the knowledge of how to make and stabilise emulsions, the concept of instantly structured creamy emulsions was able to be further developed. The instant emulsions were formed using an instantaneous emulsification process which involves simply adding cold water to an oil mixture containing emulsifiers and thickening agents followed by mixing the mixture by hand shaking. Thus, the preparation process of the instant emulsions is more convenient and simple because no electrical equipment was involved during the preparation and storage of the products so that the emulsions could be made anywhere. In addition, the development of instant emulsions has many potential applications with different flavours and colours. Although some researches have been conducted on the development of instant emulsions, the effect of different types and concentrations of polysaccharides and surfactants on the formation and physicochemical properties of instant creamy emulsions has not been investigated thoroughly. Therefore, this study was carried out to fill some of the existing gaps.

# Chapter 3 Materials and Methods

The aim of this study was to investigate the effects of different ingredients on the formation and physicochemical properties of an instant emulsion, which was simply made by a relatively low shear generated through hand shaking. Thus, based on a standard formulation created from a number of preliminary trails, a series of formulations with some modifications were designed by varying the types and concentrations of the major ingredients, such as starches, non-starch hydrocolloids and emulsifiers.

In this chapter, the general materials and the procedures used in preparing instant emulsion are described. The methodologies employed to characterise the properties of the instant emulsions are also discussed. However, the specific formulations for investigating the influence of each key ingredient would be further explained and described in each experimental section of this thesis (Chapters 4, 5 and 6).

## 3.1 Materials

The ingredients used in preparing the instant emulsion samples were as follows: 40% w/w canola oil (Value, New Zealand), 4% w/w food starch (Ingredion ANZ Pty Ltd., New Zealand), 0.2% w/w xanthan gum (Danisco, New Zealand), 0.3% w/w citric acid (Hawkins Watts Ltd., New Zealand), 0.5% w/w egg yolk powder (LM Wright & Co., New Zealand), 0.5% w/w Tween 80 (Scharlau, Spain), and 54.5% w/w distilled water (Millipore RiOs 8, USA). This base formulation, which is also shown in Table 3.1, was based on some preliminary trials and workshops (not shown in this thesis).

## 3.2 Preparation of Instant Emulsions

In this study, 200 g of instant creamy emulsion samples were prepared. A 400 mL plastic container with a sealed lid (Thermo Fisher, New Zealand) shown in Figure 3.1 was used to mix all the ingredients for the process of emulsification. Based on the percentage weight of each ingredient as described above, the formulation for preparing 200 g of sample was calculated which is shown in Table 3.1. The instant emulsion sample was prepared in a three-step procedure. Firstly, all the dry ingredients (starch, xanthan gum, citric acid, egg yolk powder) were weighed into the plastic container and mixed thoroughly without lumps using a stainless spoon. Secondly, oil was added to the dry mixture. After closing the container, it was shaken thoroughly by hand via quickly moving the container up and down

to form a slurry. Lastly, distilled water mixed with Tween 80 at 30 °C was slowly poured into the slurry. The container was then sealed with the lid again and shaken vigorously by hand for 2 minutes. In this project, the shaking of the plastic container containing all the ingredients by hand was tried to be consistent, so that the shear force generated through the hand shaking to breakdown the oil phase for the instantaneous emulsification process was relatively constant.

**Table 3.1 The standard formulation for preparing 200 g of instant emulsion sample.**

| Ingredients | Weight (g) | % (w/w) |
|---|---|---|
| Canola oil | 80 | 40 |
| Starch | 8 | 4 |
| Xanthan gum | 0.4 | 0.2 |
| Citric acid | 0.6 | 0.3 |
| Egg yolk powder | 1 | 0.5 |
| Tween 80 | 1 | 0.5 |
| Water | 109 | 54.5 |
| Total | 200 | 100 |

**Figure 3.1 The plastic container used in this research project to make instant emulsions.**

### 3.3 Visual Observation of Instant Emulsions

After the preparation of instant emulsions, a small amount of each sample was transferred into a petri dish and then observed visually to examine the presence of phase separation, free oil, lumps, etc. Photos of samples were also taken for the purpose of comparison between samples.

### 3.4 pH Measurement of Instant Emulsions

The pH of emulsion samples was measured at 20 °C in triplicate by using a pH meter (Sartorius, Germany) after the pH meter was calibrated against two standard pH buffer solutions (pH 4.0 and pH 7.0).

### 3.5 Emulsion Stability

A 40 g of sample was weighed and transferred into a 50 mL centrifuge test tube (Thermo Fisher Scientific, New Zealand). Then the tube was tightly sealed with plastic cap and centrifuged under 3792 g for 10 minutes at 20°C by using a Sigma 6-16KS centrifuge (Sigma-Aldrich Inc., Germany). After centrifugation, the stability of emulsion samples was visually evaluated by identifying the presence of free oil, phase separation, aggregation and creaming in the tube.

### 3.6 Viscosity Analysis

The viscosity of emulsion samples was analysed by using a rheometer (AR550 advanced rheometer, TA Instruments, USA). The instrument was controlled by the Rheology Advantage Instrument Control AR software (version 5.8.2, TA Instruments Ltd., USA) and the data was collected and analysed by the Rheology Advantage Data Analysis software (version 5.7.0, TA Instruments Ltd., USA). The measurement of flow properties of emulsion was carried out at 20˚C using a 2˚ stainless steel plate (diameter of 40 mm). A circulating water bath at 10˚C was connected to the rheometer for ensuring the constant temperature. For each test, 1 ml of sample was loaded onto the Peltier plate by a pipette, then the rheometer with the filled sample was initially temperature equilibrated for 2 minutes. The sample was then sheared with a programmed continuous sequence in which the shear rate was increased linearly from 2.0 to 100.0 $s^{-1}$ over a 5-minute period. Shear

stress-shear rate data were collected continuously at 10 second intervals throughout the test. Each sample from the replicated experiments was analysed in triplicate.

In order to better describe the rheological properties of the emulsion samples, the obtained shear stress-shear rate data were fitted to the Power-Law model by using Microsoft Excel (version 15.32, Microsoft, USA). The model was described by the equation below, where $\delta$ is the shear stress in Pa, $\gamma$ is the shear rate in $s^{-1}$, $n$ is the flow behaviour index and $K$ is the consistency coefficient in $Pa \cdot s^n$.

$$\delta = K \cdot \gamma^n$$

### 3.7 Texture Profile Analysis

The textural properties of instant emulsion samples were analysed using a texture analyser (TA-XT plus, Stable Micro Systems Ltd., UK) with a 5-kg load cell. Back extrusion with a 35 mm disc probe was used to analyse the samples. A 60 g of sample was placed in an acrylic cylindrical container (40 mm internal diameter x 75 mm height) to give a height of 45 mm. Then the filled container was tapped to remove air bubbles. The measurement cycle was set to apply at a constant crosshead velocity of 5 mm/s, travelled to a sample depth of 40 mm and returned to the original position. Through the measurement, a force-time curve was generated by the Exponent Software (version 6,1,15,0, Stable Micro Systems Ltd., UK), through which the values of texture attributes, including firmness, cohesiveness, consistency and index of viscosity, were measured and obtained. For each sample, three experimental runs with repetition were performed and the average value with its standard deviation was calculated from the six measurements.

### 3.8 Particle Size and Particle Size Distribution

The instant emulsion sample was too viscous and concentrated to analyse its particle size directly. Therefore, a 10-times dilution was prepared for each sample. This was done by diluting 3 g of sample with 27 g of 0.05 M phosphate buffer solution (pH = 3), then the sample was stirred gently by using a magnetic stirring bar for 20 minutes to ensure uniform mixing and dispersion. The 0.05 M phosphate buffer solution was prepared by dissolving 0.30 g of phosphoric acid ($H_3PO_4$) and 2.98 g of potassium dihydrogen phosphate ($KH_2PO_4$)

in 500 mL of distilled water by using a 500 mL volumetric flask. Then the pH of the solution was adjusted to pH 3 using a 0.1 M phosphoric acid.

The mean particle size and particle size distribution of emulsion samples were analysed by utilising a Malvern Mastersizer (Hydro 2000sm, Malvern Instruments Ltd., UK). The refractive indices used for the oil and water phases were 1.465 and 1.33, respectively, and the absorption of the particle was set as 0.001. The measurement of particle size was conducted in triplicate from two replicate samples.

### 3.9 Microscopic Observation

Emulsion samples were observed at 5x, 10x and/or 40x magnification using a light microscopy (ZEISS Axiostar Plus binocular microscope, Carl Zeiss, Germany) attached with an AxioCam MRc digital camera. The images of emulsion samples taken were analysed by AxioVision SE64 Rel. 4.9.1 software (Carl Zeiss, Germany).

Emulsion samples were also analysed by using a confocal laser scanning microscopy (CLSM). A small amount of sample was placed in a cavity slide and 50 µL each of 0.2 g/L Nile Red and Fast Green were added before being covered with a coverslip. Imaging was carried out using the Leica DM6000B SP5 confocal laser scanning microscope system running LAS AF software (version 2.7.3.9723, Leica Microsystems CMS GmbH, Germany). Images were acquired with an HCX PL APO CS 20x (N.A. 0.70), 40x oil (N.A. 1.25) and 63x oil (N.A. 1.40) lens. Nile Red and Fast Green were sequentially imaged through excitation at 488 nm (argon laser) and 633 nm (HeNe 633 laser), respectively, and emission collection at 498-569 nm and 643-787 nm, respectively.

### 3.10 Data Analysis

The results were statistically analysed using the IBM SPSS Statistics Program (version 25, IBM Corporation, USA). The one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test were used to examine differences between the mean values at a significance level of $p < 0.05$. All the measurements (pH values, viscosity, textural attributes, particle size) were carried out at least in duplicate for each sample from duplicate experiments.

# Chapter 4 Effects of Type and Concentration of Starches on the Formation and Properties of Instant Creamy Emulsions

## 4.1 Abstract

In this research study, instant creamy O/W emulsions containing 40% w/w oil were formulated with seven different types of starch at different concentrations (0, 1, 2, 4 and 6% w/w), and the emulsions were prepared through an instantaneous emulsification process which involves mixing the ingredients in a closed plastic container by simple hand shaking. After the emulsion preparation, some physicochemical properties of the emulsion samples, such as visual appearance, pH, rheological and textural properties, particle size and particle size distribution, microstructure and emulsion stability, were analysed and characterised to determine the effects of different types and concentrations of starch on the formation and properties of instant creamy emulsions. The results revealed that the properties of the instant emulsions were significantly influenced by the type and concentration of the starch used. In order to obtain desirable rheological and textural properties, the starch added into the formulation for making instant emulsions must be a pregelatinised or cold-water-swelling starch which can disperse in cold water to form a thick, creamy emulsion containing 40% oil. In term of the effect of different types of starch, it was found that instant emulsion samples formulated with starch ingredients with smaller granular sizes, such as "Ultra-Tex-4" and "Ultra-Sperse-M", had smaller oil droplets (49 μm and 59 μm), respectively. On the other hand, the larger oil droplets (98 μm and 203 μm) of instant emulsions were formed when prepared without adding starch or with starch that had a larger granular size of starch "Quik-Tex 3331", respectively. In addition, it was identified that the modified cold-water-swelling waxy maize starch "Ultra-Tex-4" was the most suitable starch to be incorporated into the formulation of making instant creamy emulsions among all seven starches used in this research. The instant emulsion formed with 4% w/w of starch "Ultra-Tex-4" had the smallest oil droplet in diameter as 49 μm, as well as the most uniform mono-modal particle size distribution with a span value of 1.73. Compared to the other instant emulsions formed with other types of starch at the same concentration (4% w/w), the "Ultra-Tex-4" stabilised emulsion showed more shear thinning flow behaviour coupled with higher values of all the textural parameters in terms of firmness, consistency, cohesiveness and index of viscosity. This emulsion sample also exhibited better emulsion stability than the other samples formulated with different types of starch against creaming

and phase separation. In addition, the concentration of starch "Ultra-Tex-4" also showed a significant impact on the properties of the instant creamy emulsion. When increasing the concentration of the starch from 0 to 6%, the viscosity of the emulsion increased markedly. The mean particle diameter (D[4,3]) of oil droplets in this emulsion was decreased from around 72 μm to 47 μm when the concentration of "Ultra-Tex-4" was increased from 1 to 4% w/w with a shift from a bi-modal to mono-modal particle size distribution. However, no significant change was observed when the concentration of the starch was continuously increased to 6% w/w whereas the bi-modal particle size distribution was observed again. Furthermore, relatively better emulsion stability was obtained from the instant emulsion formed with 4% of "Ultra-Tex-4", as no formation of phase separation, free oil and creaming were observed visually. The results indicate that the optimum concentration of the starch "Ultra-Tex-4" to be incorporated into the instant creamy emulsion was 4% w/w.

## 4.2 Introduction

Mayonnaise is an O/W food emulsion consisting of vegetable oil, egg yolk, vinegar and other seasonings (e.g. mustard, salt) (Liu et al., 2007). Generally, commercial mayonnaise has a high content of oil for 70-80%, which significantly contributes to the high viscosity of mayonnaise and imparts to the product a unique flavour, creamy texture and thicker mouthfeel (Depree & Savage, 2001). Owing to its high level of oil content, mayonnaise is generally regarded as a "high-fat" and "high-caloric" food product (Puligundla et al., 2015). However, nowadays, consumers are more concerned about health issues (e.g. obesity, cardiovascular disease, cancer) related with the overconsumption of fat. Consequently, there is an increasing demand for mayonnaise or mayonnaise-type dressings with a reduced fat content (Liu et al., 2007).

Fat plays important functional roles in a high-fat emulsion product like mayonnaise containing 70-80% oil (e.g. appearance, flavour, texture, viscosity, shelf stability). Thus, the reduction of oil content can cause an adverse effect on the properties of emulsion (Depree & Savage, 2001; Nikzade et al., 2012). It is difficult to imitate the quality of full-fat mayonnaise when producing a low-fat version of mayonnaise. One possible approach is to employ the fat substituents in specific quantities to produce an emulsion with a texture close to that of traditional full-fat mayonnaise (Liu et al., 2007). One of the most commonly used fat substituents is starch, especially modified starch, due to its inexpensiveness, tastelessness and the unique creamy texture (Liu et al., 2007; Puligundla et al., 2015). The

key function of starch is to increase the viscosity of the emulsion continuous phase, thus conferring the thickened, creamy textural properties and stabilising the reduced-fat mayonnaise for longer shelf life (Taggart & Mitchell, 2009).

Some research studies have been conducted to characterise the reduced-fat mayonnaise formulated with native or modified starches (Dolz et al., 2004; Lee et al., 2013; Puligundla et al., 2015; Thaiudom & Khantarat, 2011). However, the emulsion samples in most studies were prepared through a homogenisation process by employing the mechanical homogenisers (e.g. high shear mixer, high pressure homogeniser, microfluidizer, colloid mills). There is very limited published literature focused on the utilisation of native and modified starch ingredients in an instant low-fat mayonnaise-like emulsion, which is formed using an instantaneous emulsification process that involves simply adding cold water into an oil slurry containing emulsifiers and dry ingredients (e.g. thickening agents) and mixing the resulting mixture of oil and water with a relatively low shear generated by hand shaking. Farrer et al. (2003) patented the preparation of a spreadable instant emulsion containing around 50% oil using a cold-water-swelling starch (e.g. Ultra-Tex-4, Ultra-Sperse M, Instant Clearjel) as the thickening agent. However, the effects of starch ingredients on the formation and properties of instant emulsions have not been investigated. Therefore, the objective of this research was to investigate the effects of different types and concentrations of starch on the formation and properties of low-fat instant creamy emulsions containing 40% w/w oil prepared by hand shaking. The instant emulsion samples prepared were analysed for their visual appearance, pH, viscosity, particle size and particle size distribution, microstructure, textural properties and emulsion stability.

## 4.3 Materials and Methods

### 4.3.1 Materials

Seven different types of starch ingredients as shown in Table 4.1 were used to determine their effects on the formation and properties of an instant creamy emulsion. These starches were denoted by sample codes as ST1, ST2, ST3, ST4, ST5, ST6 and ST7. These starches were provided by Ingredion ANZ Pty Ltd. (New Zealand).

These starch ingredients were selected because they have been used in previous studies for preparing the instant emulsions (Farrer et al., 2003), or based on the technical advises from the starch ingredient supplier. According to the product specification provided by the starch

ingredient suppliers, all the starches have the functional properties to be applied in making salad dressings or emulsified sauces, for example, good resistance to heat, acid and shear, building viscosity with or without heating treatment, and offering the products a rich and creamy texture. In addition, all the starches except for ST3 were pregelatinised or cold-water-swelling starches that are cold-water soluble. It should be noted that starch ST4 (Novation 5600) is referred to as a functional native waxy maize starch and is able to produce viscosity in cold water to thicken and stabilise food systems, thus meeting clean labelling criteria (Ingredion, 2015). ST1, ST2 and ST5 are hydroxypropyl distarch phosphate (E1442) which are modified by cross-linking the starch with sodium trimetaphosphate or phosphorus oxychloride and etherified with propylene oxide (EFSA Panel on Food Additives et al., 2017). ST3 and ST6 are acetylated distarch adipate (E1422) which are obtained by cross-linking and esterification with adipic acid and acetic anhydride (Tian et al., 2018). ST7 is starch sodium octenyl succinate (E1450) which is produced by starch esterification with ocetenylsuccinic anhydride (EFSA Panel on Food Additives et al., 2017). Based on the product specifications provided by the starch ingredient supplier, the functional features of these seven starch ingredients are summarised in Table 4.2. In addition, the other ingredients (e.g. oil, acid, egg yolk powder, etc.) which were also used in preparing the instant emulsions are described in Chapter 3.1 (Section 3.1).

**Table 4.1 List of seven different starches used to investigate their effects on the formation and properties of instant creamy O/W emulsions.**

| Sample | Product Code | E Number | Source | Cold-water soluble | Chemical modified |
|---|---|---|---|---|---|
| ST 1 | Ultra-Tex-4 | E1442 | Waxy maize | Y | Y |
| ST 2 | Ultra-Sperse-M | E1442 | Waxy maize | Y | Y |
| ST 3 | Purity HPC | E1422 | Waxy maize | N | Y |
| ST 4 | Novation 5600 | None | Waxy maize | Y | N |
| ST 5 | Quik Tex 3331 | E1442 | Tapioca | Y | Y |
| ST 6 | Instant FTD 176 | E1422 | Waxy maize | Y | Y |
| ST 7 | N-Creamer 46L | E1450 | Waxy maize | Y | Y |

"Y" indicates yes and "N" indicates no.

**Table 4.2 Functional features and applications of seven different starches used to investigate their effects on the formation and properties of instant creamy O/W emulsions (Ingredion, 2020).**

| Sample | Properties | Applications |
|---|---|---|
| ST 1 | Cold water swelling with intact starch granule;<br>Fine powder to hydrate quickly;<br>Good tolerance to heat, acid and shear;<br>Excellent freeze-thaw stability. | Cold processed salad dressings or emulsified sauces;<br>Dry mixed sauces and gravies. |
| ST 2 | Disperse easily in hot or cold water without lumping;<br>Yield a smooth, short texture with excellent sheen and clarity;<br>Extremely resistance to heat, shear and pH;<br>Impart a rich, creamy mouthfeel to prepared foods;<br>Excellent cold temperature storage stability. | Instant food preparations. |
| ST 3 | Cook-up starch;<br>Very good freeze thaw stability;<br>Good resistance to low pH and low temperature;<br>Impart exceptional clarity and are smooth, short-textured and heavy-bodied. | Frozen foods. |
| ST 4 | Clean labelling as simple “starch”;<br>Develop viscosity in cold or hot water;<br>Excellent dispersion characterisation;<br>Bland flavour profile;<br>Moderate process tolerance to heat, shear and acid;<br>Improved texture and stability. | Dry mix soups and sauces;<br>Dressings. |
| ST 5 | Cold water soluble;<br>High resistance to heat, shear and acid;<br>Outstanding freeze thaw stability;<br>Impart a rich and creamy mouthfeel and provide a smooth and heavy body. | Instant salad dressings. |
| ST 6 | Cold water soluble;<br>Freeze thaw resistance;<br>Impart a smooth, short texture gel with no tendency to retrograde.<br>Recommend in food systems down to pH 4.0. | Instant sauce;<br>Instant soup and dessert. |
| ST 7 | Cold water soluble;<br>Designed to bind water and fat/oil;<br>Acid and shear tolerance. | Dressings and emulsified sauces. |

### 4.3.2 Sample Preparation

#### 4.3.2.1 Starch Solution

A 6.8% w/w starch solution was prepared using each starch ingredient. The 6.8% w/w starch solution was calculated based on the aqueous phase (54.5 g) of the standard formulation containing 4% starch as described in Section 3.2, excluding oil and some other ingredients, by only adding 4 g of starch and 0.3 g of citric acid into 54.5 g of distilled water. Therefore, the percentage of starch was calculated as:

$$\%\ starch = 4 \div (4 + 0.3 + 54.5) \times 100 = 6.8\ \%$$

A 400 mL plastic container (Figure 3.1) was used for preparing the starch solution. After the starch, citric acid and distilled water were weighed into the container, the plastic container was closed securely with a lid and shaken vigorously by hand for 1 minute for hydration and dissolution.

#### 4.3.2.2 Instant Emulsions with Different Types and Concentrations of Starch

Instant emulsion samples were prepared based on the formulation and method as described in Section 3.2. However, seven different types of starch ingredients described above were used at different concentrations (0, 1, 2, 4, 6%) as shown in Table 4.3 to investigate their effects on the formation and characteristics of instant emulsions.

**Table 4.3 Formulations of instant emulsions prepared at different concentrations of starch (0, 1, 2, 4, 6% w/w).**

| Ingredients | Concentration (% w/w) | | | | |
|---|---|---|---|---|---|
| Canola oil | 40 | 40 | 40 | 40 | 40 |
| **Starch** | **0** | **1** | **2** | **4** | **6** |
| Xanthan gum | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Citric acid | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Egg yolk powder | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Tween 80 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Water | 58.5 | 57.5 | 56.5 | 54.5 | 52.5 |
| Total | 100 | 100 | 100 | 100 | 100 |

### 4.3.3 Analysis of Samples

The starch solution and the instant emulsion samples formulated with different types and concentrations of starches were analysed in various aspects, including their pH, visual appearance, mean particle size and particle size distribution, microstructural features, emulsion stability, rheological and textural properties as described in the Materials and Methods section of Chapter 3.

The mean particle diameter of starch solution samples was analysed by utilising a Malvern Mastersizer. The refractive indices used for the starch and water phases were 1.5 and 1.33, respectively, and the absorption of the particle was set as 0.001. The method used for analysing starch solution samples was the same as the method used for analysing instant emulsion samples described in Section 3.8 of this thesis.

### 4.3.4 Data Analysis

All the measurements were carried out at least twice for each sample from duplicate experiments. The results were statistically analysed using the IBM SPSS Statistics Program (version 25, IBM Corporation, USA). The one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test were used to examine differences between the mean values at a significance level of $p < 0.05$.

## 4.4 Results and Discussions

### 4.4.1 Characteristics of Starch Solutions

Initially, the properties of starch solutions prepared with seven types of starch ingredients were characterised to understanding how the formation and properties of instant emulsions would be affected by these different starch ingredients. Therefore, the rheological properties, particle size and microstructure of these seven starches were examined and compared.

Figure 4.1 shows a picture of seven different starches existing in a dry powdery form as starch ingredients for their visual appearance. It was observed that ST1 (Ultra-Tex-4), ST3 (Purity HPC), ST6 (Instant FTD 176) and ST7 (N-Creamer 46L) were whitish in colour and a very fine powder, while ST2 (Ultra-Sperse-M) and ST5 (Quik-Tex 3331) were a coarse white powder and ST4 (Novation 5600) was a coarse powder with a slight yellow

tint. As listed in Table 4.1, all the starches, except for ST3, were cold-water soluble, which implies that these starches could disperse into cold water to produce viscosity instantly without a thermal heat treatment whereas ST3 could not be soluble in cold water. This was confirmed visually by simply mixing each starch ingredient with cold water as shown in Figure 4.2. When dispersing 6.8% w/w starch in cold water, ST1, ST2, ST4, ST5 and ST6 starch samples formed an optically opaque thicken viscous solution which can be also described as starch pastes. On the other hand, a liquid suspension was obtained when starch ST3 was mixed with cold water and a white sediment was formed at the bottom of the aqueous solution. This was because the starch ST3 was not a pre-gelatinised or cold-water-swelling starch. ST3 could only dissolve in hot water as it requires heat for gelatinisation to produce viscosity. As for the ST7 starch (N-Creamer 46L), when it was mixed with cold water, an optically transparent thin liquid solution was observed with a layer of foam on the top of the solution. This could be due to the surface-active properties of this starch which is known to be chemically modified as sodium octenyl succinate starch (ST7) (Thaiudom & Khantarat, 2011).


**Figure 4.1 Appearance of seven different types of starch ingredients in a dry powder form.**

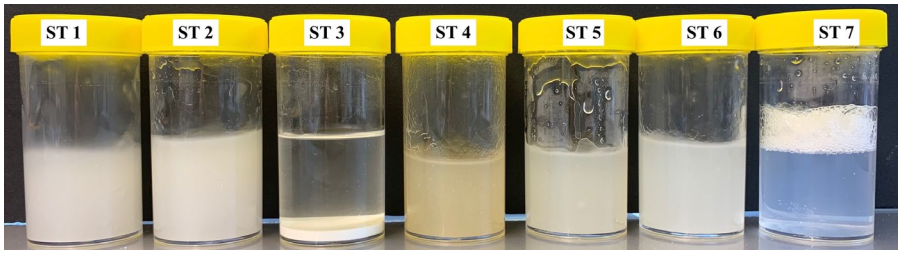



**Figure 4.2 Image of the starch solution (6.8% w/w) prepared from seven different types of starch by mixing with cold water.**

The structure of the starch solutions was also examined visually using an optical light microscope. The microscopic images taken are shown in Figure 4.3 although they are not clearly visible. It was observed that the ST3 sample (Purity HPC) was the only starch maintaining its granular integrity unlike all the others (ST1, ST2, ST4, ST5, ST6 and ST7) had highly swollen hydrated forms often called ghosts. ST1 (Ultra-Tex-4) and ST2 (Ultra-Sperse-M) showed a similar microstructural feature with similar particle sizes. These two starches are both cold-water-swelling starches (CWS) that have lost their crystallinity but retained their granular forms known as ghosts (BeMiller, 2003). The CWS starches could be prepared by heating the starch in an aqueous alcohol solution with sufficient water to allow gelatinisation and sufficient alcohol that granule integrity is maintained (BeMiller, 2003). This modification reveals the CWS starches to have some advantages, including greater viscosity, more homogeneous texture with higher clarity and more processing tolerance, compared to standard pregelatinised starches (Majzoobi et al., 2015). In contrast to ST1 and ST2, the complete granular disruption was observed for the pregelatinised starches (PG) including ST4 (Novation 5600), ST5 (Quik-Tex 3331) and ST6 (Instant FTD 176). Micrographs of PG starches exhibited irregular shapes and no intact starch granules could be found in the images. The presence of relatively large aggregates was observed from the images of ST4, ST5 and ST6 samples. As for the ST7 starch (N-Creamer 46L), a very little amount of starch was seen under the microscope, even though the concentration of ST7 solution was the same (6.8% w/w) as the others. This might be because most of the starch granules were trapped in the foam rather than being dispersed into the aqueous solution. Overall, some differences in the microstructural features were observed among the different starches used in this study. This can be attributed to differences in the plant source of starch as well as the modification methods applied in the production of starch ingredients.

In this study, it was attempted to quantify the particle size of dispersed starches in cold water using an instrumental particle size analyser (Mastersizer 2000). The results shown in Table 4.4 were in agreement with the results of visual observation shown in Figure 4.3. The particle size of starch (e.g. granular, ghosts, aggregates) in the starch solution containing ST4 and ST5 starches was the largest being around 200 μm, followed by ST6 at approximately 161 μm. ST1 and ST2 had a mean particle size of 42 and 55 μm, respectively. The particle size of ST3 was the smallest among all the starches (16 μm). However, the particle size of ST7 starch in solution was not able to be measured by the particle size

analyser used. One possible reason was that the measurement was carried out by taking a portion of sample from the transparent liquid phase of the starch solution, which might not contain a sufficient amount of starch particles to be well detected by a laser diffraction particle size analyser.

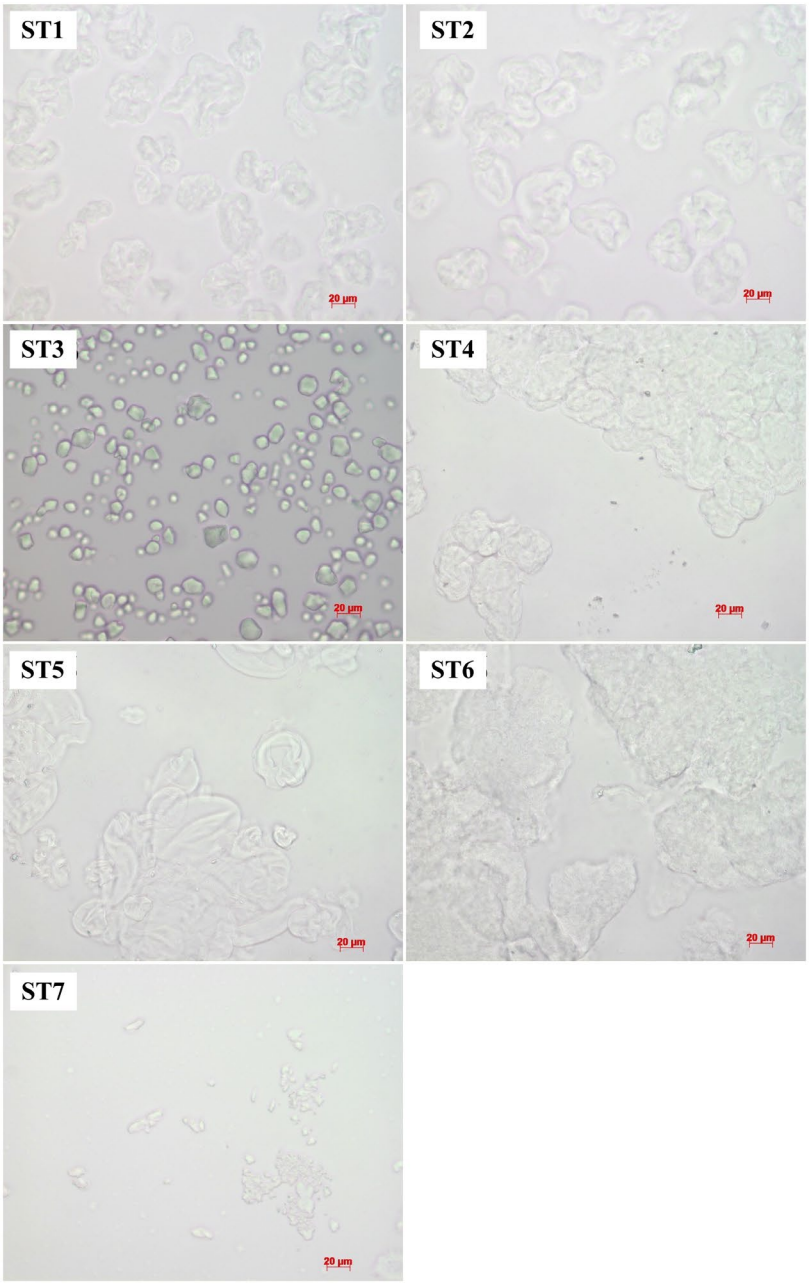



**Figure 4.3 Light microscopic images of starch solutions (6.8% w/w) with seven different modified starches (scale bar 20 μm).**

**Table 4.4 The mean particle diameter, D[4,3] (μm) of seven different starches in aqueous solution (6.8% w/w starch).**

| ST1 | ST2 | ST3 | ST4 | ST5 | ST6 | ST7 |
|---|---|---|---|---|---|---|
| $42 \pm 3.16^{d}$ | $55 \pm 8.21^{c}$ | $16 \pm 0.46^{e}$ | $202 \pm 18.24^{a}$ | $205 \pm 15.85^{a}$ | $161 \pm 11.02^{b}$ | N/A |

[a, b, c, d, e] Different letters represent significant differences ($p < 0.05$).

The rheological properties of starch solutions were analysed by characterising the relation between the shear rate and shear stress. As shown in Figure 4.4, the viscosity of ST6 sample was the highest among all the starches at the low or high shear rates (ranging from 5 $s^{-1}$ to 100 $s^{-1}$) and exhibited a strong non-Newtonian and shear-thinning flow behaviour. Its viscosity decreased sharply with increasing shear rate at a low level of shear rates and then tended to reach a relatively stable value when the shear rate was high. A similar behaviour was also observed for ST1, ST2 and ST5 samples with no significant difference in their viscosity values and with all shear-thinning flow behaviours. The observed shear-thinning behaviour of starch dispersions have also been reported by a number of studies (da Silva et al., 1998; Juszczak et al., 2012; Rao & Tattiyakul, 1999). The shear-thinning property of a thicken starch dispersion can be attributed to several molecular events that occur when the shear is applied to a starch dispersion, such as alignment and stretching of the starch polymers along the shear field, disentanglement of the starch molecules and gradual disruption of a starch network holding polymers together, which results in a decline of viscosity was observed (Ma et al., 2018; McClements, 2016).

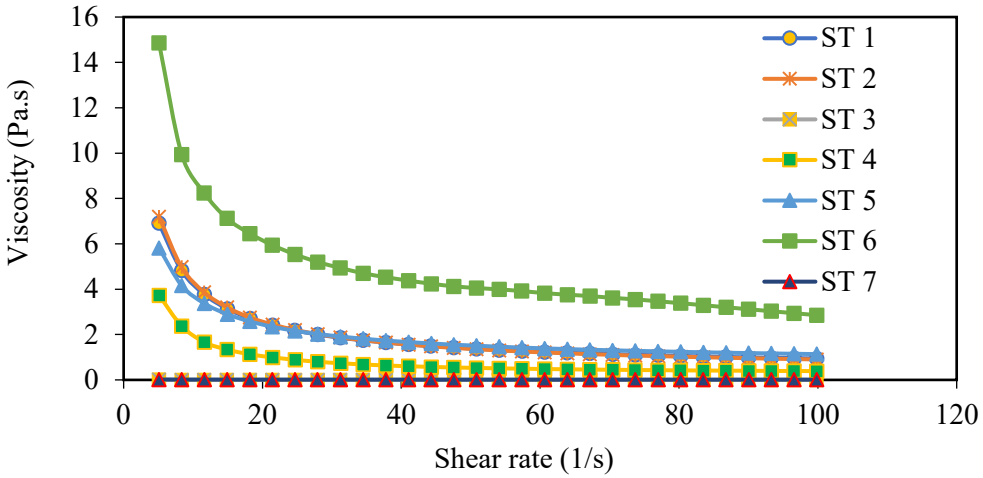



**Figure 4.4 Viscosity versus shear rate rheograms of seven different starch solutions (6.8% w/w starch).**

The viscosity of ST4 (Novation 5600) was observed to be slightly lower than the viscosity of the other starch samples except for ST3 and ST7. This ST4 starch was the only starch used in this study that is not modified chemically. However, this starch is known as a functional native starch that can be easily dispersed and swollen in cold water (Ingredion, 2020). In case of ST3 and ST7 samples, the viscosity of these samples was the lowest and had no significant viscosity decrease with increasing shear rate. This was in agreement with the previous observations as described in the above that the ST3 starch (Purity HPC) was not a cold-water soluble starch, thus not suitable for its utilisation as a thickening agent where a thermal heat treatment is not applied during the process. The ST7 starch (N-Creamer 46L) showed a pronounced surface-active property but had a very poor thickening property. As a result, the ST7 starch is used as a surfactant or a foaming agent rather than a viscosity modifier

### 4.4.2 Characteristics of Instant Emulsions with Different Types of Starch

#### 4.4.2.1 Appearance and pH

Instant emulsions containing 40% oil were prepared with seven different types of starches, such as ST1, ST2, ST3, ST4, ST5, ST6 and ST7, by simply shaking the mixture of dry and liquid ingredients by hand. These emulsion samples are designated hereafter as EST1, EST2, EST3, EST4, EST4, EST5, EST6 and EST7, respectively. The concentration of starch used initially was 4% w/w based on the standard formulation as shown in Table 3.1. After preparation, the appearance of emulsion samples was visually monitored. A picture of the emulsion samples taken is shown in Figure 4.5. Based on the results of visual observation, the O/W instant emulsions formulated with different starches were able to be produced efficiently more or less by hand shaking. Overall, the emulsion samples were semisolid that exhibited a white creamy texture with considerable viscosity, except for EST3 and EST7 which were a liquid-like, but they also differed to some extent in their appearance and textual properties. These emulsion samples (EST1, EST2, EST4, EST5 and EST6) had a heavy creamy structure like mayonnaise and remained without flow or motion at rest. However, the emulsions EST4 and EST5 seemed to have a gritty texture with the visible oily surface, indicating that the emulsification process was not effective and the oil added into the formulation was not fully emulsified.

For the emulsion samples EST3 and EST7, a liquid-like appearance with a thin texture was obtained that could flow freely. This can be related to the observed low viscosity

measurement of the corresponding starch solutions (ST3 and ST7) as already described in the above. This was because for the emulsion EST3, the added starch could not be soluble in cold water, thus the continuous phase of its emulsion was not thickened. As a result, the liquid-like emulsion was formed with the aid of emulsifiers (egg yolk powder and Tween 80) in the formulation. However, the emulsion was highly instable and broken down rapidly due to its low emulsion viscosity coupled with the presence of free oil. The undissolved starch granules moved downward to form precipitates at the bottom of the emulsion while the oil droplets moved upwards rapidly without restriction due to the thin continuous phase. Thus, large visible free oil droplets were observed on the surface of the liquid sample with a relatively strong oily smell. The sample EST7 showed a thin but more consistent texture compared to the sample EST3. This could be attributable to the surface-active properties of the starch (ST7) as already described above.

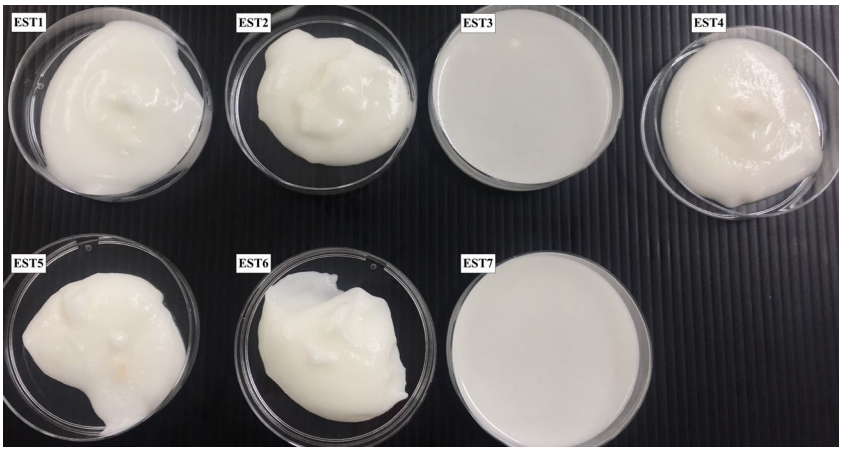



**Figure 4.5 Instant emulsion samples containing 40% w/w oil prepared with seven different types of starch at 6.8% w/w.**

The pH values of the seven emulsion samples measured are summarised in Table 4.5. Due to the citric acid added into the formulation, all the samples were acidic with a pH ranging from 2.7 to 3.1. From the microbiological safety point of view, the pH of mayonnaise and salad dressings is recommended to be below 4.1 to effectively prohibit the growth of microorganism, such as *Salmonella*, to prevent foodborne illness (Smittle, 1977). On the other hand, pH also plays an important role in determining the stability of emulsions stabilised by proteins (Depree & Savage, 2001). For example, when the pH is close to the isoelectric point of a protein molecule, the electrical net charge of the protein becomes a

net zero charge. The emulsion oil droplets stabilised by the protein emulsifiers will then lose their electrostatic repulsive forces, resulting in the flocculation and aggregation of the emulsion oil droplets (Chalamaiah et al., 2017; Depree & Savage, 2001). In this study, the pH of the instant emulsions would mainly affect the functionality of egg yolk proteins but had no effect on the functionality of a small molecule non-ionic surfactant (Tween 80), and this was further investigated in Chapter 6.

**Table 4.5 pH values of the instant emulsions prepared with seven different starch ingredients at 4% w/w starch concentration.**

| Sample | pH |
|---|---|
| EST 1 | 2.75 ± 0.05 |
| EST 2 | 2.82 ± 0.03 |
| EST 3 | 2.96 ± 0.08 |
| EST 4 | 2.85 ± 0.08 |
| EST 5 | 2.87 ± 0.06 |
| EST 6 | 3.07 ± 0.02 |
| EST 7 | 2.90 ± 0.04 |

#### 4.4.2.2 Rheological Properties

The viscosity measurement from seven different instant emulsions are presented in Figure 4.6. The apparent viscosity of all the samples decreased with the increasing shear rate over the whole range of shear rates used (5-100 $s^{-1}$). The flow curves indicated that all the emulsion samples, except for EST3 and EST7 samples, had a non-Newtonian and shear-thinning behaviour. The results were similar and corresponding to the viscosity measurement of starch solutions as described earlier in the above. Emulsions, such as mayonnaise and salad dressings, containing different types of starch have been reported to be a non-Newtonian fluid (Dolz et al., 2004; Rahmati et al., 2015; Teklehaimanot et al., 2013; Thaiudom & Khantarat, 2011).

In fact, the viscosity of emulsion samples decreased from a high value at low shear rate to a relatively low constant value at high shear rate. Among all the samples, EST6 exhibited the highest viscosity which was followed by EST1, EST2 and EST5 whose flow curves had overlaps along the range of some shear rates studied. The viscosity of EST4 was lower than

the emulsion samples EST1, EST2, EST5 and EST6. The viscosity of EST3 and EST7 was the lowest along the whole range of shear rates. As shown in Table 4.6, the viscosity of instant emulsion samples was reported at 20 $s^{-1}$ shear rate to simulated the shear rates that foods experience in the mouth during mastication (Chung et al., 2012). The viscosity of EST6 was higher than the other emulsions ($p < 0.05$). However, no significant difference was observed between the EST1, EST2 and EST5 samples ($p > 0.05$).

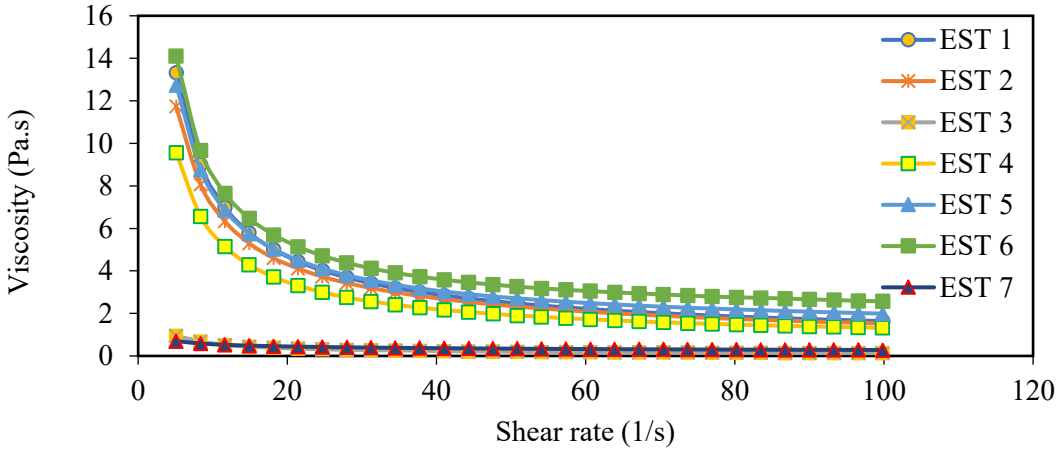



**Figure 4.6 Viscosity versus shear rate rheograms of seven instant emulsion samples containing different starch ingredients (4% w/w).**

When compared the flow curves between the instant emulsions (Figure 4.6) and starch solutions (Figure 4.4), it was observed that the viscosity of emulsion EST6 was similar to the viscosity of ST6 starch paste at the entire range of shear rate. This showed that the rheological properties of emulsion EST6 were predominated by the properties of its starch component (ST6). However, the viscosities of EST1, EST2, EST4 and EST5 emulsion samples were increased markedly when compared to the starch solutions at all shear rates. The possible reason for the enhancement of viscosity might be due to the intermolecular interaction between the starch molecules and xanthan gum in these emulsion systems. This finding seemed to be in accordance with those reported by some previous researches, which utilised a starch-gum combination to improve the rheological properties of mayonnaise and salad dressings (Dolz et al., 2004; Rahmati et al., 2015; Wang et al., 2016).

Furthermore, to identify the flow characteristics of instant emulsion samples, their flow curves were fitted to the Power Law model and the parameters are summarised in Table 4.6. The high coefficients of correlation ($r^2$ values of 0.97-0.99) showed the adequacy of Power Law model to describe the flow behaviour of the instant emulsion samples. The flow behaviour index ($n$) confirmed the shear thinning behaviour for all the samples, as the pseudoplastic fluid has a $n$ value smaller than 1 (Santana et al., 2015). The most shear thinning behaviour was observed from the samples EST1 and EST2 with the smallest values of flow behaviour index (0.31 and 0.33).

**Table 4.6 Power law parameter values of instant emulsion samples with different starch ingredients (4% w/w) and the shear viscosity of samples measured at a 20 $s^{-1}$ shear rate.**

| Sample | Shear Viscosity (Pa·s) | $k$ (Pa·$s^n$) | $n$ | $r^2$ |
|---|---|---|---|---|
| EST 1 | $4.45 \pm 0.23^{b}$ | 37.98 | 0.31 | 0.99 |
| EST 2 | $4.22 \pm 0.36^{b}$ | 33.02 | 0.33 | 0.99 |
| EST 3 | $0.34 \pm 0.07^{d}$ | 2.42 | 0.40 | 0.99 |
| EST 4 | $3.31 \pm 0.16^{c}$ | 25.00 | 0.35 | 0.98 |
| EST 5 | $4.51 \pm 0.39^{b}$ | 29.60 | 0.40 | 0.98 |
| EST 6 | $5.14 \pm 0.46^{a}$ | 28.27 | 0.46 | 0.97 |
| EST 7 | $0.44 \pm 0.09^{d}$ | 1.11 | 0.70 | 0.99 |

[a, b, c, d] Different letters within the same column represent significant differences ($p < 0.05$).
$k$: flow consistency coefficient, $n$: flow behaviour index, $r^2$: coefficient of correlation.

#### 4.4.2.3 Particle Size and Particle Size Distribution

The particle size distribution of oil droplets of instant emulsions containing 40% oil which were prepared with seven different starches (4% w/w) are presented in Figure 4.7. The values of d(0.1), d(0.5) and d(0.9) and the volume mean diameter D[4,3] are summarised in Table 4.7. The d(0.1), d(0.5) and d(0.9) values refer to the portion of particles with diameters smaller than this value is 10%, 50% and 90%, respectively.

As shown in Figure 4.7, the particle size distribution of the instant emulsion made without adding starch (denoted as ENST) was plotted as a control sample. Its particle size distribution curve was bimodal with one main peak at around 55 μm and another small peak at around 300 μm. The second peak might originate from the aggregation of oil

droplets. As there was no starch added to thicken the continuous phase, the movement of oil droplets in the emulsion system could not be prevented efficiently. Thus, the flocculation and coalescence of oil droplets might occur after the sample preparation. The volume mean diameter of this control emulsion was 98 μm which was relatively smaller in size than the other emulsions except for the EST1 and EST2 emulsions.

It is interesting to notice that among all the emulsion samples, the relatively most "symmetric" particle size distribution with the narrowest distribution width was observed from the two samples EST1 and EST2. These two samples had the smaller span values of 1.73 and 2.13, respectively, compared to the other emulsion samples. The results indicated that the size of oil droplets in the EST1 and EST2 emulsion samples were more uniform then the other samples. In addition, the EST1 and EST2 emulsion samples had the smallest mean particle size of 49 and 59 μm in diameter, respectively, compared to the other samples. The mono-modal distribution observed from both samples had one single peak at around 40 μm, which was smaller than the main peak of the control sample ENST (55 μm). Furthermore, the second peak of ENST at around 300 μm was reduced in the particle size distribution curve of EST2, and gradually flattened in the distribution curve of EST1. These changes suggested that smaller droplets could be produced under the same shear condition by adding the ST1 or ST2 starch into the formulation of the instant emulsions. Especially with ST1 starch, an emulsion with finer and more uniform oil droplets was formed. This finding could be also proved by monitoring the d(0.1), d(0.5) and d(0.9) values and volume mean diameter D[4,3] of the samples. The mean particle diameters of EST1 and EST2 were similar and both significantly smaller than the D[4,3] of the control sample prepared without adding starch (Table 4.7). Samples EST1 and EST2 also showed the smallest values of span, which indicates the width of particle size distribution for EST1 and EST2 were the smallest.

For the emulsion samples of EST4, EST5 and EST6, the bimodal distribution with two clear peaks at around 30-40 μm and round 240-360 μm was observed from the particle size distribution curves (Figure 4.7). This signified that these samples were polydisperse emulsions containing droplets in both small and larger sizes. Among them, the sample EST5 showed the largest value of D[4,3], which was associated with the large-diameter group of particles in the emulsion. The percentiles of EST5 indicated that 50% of the droplets was larger than 166 μm and 10% of the droplets was larger than 456 μm, which

was significantly larger than the particle size of the other samples. Samples EST4 and EST6 showed similar particle characteristics, as no significant difference was found on their mean diameter sizes and percentiles. The D[4,3] of EST4 and EST6 were around 150 μm and 160 μm, respectively (Table 4.7).

Samples such as EST3 and EST7 also showed a bimodal particle size distribution. The mean particle sizes of the emulsions (D[4,3]) were about 130 μm and 175 μm, respectively. It is interesting to note that the D[4,3] of EST3 sample which contained starch ST3 (Purity HPC) that was not soluble in cold water was significantly smaller than the D[4,3] of EST4, EST5, EST6 and EST7. However, the mean particle size of the EST7 sample containing ST7 starch (N-Creamer 46L) known to have the surface-active properties was not smaller than the other emulsion samples except for EST5. It can be thought that the mean particle size could be smaller when the viscosity of an emulsion is lower due to its relative higher efficiency of oil disruption during shaking. However, this pattern was not observed consistently because the very limited viscosity of an emulsion, such as EST3 and EST7, could not retard the movement of oil droplets in the emulsion system, which could cause the droplets to come into close contact and lead to the enhanced droplet aggregation (McClements, 2016). The presence of both small and larger droplets in the emulsion systems led to the largest values of span, leading to the broadest particle size distributions of EST3 and EST7 among all the samples (Table 4.7).

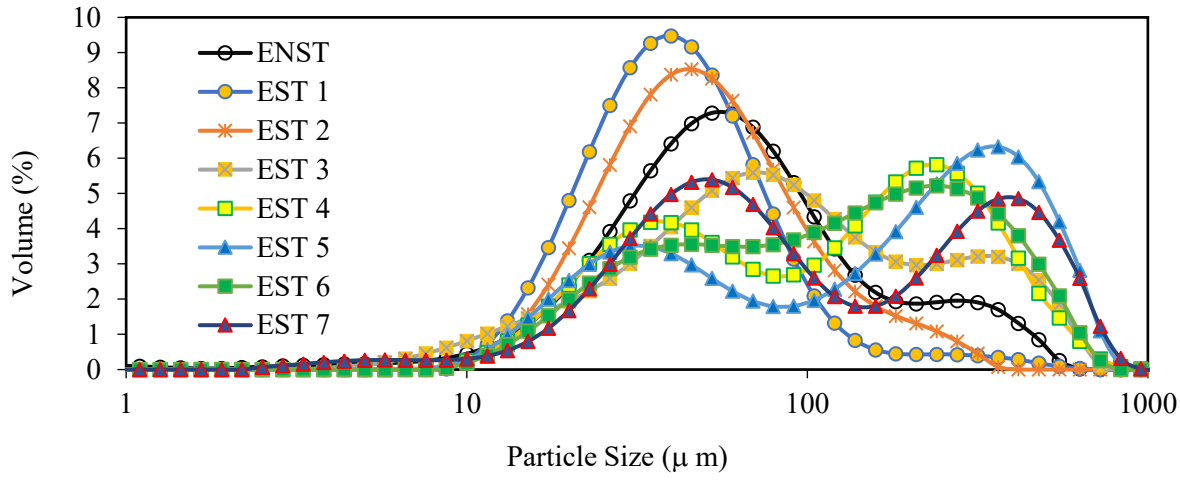



**Figure 4.7 Particle size distribution of oil droplets in instant emulsions without adding starch (ENST) and with seven different types of starch (4% w/w).**

**Table 4.7 Particle size of instant emulsion samples with different types of starch ingredients (4% w/w).**

| Sample | D [4, 3] (μm) | d (0.1) (μm) | d (0.5) (μm) | d (0.9) (μm) | Span |
|---|---|---|---|---|---|
| ENST | $98 \pm 4.95^{e}$ | $23 \pm 2.15^{a}$ | $62 \pm 4.53^{d}$ | $245 \pm 40.12^{c}$ | $3.60 \pm 0.82$ |
| EST 1 | $49 \pm 4.92^{f}$ | $18 \pm 0.44^{d}$ | $38 \pm 0.89^{e}$ | $84 \pm 9.38^{e}$ | $1.73 \pm 0.22$ |
| EST 2 | $59 \pm 4.40^{f}$ | $20 \pm 0.67^{c}$ | $45 \pm 1.87^{e}$ | $117 \pm 15.09^{d}$ | $2.13 \pm 0.27$ |
| EST 3 | $129 \pm 8.47^{d}$ | $20 \pm 1.34^{c}$ | $77 \pm 5.35^{c}$ | $337 \pm 23.26^{b}$ | $4.16 \pm 0.29$ |
| EST 4 | $150 \pm 14.84^{c}$ | $23 \pm 1.48^{b}$ | $112 \pm 12.33^{b}$ | $343 \pm 46.67^{b}$ | $2.89 \pm 0.54$ |
| EST 5 | $203 \pm 12.33^{a}$ | $22 \pm 1.02^{b}$ | $166 \pm 30.96^{a}$ | $456 \pm 21.32^{a}$ | $2.69 \pm 0.41$ |
| EST 6 | $160 \pm 12.33^{c}$ | $25 \pm 1.84^{a}$ | $118 \pm 4.93^{b}$ | $365 \pm 35.58^{b}$ | $2.89 \pm 0.20$ |
| EST 7 | $172 \pm 18.45^{b}$ | $24 \pm 1.18^{a}$ | $81 \pm 9.68^{c}$ | $446 \pm 38.64^{a}$ | $5.25 \pm 0.23$ |

[a, b, c, d, e, f] Different letters within the same column represent significant differences ($p < 0.05$).
D[4,3]: volume-weighted mean diameter; d(0.1), d(0.5) and d(0.9): the diameters where 10%, 50% and 90% of the particles are smaller than the values.

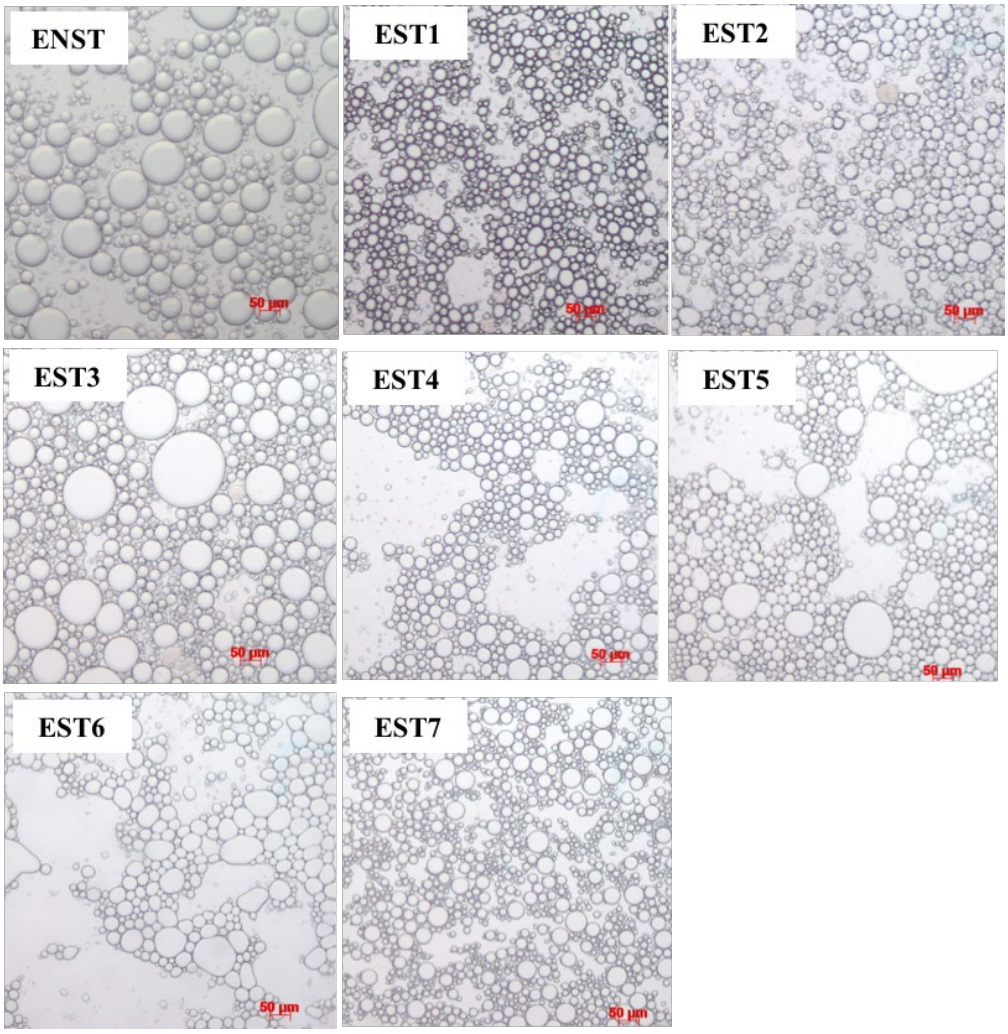
#### 4.4.2.4 Microstructure of Emulsions

The microstructure of the instant emulsion samples formulated with different starches were visualised by utilising two different microscopic techniques, namely digital light microscopy and confocal laser scanning microscopy (CLSM). The captured images are presented in Figure 4.8A and B, which revealed the extent of droplet flocculation and droplet size being different between the different emulsion samples.

The light microscopic images presented the presence of spherical oil droplets surrounded by a continuous aqueous phase. This observation was further supported by the CLSM images, in which the stained red oil droplets were dispersed in the continuous phase. It was obviously observed that all the samples were polydisperse emulsions with a broad range of oil droplet sizes. Among all the samples, the smallest oil droplets with the most uniformity was observed from the emulsion sample EST1, which was followed by the emulsion sample EST2 that contained a few oil droplets in larger sizes. For both emulsions EST1 and EST2, some degree of droplet flocculation was observed. However, due to the effect of starch as a thickening agent, the movement of the flocculated droplets could be restricted by the viscous continuous phase of the emulsion. Thus, the creaming process being promoted by the droplet flocculation would be retarded. On the other hand, the unevenly distributed

emulsion systems with a greater polydispersity were observed from the emulsion samples EST4, EST5 and EST6 in which oil droplets with large and small sizes were observed. This was also explained by the bimodal particle size distribution with two major peaks of these samples as described in the previous section (Figure 4.7).

(A)



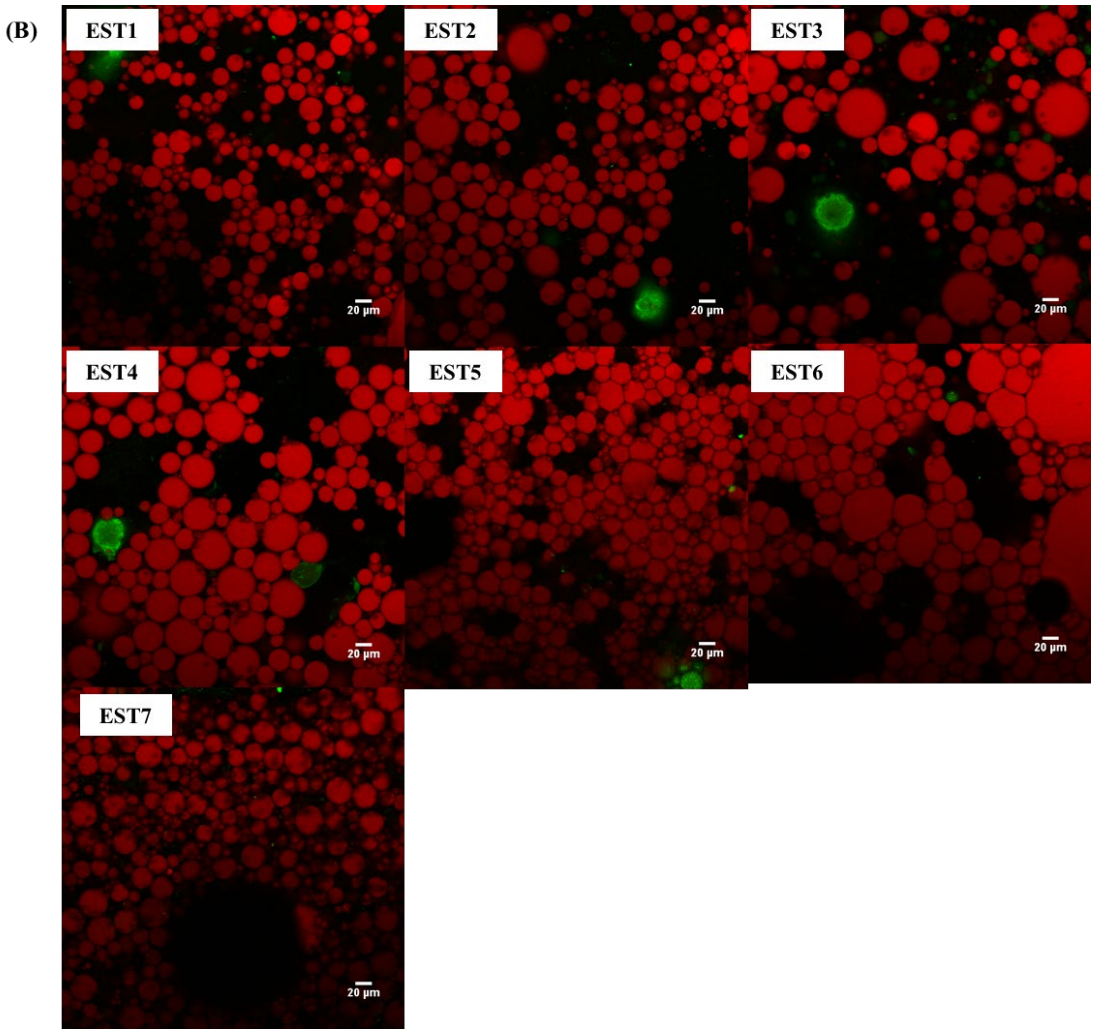



**Figure 4.8 (A) light microscopic images (scale bar 50 µm) and (B) CLSM images (scale bar 20 µm) of instant emulsions formulated without adding starch (ENST) and with seven different types of starch (4% w/w).**

In case of the EST3 and EST7 samples that were different from the other samples as aforementioned, no pronounced clusters of oil droplets were observed which might be due to the relatively low viscosity of the samples because both starches ST3 (Purity HPC) and ST7 (N-Creamer 46L) did not thicken the continuous phase of these instant emulsions when dispersed in cold water. The same phenomenon was also observed from the control sample without adding starch (ENST).

### 4.4.2.5 Stability of Emulsions

The stability of instant emulsions, in terms of free oil, creaming, phase separation and sedimentation, was assessed by centrifuging the emulsion samples at a relatively low speed (3792 g for 10 minutes). A picture of the emulsions taken after centrifugation is presented

in Figure 4.9. A thin layer of free oil was observed at the top of all the samples after centrifugation. This might be due to the nature of all the emulsions which were instantly structured by a low shear condition generated by hand shaking even though the mixture of ingredients was shaken vigorously by hand. This implies that the hand shaking wasn't strong enough to breakdown the oil phase into small droplets and a small portion of oil might not have been emulsified. As a result, all the instant emulsions formed a relatively much larger mean particle size with the broad range of particle size distribution compared to an emulsion that could be produced by high shear mixers, such as high pressure homogeniser and microfluidizer.

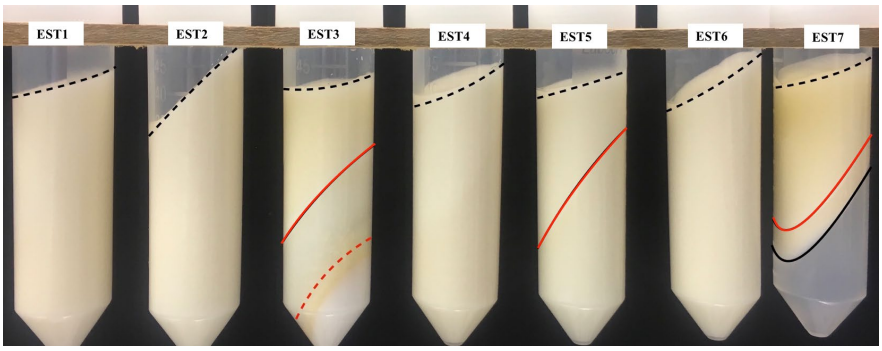



**Figure 4.9 A picture of instant emulsion samples containing seven different types of starch taken after centrifugation to observe the stability of emulsions against the formation of phase separation, free oil, etc. (----- indicates the boundary of free oil and cream layer; ——— indicates the boundary between cream layers; ——— indicates the boundary of cream layer and serum phase; ----- indicates the boundary of serum phase and sediment).**

Except for the thin layer of free oil observed on the surface of the samples, no other visible emulsion instability was detected from the emulsion samples EST1, EST2, EST4 and EST6. However, phase separation and creaming were observed form some emulsion samples (EST3, EST5 and EST7), especially EST3 and EST7. In sample EST3 containing a cook-up starch (Purity HPC starch) which requires heat treatment for its starch gelatinisation and thickening effect, an optically opaque creamed layer was observed on top of a less opaque middle layer as well as a layer of sediments containing the undissolved starch granules at the bottom of the test tube. In sample EST5, a creamy upper layer and a liquid layer with some turbidity in appearance like a very dilute emulsion, were obtained. On the other hand, in case of the EST7 containing a cold-water soluble starch with an emulsifying property (N-Creamer 46L), three layers, such as a creamy upper layer, an intermediate dilute

emulsion layer and a bottom serum phase, were discerned. The formation of phase separation in the emulsions could be considered as a sign of emulsion destabilisation which is facilitated by the emulsion creaming (Chivero et al., 2016; McClements, 2016). The larger extent of emulsion destabilisation observed from EST3 and EST7 samples was due to the type of starch ingredients with low viscosity and no thickening properties as shown in Figure 4.2. Thus, the oil droplets could move freely in the emulsion system promoting creaming and leading to the phase separation under the effect of the centrifugal force. On the other hand, even though the viscosity of the emulsion EST5 was relatively high to prevent the movement of oil droplets in the emulsion system, the presence of phase separation in this emulsion sample after centrifugation could be attributed to the presence of oil droplets in large particle sizes. As shown in Table 4.7, the largest mean particle diameter was observed from the emulsion EST5 (203 μm), which was significantly larger than the mean diameters of all the other emulsion samples. The larger droplets are more susceptible to gravitational separation than small ones, therefore, leading to rapid creaming with visualised phase separation in the emulsion sample EST5.

#### 4.4.2.6 Textural Properties of Emulsions

The texture parameter values of instant emulsions were measured, and the results are summarised in Table 4.8. Among all the samples, the highest values of firmness, consistency, cohesiveness and index of viscosity were all obtained from sample EST1, compared with the other instant emulsions ($p < 0.05$). This might be due to the finer particle size (49 μm) and more uniform particle size distribution (span value 1.73) of the instant emulsion (Table 4.7) coupled with its relatively high viscosity (Figures 4.4 and 4.6) under the effect of starch ST1 (Ultra-Tex-4) (Table 4.2). As stated by Liu et al. (2007), the smaller size of oil droplets could lead to a greater contact surface area between the droplets in the emulsion. Thus, the viscosity of the emulsion could be increased and a firmer emulsion could also be formed.

The second highest values for all the texture parameters were obtained from the emulsions EST2, EST5 and EST6. There was no significant difference found between the thickness (consistency), stickiness (cohesiveness) and resistance to deformation (firmness) between these instant emulsion samples ($p > 0.05$). This was followed by the sample EST4, which showed the significantly lower values in terms of all the texture parameters than the samples aforementioned. The lowest values of all the texture parameters were obtained from

samples EST3 and EST7, which could be associated with the low viscosity of the emulsion samples. It should be mentioned that the emulsion viscosity index shown in Table 4.8 was corresponding to the viscosity measurement results as shown in Figure 4.6. The textural attributes of food emulsions are directly related to their rheological properties (McClements, 2016). The effect of viscosity on the texture parameters reported by a number of studies indicates that increasing the viscosity of emulsion leads to the higher values of texture attributes (Amin et al., 2014; Khushbu & Sunil, 2018; Liu et al., 2007).

**Table 4.8 Texture parameter values of the instant emulsion samples containing seven different types of starch (4% w/w).**

| Sample | Firmness (g) | Consistency (g·s) | Cohesiveness (g) | Index of Viscosity (g·s) |
|---|---|---|---|---|
| EST 1 | $117.14 \pm 4.36^{a}$ | $771.42 \pm 25.94^{a}$ | $-96.91 \pm 0.74^{d}$ | $-305.68 \pm 5.69^{d}$ |
| EST 2 | $102.48 \pm 5.27^{b}$ | $685.36 \pm 37.43^{b}$ | $-86.47 \pm 4.33^{c}$ | $-274.24 \pm 13.03^{c}$ |
| EST 3 | $17.25 \pm 1.33^{d}$ | $114.57 \pm 3.21^{d}$ | $-6.73 \pm 0.51^{a}$ | $-2.30 \pm 0.56^{a}$ |
| EST 4 | $87.72 \pm 5.51^{c}$ | $581.16 \pm 42.59^{c}$ | $-70.54 \pm 5.28^{b}$ | $-226.77 \pm 14.07^{b}$ |
| EST 5 | $105.96 \pm 6.64^{b}$ | $695.65 \pm 59.03^{b}$ | $-90.92 \pm 5.87^{c}$ | $-285.96 \pm 22.69^{c}$ |
| EST 6 | $105.91 \pm 11.94^{b}$ | $693.21 \pm 80.95^{b}$ | $-88.70 \pm 8.84^{c}$ | $-280.44 \pm 25.06^{c}$ |
| EST 7 | $15.95 \pm 2.12^{d}$ | $109.77 \pm 5.14^{d}$ | $-6.77 \pm 0.70^{a}$ | $-2.31 \pm 0.62^{a}$ |

$^{a, b, c, d}$ Different letters within the same column represent significant differences ($p < 0.05$).

### 4.4.3 Effects of Different Starch Concentrations on Instant Emulsions

Based on the overall results as discussed in the previous sections, two types of starch ingredients, such as ST1 (Ultra-Tex-4) and ST6 (Instant FTD 176), were selected to further investigate the effects of their concentrations on the formation and physicochemical properties of the instant creamy emulsions. ST1 was chosen because of its outstanding ability to form and stabilise the instant emulsions with the relatively uniform particle size distribution, in which the mono-modal particle size distribution with the smallest values of D[4,3] and span was observed. ST6 was chosen because of its ability to build relatively higher viscosity instantly in the preparation of instant O/W emulsions. In other words, the shear viscosity of the instant emulsion samples prepared with ST6 (EST6) was observed to be the highest along the whole range of shear rates studied in this research. Also, the instant emulsion samples formulated with ST1 and ST6 showed good emulsion stability coupled with the relatively higher values for all the textural parameters. Therefore, the instantly

structured low-fat O/W emulsion was prepared with different concentrations of starch ST1 and ST6 (0, 1, 2, 4 and 6% w/w), respectively. The physicochemical properties of the formed instant emulsions were then characterised as discussed in the following sections.

#### 4.4.3.1 Visual Appearance

As shown in Figure 4.10, the whitish creamy emulsions were instantly formed by hand shaking regardless of the concentrations of starch used. However, it was observed that the thickness of the instant emulsion was largely dependent on the starch concentrations as expected. Especially when over 2% of starch was added into the formulation, the appearance of the instant emulsion was changed from a low-viscosity fluid to a viscoelastic semi-solid like. This trend was observed for both EST1 and EST6. However, when the starch concentration was too high, free oil was readily noticeable on the surface of the samples (EST1-6% and EST6-6%). This might be resulting from the highly viscous continuous phase of the emulsion under the effect of a very high concentration of starch, which makes the process of oil disruption and droplet dispersion less efficient, therefore, resulting in the presence of the non-emulsified free oil on the surface of the emulsion samples.

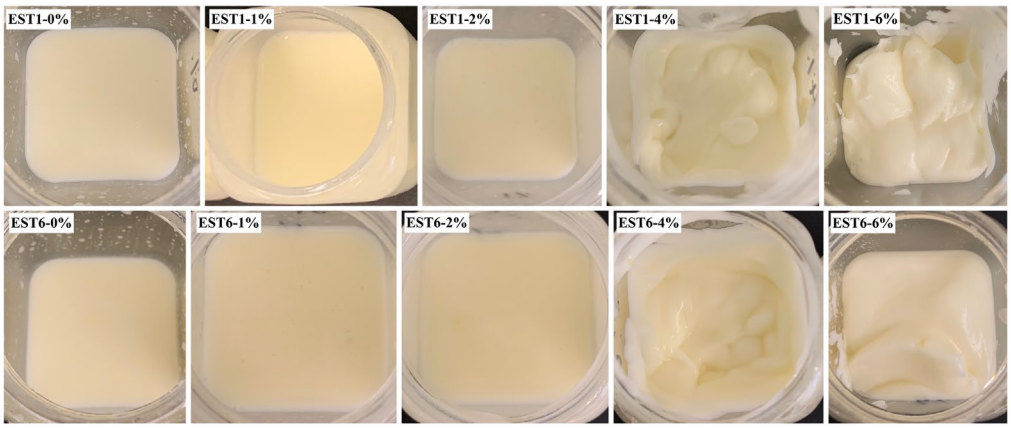



**Figure 4.10 Appearance of instant emulsion samples (EST1 and EST6) prepared with 0, 1, 2, 4 and 6% w/w of starch ST1 (images in the top row) and starch ST6 (images in the bottom row), respectively.**

#### 4.4.3.2 Rheological Properties of Emulsions

Figures 4.11A and B represent the flow rheograms of the instant emulsion samples formulated with various concentrations (0, 1, 2, 4 and 6% w/w) of ST1 and ST6 starches,

respectively. For all the samples, the non-linear relationship was observed between the viscosity and the shear rate. The viscosity of instant emulsions was reduced with the increasing shear rate over the range of 0-100 $s^{-1}$. In other words, the non-Newtonian pseudoplastic flow characteristic was observed for all the instant emulsion samples, especially the emulsions containing 4 and 6% starch. As expected, a higher viscosity was also observed with an increase in starch concentration for both ST1 and ST6 starches. However, the level of viscosity was significantly greater when the concentration of starch was increased from 4 to 6%. The apparent viscosities of EST1 and EST6 emulsion samples, which is at the shear rate that foods experience in the mouth during mastication (20 $s^{-1}$) (Chung et al., 2012), are listed and compared in Tables 4.9. The results also confirmed that the apparent viscosity of the instant emulsions increased significantly with the increasing concentrations of starch for both ST1 and ST6 ($p < 0.05$).

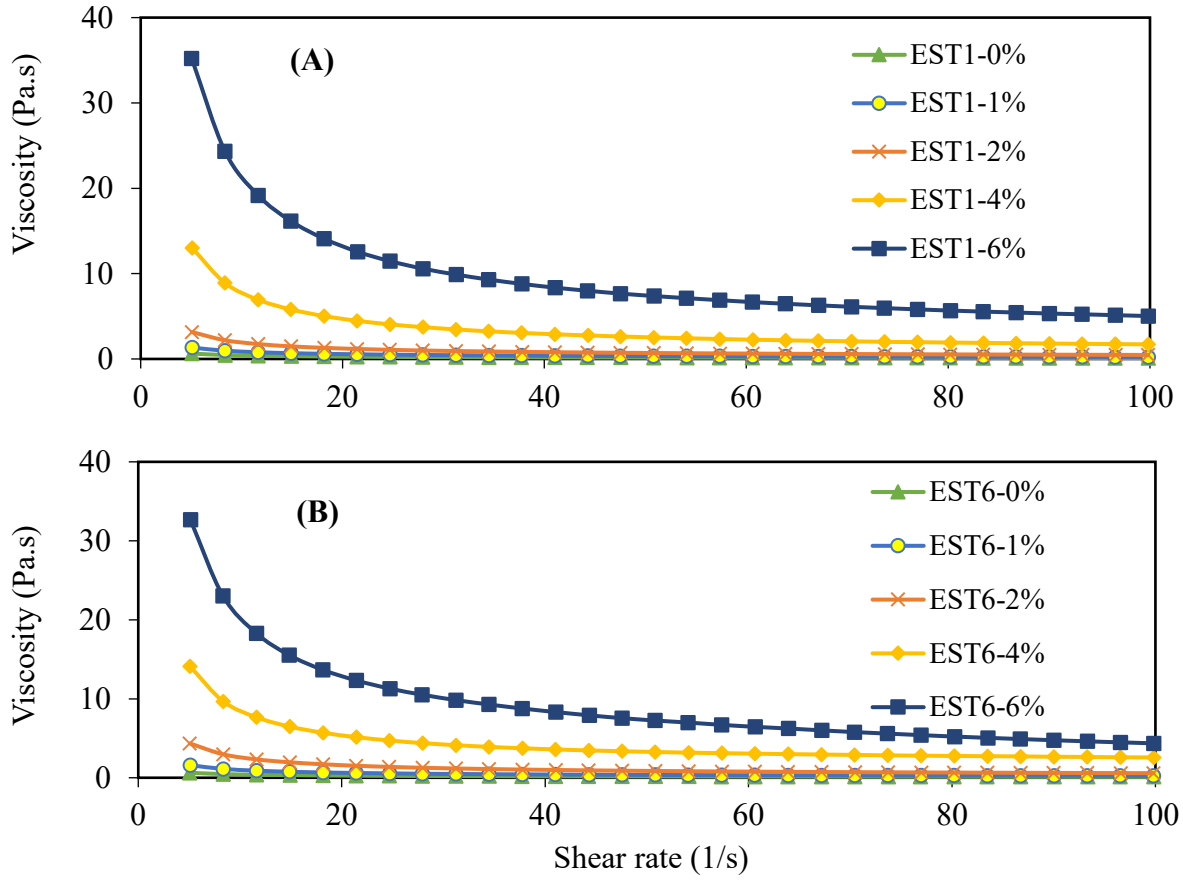



**Figure 4.11 Viscosity versus shear rate rheograms of instant emulsion samples prepared with (A) starch ST1 and (B) starch ST6, at different concentrations (0, 1, 2, 4, and 6% w/w).**

The flow behaviour data of samples were fitted into the Power Law model. The coefficient of determination ($r^2$) was in the range of 0.97-0.99, indicating a high correlation. Flow behaviour index values ($n$) of the samples were determined to be in the range of 0.3-0.5. As stated by Santana et al. (2015), a fluid is classified as a pseudoplastic fluid if its flow behaviour index is smaller than 1, hence, the apparent viscosity decreased when the shear rate is increased and it is also referred to as "shear thinning" (McClements, 2016). Thus, the $n$ values of these emulsion samples confirmed the observations based on the flow curves (Figures 4.11A and B) as discussed previously that all the samples exhibited the shear thinning flow behaviour. Furthermore, there was a decreasing trend for the flow behaviour index of EST1 with the increasing concentration of ST1 starch. This implies that the flow characteristics of the EST1 emulsion became more pseudoplastic with higher concentration of starch ST1 added into the formulation. A similar trend was noted for the EST6 emulsions as well, except the sample made with 4% of ST6 (EST6-4%), which showed the highest flow behaviour index value. This might be due to some differences in the conditions used as the sample EST6-4% was prepared in a different batch from other samples. Moreover, the consistency index ($k$) of the instant emulsions increased when the starch concentration increased for both EST1 and EST6 emulsions. This could be explained by the increasing viscosity of the samples that resulted from the increasing addition of starch into the formulations, as the consistency index was considered as an indicator of the samples viscous nature and a high consistency index ($k$) indicates the high viscosity of sauces (Krystyjan et al., 2012). Overall, when the concentration of starch increased, the flow behaviour index ($n$) decreased while the consistency index ($k$) increased. Similar results have been reported by Wu et al. (2009) that when the concentration or molecular weight of hydrocolloids added into an emulsion increased, the consistency index ($k$) of the emulsion increased and the flow behaviour index ($n$) decreased.

**Table 4.9 Power law parameter values of instant emulsion samples formulated with starch ST1 (EST1) and starch ST6 (EST6) at different concentrations (0, 1, 2, 4 and 6% w/w) and the shear viscosity of samples measured at a 20 $s^{-1}$ shear rate.**

| EST1 | Shear Viscosity (Pa·s) | *k* (Pa·$s^n$) | *n* | $r^2$ |
|---|---|---|---|---|
| EST1-0% | $0.26 \pm 0.01^{e}$ | 1.52 | 0.43 | 0.99 |
| EST1-1% | $0.55 \pm 0.04^{d}$ | 3.23 | 0.43 | 0.99 |
| EST1-2% | $1.17 \pm 0.08^{c}$ | 8.02 | 0.38 | 0.99 |
| EST1-4% | $4.47 \pm 0.05^{b}$ | 35.69 | 0.33 | 0.99 |
| EST1-6% | $12.56 \pm 0.25^{a}$ | 90.84 | 0.36 | 0.99 |
| | | | | |
| EST6 | | | | |
| EST6-0% | $0.26 \pm 0.01^{e}$ | 1.52 | 0.43 | 0.99 |
| EST6-1% | $0.61 \pm 0.02^{d}$ | 4.09 | 0.38 | 0.99 |
| EST6-2% | $1.52 \pm 0.06^{c}$ | 11.79 | 0.34 | 0.99 |
| EST6-4% | $5.14 \pm 0.46^{b}$ | 28.27 | 0.46 | 0.97 |
| EST6-6% | $12.32 \pm 0.46^{a}$ | 94.27 | 0.34 | 0.99 |

$^{a, b, c, d, e}$ Different letters within the same column represent significant differences ($p < 0.05$).
*k*: flow consistency coefficient, *n*: flow behaviour index, $r^2$: coefficient of correlation.

#### 4.4.3.3 Particle Size and Particle Size Distribution

The particle size distribution curves of the instant emulsions with 0, 1, 2, 4 and 6% (w/w) of starch ST1 are shown in Figure 4.12A. It was clearly seen that all the EST1 samples had the bimodal distributions with two ranges of droplet population. When the starch concentration increased from 0 to 6%, the smaller-diameter peak shifted from around 70 μm towards a smaller particle size at around 40 μm. The volume of this population also increased with increasing ST1 concentration. At the same time, the second peak of the size distribution curve was gradually flattened and shifted from approximately 400 μm to around 260 μm when the starch concentration increased from 0 to 4%. However, when the starch level continuously increased from 4 to 6%, the volume of this larger-diameter peak increased again. The possible reason for the "recovery" of large particles might be that the high viscosity resulting from 6% of starch had reduced the efficiency of oil disruption and emulsification processes, leading to the formation of some larger oil droplets as well as the droplets aggregation caused by flocculation and coalescence.

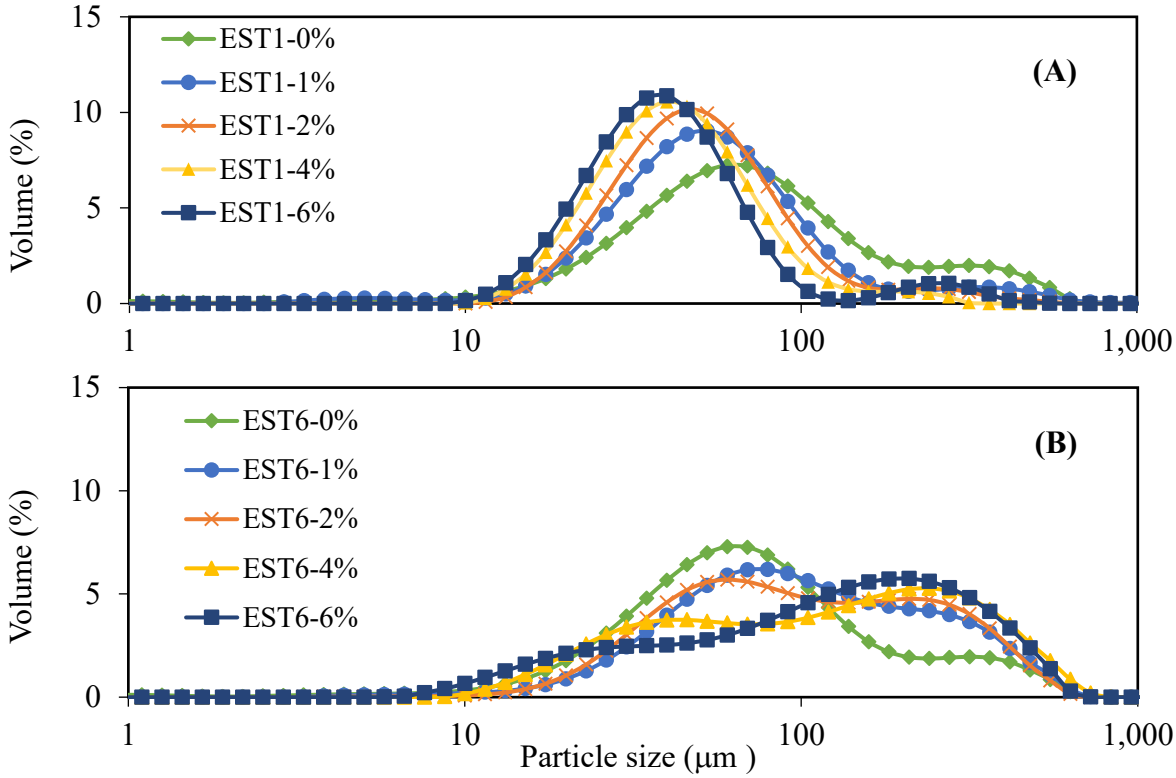



**Figure 4.12 Particle size distribution of oil droplets in instant emulsions prepared with (A) starch ST1 and (B) starch ST6 at different concentrations (0, 1, 2, 4 and 6% w/w).**

More evidences could be obtained from Table 4.10. For the emulsion EST1, the values of d(0.1), d(0.5) and d(0.9) were all reduced in a significant level with the increasing concentration of ST1, which also led to a significant decrease of the width of distribution (span). In addition, a significant reduction in the volume mean diameter (D[4,3]) was also observed as the mean size of the system decreased from 98 μm when no starch was added, to around 50 μm when 4-6% ST1 was added. These changes signified that there were both small and large droplets presenting in the EST1 emulsion systems. With the increasing concentration of ST1, the volume of particles in the small-size range increased whereas the volume of particles in the larger-size range decreased. Thus, the emulsions with finer and more uniform particle sizes were produced.

In contrast to ST1, the increasing concentrations of ST6 had a reversed effect on the particle size distribution of emulsion EST6. As shown in Figure 4.12B, all the EST6 samples had the broad bimodal particle size distribution. With the increasing concentration of ST6 added into the formulation, the volume of particles in small size range decreased while the volume of larger particles seemed to increase. The particle size characteristics of EST6 samples are summarised in Table 4.10. There was no significant change in d(0.1) values with the

increasing level of ST6 starch, whereas the values of d(0.5) and d(0.9) were increased significantly. This led to a significantly increase in D[4,3] as the volume mean diameter was more sensitive to the presence of large particles in the size distribution. The D[4,3] was around 98 μm when the emulsion formulated without starch but its size was increased to around 150 μm when the emulsions containing 4-6% of ST6.

**Table 4.10 Particle size of instant emulsion samples formed with starch ST1 (EST1) and starch ST6 (EST6) at different concentrations (0, 1, 2, 4 and 6% w/w).**

| EST1 | D [4, 3] (μm) | d (0.1) (μm) | d (0.5) (μm) | d (0.9) (μm) | Span |
|---|---|---|---|---|---|
| EST 1-0% | 98 ± 4.95$^{a}$ | 23 ± 2.15$^{a}$ | 62 ± 4.53$^{a}$ | 245 ± 40.12$^{a}$ | 3.60 ± 0.82 |
| EST 1-1% | 72 ± 8.32$^{b}$ | 22 ± 0.13$^{b}$ | 49 ± 0.29$^{b}$ | 119 ± 8.94$^{b}$ | 2.00 ± 0.18 |
| EST 1-2% | 59 ± 3.39$^{c}$ | 23 ± 0.54$^{a}$ | 45 ± 0.64$^{c}$ | 97 ± 4.90$^{c}$ | 1.65 ± 0.10 |
| EST 1-4% | 47 ± 1.05$^{d}$ | 20 ± 0.37$^{c}$ | 39 ± 0.41$^{d}$ | 78 ± 2.80$^{d}$ | 1.49 ± 0.07 |
| EST 1-6% | 49 ± 2.37$^{d}$ | 19 ± 0.34$^{d}$ | 36 ± 0.21$^{e}$ | 72 ± 3.79$^{d}$ | 1.48 ± 0.11 |
| EST6 | | | | | |
| EST 6-0% | 98 ± 4.95$^{c}$ | 23 ± 1.99$^{bc}$ | 63 ± 4.40$^{c}$ | 245 ± 48.97$^{c}$ | 3.60 ± 0.97 |
| EST 6-1% | 132 ± 14.26$^{b}$ | 32 ± 2.57$^{a}$ | 90 ± 2.15$^{b}$ | 305 ± 50.15$^{b}$ | 3.02 ± 0.47 |
| EST 6-2% | 132 ± 10.79$^{b}$ | 30 ± 1.70$^{a}$ | 91 ± 9.92$^{b}$ | 299 ± 29.66$^{b}$ | 2.98 ± 0.29 |
| EST 6-4% | 154 ± 15.64$^{a}$ | 25 ± 1.59$^{b}$ | 113 ± 9.45$^{a}$ | 351 ± 42.27$^{a}$ | 2.89 ± 0.22 |
| EST 6-6% | 150 ± 6.61$^{a}$ | 22 ± 2.94$^{c}$ | 119 ± 5.93$^{a}$ | 332 ± 18.57$^{ab}$ | 2.61 ± 0.16 |

$^{a, b, c, d}$ Different letters within the same column represent significant differences ($p < 0.05$). D[4,3]: volume-weighted mean diameter; d(0.1), d(0.5) and d(0.9): the diameters where 10%, 50% and 90% of the particles are smaller than the values.

Overall, the increasing concentration of ST1 into the emulsion could promote the formation of finer and more uniform oil droplets, while the increasing concentration of ST6 induced the formation of larger oil droplets. The different effects of two different starch molecules (ST1 and ST6) on the particle size distribution of the instant emulsions might also be resulted from some differences in their starch granular size and properties. As mentioned previously, after being dispersed into cold water, the size of starch granules in water for ST1 had a D[4,3] value at around 42 μm while it was significantly larger being around 161 μm for ST6 (Table 4.4). Also, as shown in Figure 4.3, ST1 still retained its intact starch granules after dispersion into cold water. On the other hand, the granule structure of ST6 was completely disrupted, showing as the relatively large aggregates in irregular shapes

when dispersed into cold water. Although it is not sure, the large size of ST6 starch might have promoted droplet flocculation in the emulsion system through a depletion mechanism resulting in larger D[4,3] values of the instant emulsion samples prepared with ST6 (EST6).

#### 4.4.3.4 Microstructure of Emulsions

Similar particle sizes and size distribution were observed in both light microscopic and CLSM images for the same emulsion sample. However, the microstructural features of the instant emulsions prepared with two different types of starches, ST1 and ST6, were different, in terms of the extent of droplet flocculation, droplet size and polydispersity. This indicated that the type and concentration of the starch added into the formation could influence the microstructure of the formed instant emulsions.

It was observed that there was a reduction in the oil droplet size of the EST1 emulsions with the increasing concentration of ST1 starch, and the distribution became more uniform with less polydispersity (Figures 4.13) as already discussed in the above. There were some non-stained, convoluted structures observed in the CLSM images of EST1 samples between the oil droplets (Figure 4.13B). These were considered as the dispersed starch granules, which is described in Table 4.2 as cold water swelling with intact starch granule, and the similar structural feature was also observed under the light microscopy when assessing the ST1 starch solution (Figure 4.3).

A similar trend was observed in the images of EST6 (Figures 4.14) with a decrease in the size of oil droplets as the ST6 concentration was increased. On the other hand, a significant droplet flocculation (clustering of oil droplets) seemed to occur when a high concentration of ST6 starch was added. As a result, the volume of the larger-size group in the size distribution was increased as the effective size of oil droplets detected by the particle analyser increased. The sample EST6-6% showed the highest degree of droplet flocculation and the flocculated droplets seemed to be connected to form a network. The formation of the network might provide the sample with a viscoelastic-solid texture, resulting in a different appearance from the other samples (Figure 4.10). It should also be mentioned that small particles stained with green colour were observed in the CLSM images of EST6 samples. This could be due to the presence of some lumps of undispersed egg yolk powder containing proteins.

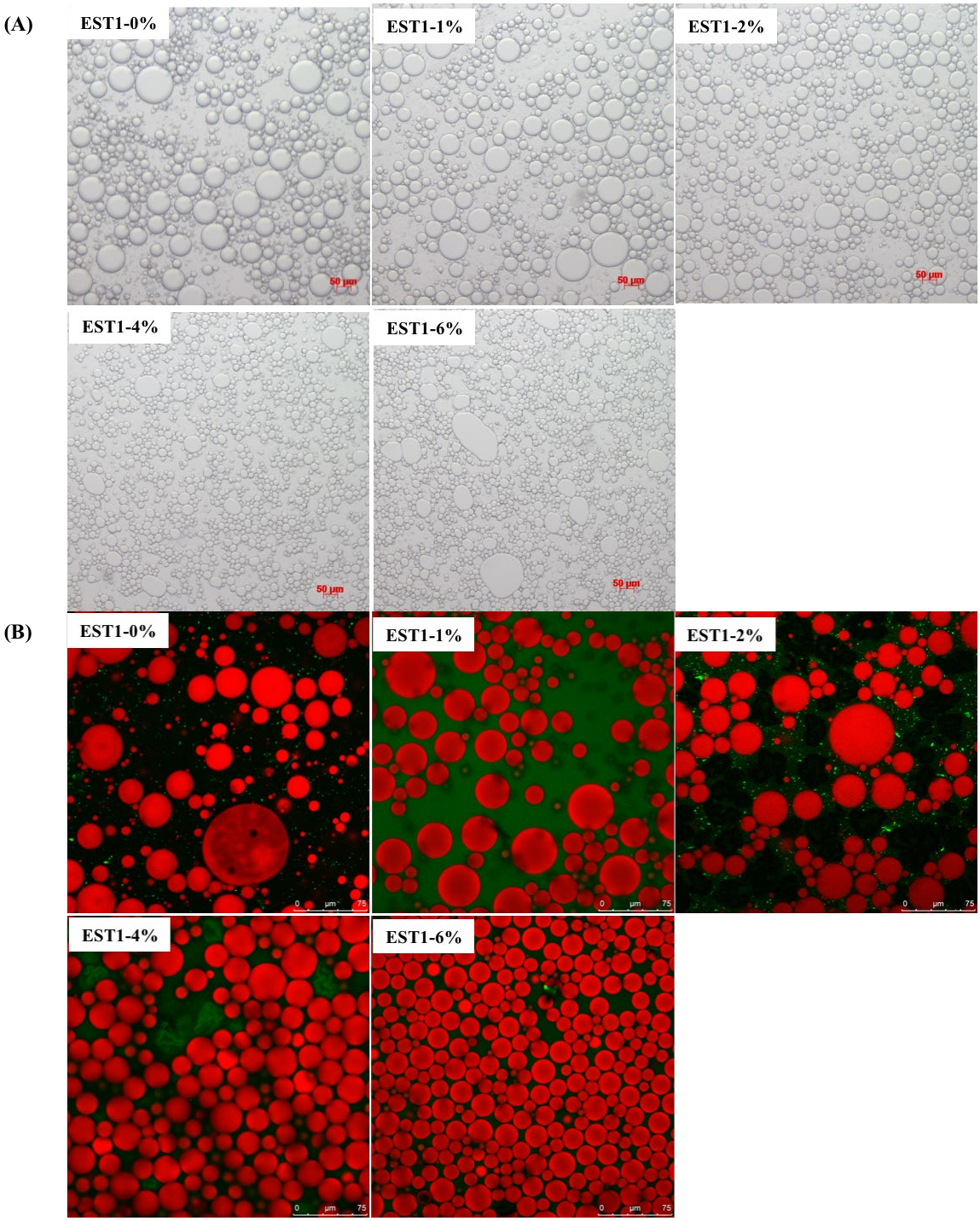



**Figure 4.13 (A) light microscopic images (scale bar 50 μm) and (B) CLSM images (scale bar 75 μm), of instant emulsions formulated with different concentrations of starch ST1 (0, 1, 2, 4 and 6% w/w).**

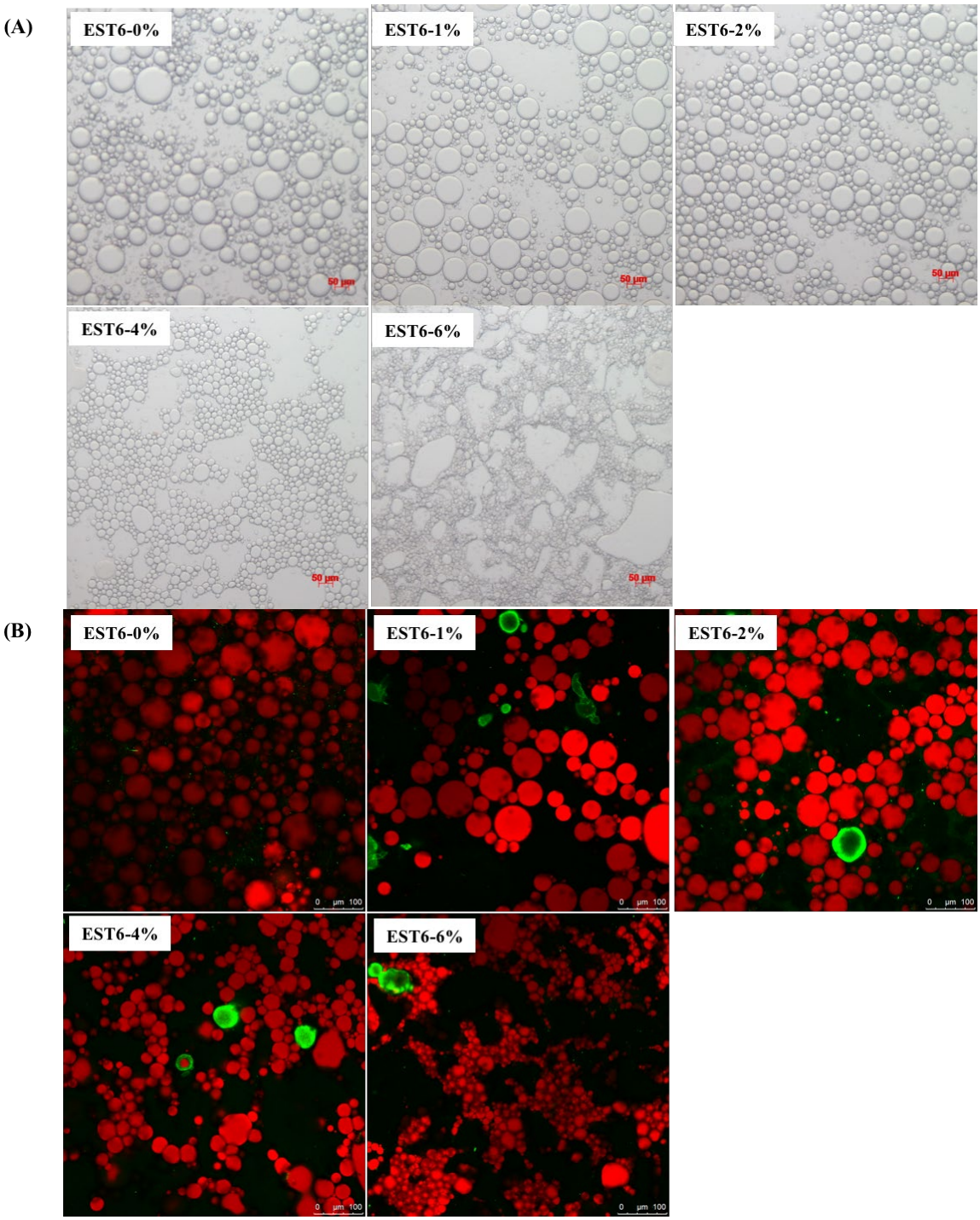



**Figure 4.14 (A) light microscopic images (scale bar 50 μm) and (B) CLSM images (scale bar 100 μm), of instant emulsions formulated with different concentrations of starch ST6 (0, 1, 2, 4 and 6% w/w).**

#### 4.4.3.5 Stability of Emulsions

The results of the accelerated stability assessment of the instant emulsions made with different concentrations of ST1 or ST6 are shown in Figures 4.15A and B, respectively. The results showed that the stability of instant emulsions could be significantly influenced by the concentration of component starches. When there was none or a small amount (0-2%) of starch added into the formulation, creaming and sedimentation occurred. Thus, oil droplets moved upward and accumulated at the top of the emulsion to form a rich cream layer, while the relatively heavy particles, such as some swollen starch granules and undissolved egg yolk powders, moved downward to form a layer of sediments at the bottom of the emulsion. In between, a serum layer could also be observed containing mainly aqueous phase with some water-soluble components in the formulation.

The best stability was observed in the emulsion samples containing 4% starches in both cases for ST1 and ST6 starches, showing that the gravitational separation was retarded when the higher concentration of starch was added into the emulsions. This would be because the increasing viscosity of the continuous phase surrounding the oil droplets decreased the velocity at which the droplets moved. Also, a three-dimensional network might have formed between the added starches and xanthan gum, which could trap the oil droplets and stabilise them against creaming (McClements, 2016). Furthermore, a more pronounced layer of pure oil although it was not very significant visually was formed on top of the sample EST4-6% and EST6-6%. This indicates that there was an upper limit on the addition of starch into the instant emulsion for desirable characteristics. Either too high or too low viscosity of the instant emulsion can cause an adverse effect on the stability and properties of the instant emulsions.

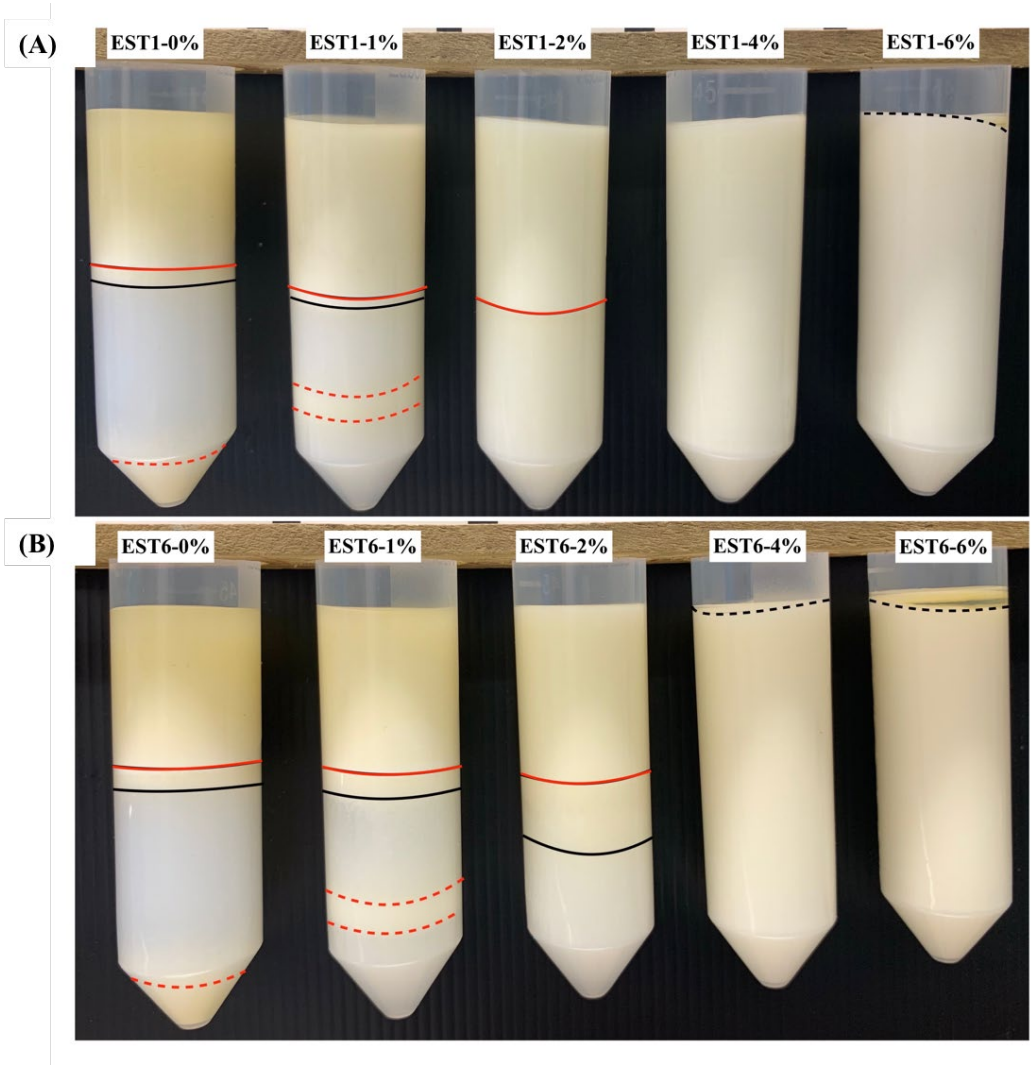



**Figure 4.15 Pictures of instant emulsion samples prepared with different types of starch (A) ST1 and (B) ST6 at different concentrations (0, 1, 2, 4 and 6% w/w). Pictures taken after centrifugation to observe the stability of emulsions against creaming, phase separation, free oil, etc. (----- indicates the boundary of free oil and cream layer; ——— indicates the boundary between cream layers; ——— indicates the boundary of cream layer and serum phase; ----- indicates the boundary of serum phase and sediment).**

### 4.4.3.6 Textural Properties of Emulsions

For both instant emulsions EST1 and EST6, the values of all the texture attributes were significantly increased with the increasing amount of starch added into the emulsions (Tables 4.11). These results were probably related to the increasing viscosity of the samples when increasing the concentration of starch in the formulations. Thus, the emulsion samples with the highest viscosity (EST1-6% and EST6-6%) showed the highest values for

all the texture parameters. However, the sample formulated with 6% ST1 (EST1-6%) had the higher values of firmness, consistency, cohesiveness and index of viscosity to some extent than the sample formulated with the same amount of ST6 (EST6-6%), even though their rheological properties were very similar to each other (Tables 4.9). This might be due to the different particle size and microstructure characteristics of these two samples, which might lead to some effect on the textural profile of the instant emulsions although it seems not very significant.

**Table 4.11 Texture parameter values of the instant emulsion samples containing starch ST1 and starch ST6 at different concentrations (0, 1, 2, 4 and 6% w/w).**

| EST1 | Firmness (g) | Consistency (g·s) | Cohesiveness (g) | Index of Viscosity (g·s) |
|---|---|---|---|---|
| EST 1-0% | 17.17 ± 0.22$^{d}$ | 112.48 ± 1.01$^{d}$ | -6.41 ± 0.08$^{a}$ | -2.15 ± 0.07$^{a}$ |
| EST 1-1% | 21.17 ± 0.87$^{d}$ | 134.31 ± 6.23$^{d}$ | -8.87 ± 0.42$^{a}$ | -10.81 ± 2.91$^{a}$ |
| EST 1-2% | 35.25 ± 2.22$^{c}$ | 224.46 ± 15.61$^{c}$ | -17.05 ± 1.68$^{b}$ | -41.04 ± 14.20$^{b}$ |
| EST 1-4% | 107.12 ± 5.32$^{b}$ | 707.88 ± 35.88$^{b}$ | -90.15 ± 4.54$^{c}$ | -287.2 ± 15.06$^{c}$ |
| EST 1-6% | 233.17 ± 14.06$^{a}$ | 1395.87 ± 69.48$^{a}$ | -230.86 ± 5.46$^{d}$ | -664.1 ± 25.52$^{d}$ |
| EST6 | | | | |
| EST 6-0% | 17.17 ± 0.22$^{d}$ | 112.48 ± 1.01$^{e}$ | -6.41 ± 0.08$^{a}$ | -2.15 ± 0.07$^{a}$ |
| EST 6-1% | 26.50 ± 1.40$^{d}$ | 156.09 ± 1.36$^{d}$ | -10.84 ± 0.11$^{a}$ | -24.44 ± 0.15$^{a}$ |
| EST 6-2% | 52.86 ± 3.56$^{c}$ | 314.45 ± 3.00$^{c}$ | -31.32 ± 0.49$^{b}$ | -105.37 ± 1.70$^{b}$ |
| EST 6-4% | 102.19 ± 7.17$^{b}$ | 667.73 ± 15.93$^{b}$ | -81.34 ± 0.22$^{c}$ | -259.42 ± 8.45$^{c}$ |
| EST 6-6% | 214.54 ± 18.65$^{a}$ | 1231.50 ± 28.21$^{a}$ | -184.23 ± 6.66$^{d}$ | -591.69 ± 37.89$^{d}$ |

$^{a, b, c, d}$ Different letters within the same column represent significant differences ($p < 0.05$).

## 4.5 Conclusions

In order for starch ingredients to be used as a thickener and stabiliser in making instant emulsions simply by hand shaking without any thermal treatment, the type of starch adopted in this application must be cold-water soluble and be able to thicken the continuous phase of the emulsion instantly to form desirable rheological properties. In this research study, instant emulsions were formed successfully but their emulsion stability and properties were affected to some extent by the type and concentration of starches used and added into the formulations.

All the instant emulsion samples had an acidic pH value ranging from 2.7 to 3.1 due to the addition of citric acid and exhibited a non-Newtonian pseudoplastic flow behaviour. Among all the samples made with seven different types of starch at a concentration of 4% w/w, the highest viscosity was observed from the emulsion formulated with ST6 starch ("Instant FTD 176") which had a mean diameter of around 160 μm. However, the most pseudoplastic flow behaviour was observed from the emulsion made with ST1 starch ("Ultra-Tex-4"), with the smallest mean diameter of 50 μm. On the other hand, by increasing the concentration of starch for both ST1 and ST6, the viscosity of the formed instant emulsions increased significantly and exhibited greater shear-thinning behaviour. The mean diameter of EST1 was reduced significantly from 98 μm to 50 μm with the increasing concentration of ST1 from 0 to 6% w/w. However, a reverse trend was observed for the instant emulsion formulated with ST6 as the mean diameter of EST6 was increased from 98 μm to 150 μm when the starch concentration was increased from 0 to 6%. In addition, the emulsion formulated with 4-6% w/w of ST1 had better textural characteristics than the emulsions formed with same amount of ST6 with the higher values of texture attributes (firmness, consistency, cohesiveness and index of viscosity). Thus, it was concluded that the starch ST1 "Ultra-Tex-4" was the most suitable starch identified among seven different types of starch used in this study which can be incorporated into the formulation for thickening and stabilising a low-fat, mayonnaise-like instant food emulsion produced by hand shaking. Based on the results of the stability assessment, it was also concluded that 4% w/w was the most appropriate concentration for ST1 starch to be incorporated into the formulation for obtaining the best emulsion stability and properties.

# Chapter 5 Effects of Types and Concentrations of Non-Starch Hydrocolloids on the Formation and Properties of Instant Creamy Emulsions

## 5.1 Abstract

In the previous Chapter 4, a low-fat, mayonnaise-like, instant creamy O/W emulsion was prepared by hand shaking using a base formulation (40% oil, 4% starch, 0.2% xanthan gum, 0.3% citric acid, 0.5% egg yolk powder, 0.5% Tween 80 and 54.5% water). The effects of different types and concentrations of modified starches were investigated. In this Chapter 5, instant creamy emulsions were also prepared using the same method and formulation but formulated with four different types of hydrocolloids, such as xanthan gum, guar gum, carboxymethyl cellulose (CMC) and lambda (λ) carrageenan, at various concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w) for their effects on the formation and physicochemical properties. The results revealed their significant impact on the viscosity, particle size and particle size distribution, stability and textural properties of instant emulsions. For all the tested emulsion samples, the increasing concentration of all types of hydrocolloids increased the emulsion viscosity and improved the emulsion stability against creaming and phase separation as well as enhanced the textural properties of the instant emulsions, in terms of firmness, consistency, cohesiveness and viscosity index. However, among all the emulsion samples, the instant emulsion prepared with CMC showed the highest viscosity and textural attribute values. In addition, the instant emulsion with CMC had the smallest mean particle diameter (around 41 μm at 0.8% w/w) of oil droplets which was reduced gradually with the increasing concentration of CMC. On the other hand, an increase in the concentration of xanthan gum and guar gum seemed to induce the formation of oil droplet flocculation. As a consequence, an increase in the mean droplet sizes from 55 μm to 64 μm and from 42 μm to 50 μm was observed, respectively, when the concentration of xanthan gum and guar gum was increased from 0.05 to 1% w/w. However, no noticeable effect of different concentrations of λ-carrageenan was observed on the mean particle diameter and particle size distribution of the instant emulsions, even though the emulsion viscosity increased with the increasing concentration of carrageenan. These results indicated that stable instant creamy emulsions were able to be produced using all four different types of hydrocolloids as stabilisers. However, their stability against creaming after centrifugation

was found to be highly affected by their concentrations used. This implies that a careful selection of hydrocolloid type and concentration is required to produce instant emulsions with desirable emulsion stability and textural properties.

## 5.2 Introduction

Polysaccharides are commonly used in food emulsions to modify the viscosity of the continuous phase of an emulsion, enhance the textural properties and mouthfeel of the emulsion products, and stabilise an emulsion for a longer shelf life (Dickinson, 2009; Wang et al., 2016). Especially in low-fat food emulsions (e.g. low-fat mayonnaise), a variety of polysaccharides (e.g. starch, xanthan gum, guar gum, cellulose, pectin, carrageenan) have been generally utilised to thicken the continuous phase of emulsions and enhance the emulsion stability (Amin et al., 2014). In Chapter 4, the effects of starch on the formation and physicochemical characteristics of a low-fat instant creamy emulsion was investigated by formulating with different types of starches at various concentrations. However, many studies have suggested that starch could be used in combination with another subsidiary non-starch hydrocolloid in the low-fat food emulsions for better functional properties (Dolz et al., 2004; Puligundla et al., 2015; Rahmati et al., 2015; Wang et al., 2016).

Xanthan gum and guar gum are the most commonly used non-starch hydrocolloids and they have also been increasingly studied as fat replacer in low-fat food emulsions (Amin et al., 2014; Lee et al., 2013; Rahmati et al., 2015; Su et al., 2010). However, all the studies have been focused on the properties of emulsions formed by using the homogenisation methods which involves the use of electrical equipment, such as KitchenAid mixer (Lee et al., 2013; Puligundla et al., 2015), stand mixer (Rahmati et al., 2015) and kitchen machine (Amin et al., 2014). There is no published literature available on the application of a combination of starch with xanthan gum or guar gum in the low-fat food emulsions which are formed through an instantaneous emulsification process involving the agitation of ingredients simply by hand shaking. In addition, there is no information about the effects of starch with CMC or λ-carrageenan on the formation and characterisation of a low-fat instant creamy O/W emulsion. Thus, the objective of this research is to investigate the effects of four types of non-starch hydrocolloids (xanthan gum, guar gum, CMC, λ-carrageenan) at various concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w) on the formation and physicochemical properties of the low-fat, mayonnaise-like, instant O/W emulsions containing 40% w/w oil. The instant emulsions prepared were characterised for the determination of visual

appearance and pH, particle size and particle size distribution, microstructure, rheological and textural properties, as well as emulsion stability.

## 5.3 Materials and Methods

### 5.3.1 Materials

Four different non-starch hydrocolloids, namely xanthan gum (GRINDSTED Xanthan 200 ANZ), guar gum (GRINDSTED Guar 250), sodium carboxymethyl cellulose (GRINDSTED Cellulose gum NP 67) denoted hereafter as CMC, and lambda (λ) carrageenan (CARAGEM 402), were supplied by Danisco (Copenhagen, Denmark). All the other ingredients (e.g. oil, acid, egg yolk powder, etc.) which were also used in preparing the instant emulsions are described in Section 3.1.

### 5.3.2 Preparation of Instant Emulsions with Different Types and Concentrations of Hydrocolloids

Instant emulsion samples were prepared based on the formulation and method as described in Chapter 3 (Section 3.2). However, four different non-starch hydrocolloid-based stabilisers (xanthan gum, guar gum, CMC and carrageenan) were used at different concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w) as shown in Table 5.1 to investigate their effects on the formation and characteristics of instant emulsions.

**Table 5.1 Formulations of instant emulsions prepared at different concentrations of non-starch hydrocolloids (xanthan gum, guar gum, CMC and λ-carrageenan).**

| Ingredients | Concentration (% w/w) | | | | | |
|---|---|---|---|---|---|---|
| Canola oil | 40 | 40 | 40 | 40 | 40 | 40 |
| Starch (Ultra-Tex-4) | 4 | 4 | 4 | 4 | 4 | 4 |
| **Non-starch hydrocolloid** | **0.05** | **0.1** | **0.2** | **0.4** | **0.8** | **1** |
| Citric acid | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Egg yolk powder | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Tween 80 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Water | 54.65 | 54.6 | 54.5 | 54.3 | 53.9 | 53.7 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |

### 5.3.3 Analysis of Samples

The instant emulsion samples prepared with different types and concentrations of non-starch hydrocolloids were analysed in various aspects, including their pH, visual appearance, mean particle diameter and particle size distribution, microstructural features, emulsion stability, rheological and textural properties as described in the Materials and Methods section of Chapter 3.

### 5.3.4 Data Analysis

All the measurements were carried out at least twice for each sample from duplicate experiments. The results were statistically analysed using the IBM SPSS Statistics Program (version 25, IBM Corporation, USA). The one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test were used to examine differences between the mean values at a significance level of $p < 0.05$.

## 5.4 Results and Discussions

### 5.4.1 Appearance and pH

Instant emulsion samples were formulated with four different non-starch stabilisers, including xanthan gum (X), guar gum (G), CMC (CL) and λ-carrageenan (C), at various concentrations from 0.05 to 1% w/w. These samples were prepared by an instant emulsification process which involves a relatively low shear force generated by hand shaking to mix the mixture of dry ingredients and oil first in a closed plastic container followed by mixing with the aqueous phase (water and Tween 80). Figure 5.1 shows the pictures of samples of the instant emulsions taken after the preparation. All the emulsion samples were formed successfully regardless of the type and concentration of the hydrocolloid stabilisers used. A white creamy smooth thick texture was observed from all the samples with its appearance similar to commercial mayonnaise. However, it was observed that the emulsions became more thickened and viscous, and formed a more "solid" like (more rigid) when formulated with a higher concentration of hydrocolloids.

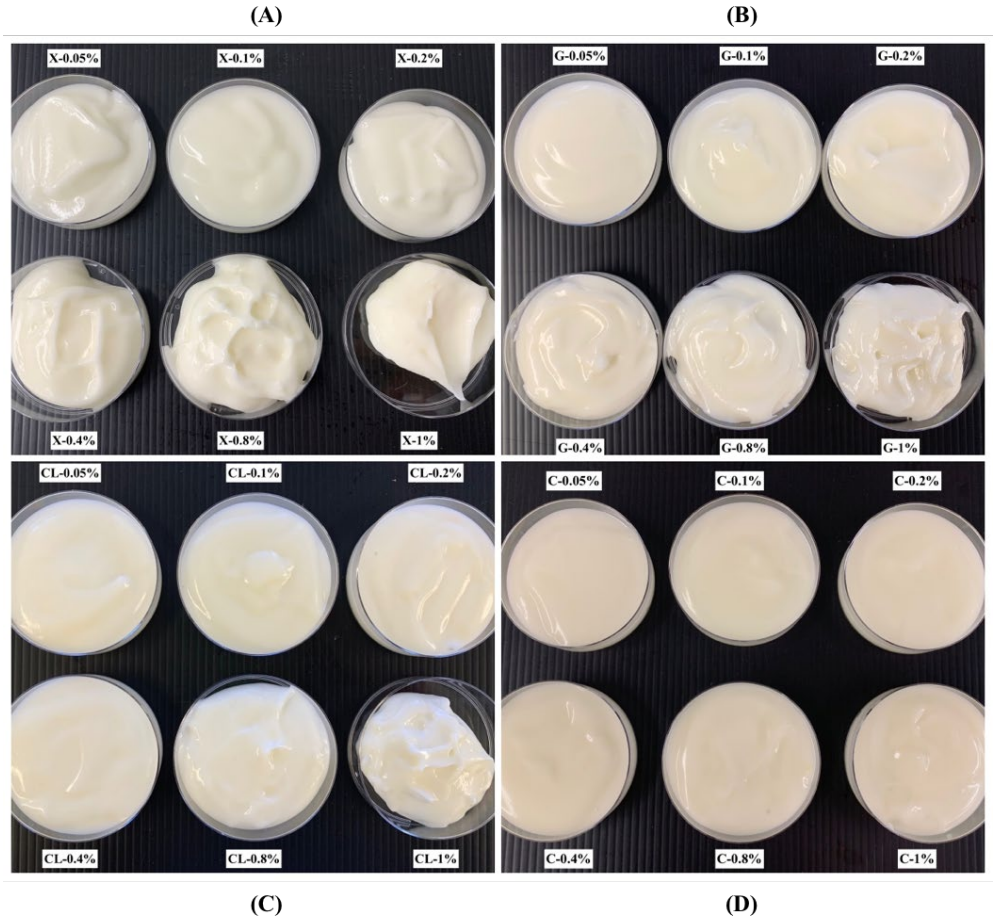



**Figure 5.1 Instant emulsion samples containing 40% w/w oil prepared with (A) xanthan gum, (B) guar gum, (C) CMC, and (D) λ-carrageenan at different concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w).**

The pH values of emulsion samples measured are summarised in Table 5.2. Due to the addition of citric acid, all the samples were acidic, and their pH values were in the range of 2.6-4.1, depending the type of hydrocolloids (stabilisers) used. It was found that the emulsion pH values increased with increasing the concentration of stabilisers for all four types of hydrocolloids but its pH increase was more pronounced especially with CMC. From the microbiological safety point of view, several studies have suggested that the pH of mayonnaise should be 4.1 or less to ensure the products free from the growth of microorganism, such as *Salmonella* (Keerthirathne et al., 2016; Smittle, 1977). All the instant emulsion samples prepared in this study were in good agreement with the recommended pH value as below 4.1.

**Table 5.2 pH values of the instant emulsion samples prepared with different hydrocolloids at different concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w).**

| Concentration | pH | | | |
|---|---|---|---|---|
| | Xanthan gum | Guar gum | CMC | Carrageenan |
| 0.05% | 2.64 ± 0.04 | 2.68 ± 0.03 | 2.78 ± 0.10 | 2.64 ± 0.07 |
| 0.1% | 2.72 ± 0.02 | 2.73 ± 0.05 | 2.60 ± 0.44 | 2.70 ± 0.01 |
| 0.2% | 2.77 ± 0.03 | 2.73 ± 0.07 | 3.12 ± 0.14 | 2.74 ± 0.07 |
| 0.4% | 2.87 ± 0.05 | 2.75 ± 0.07 | 3.27 ± 0.04 | 2.80 ± 0.13 |
| 0.8% | 3.02 ± 0.07 | 2.75 ± 0.04 | 3.83 ± 0.16 | 2.86 ± 0.15 |
| 1% | 3.09 ± 0.08 | 2.76 ± 0.06 | 4.04 ± 0.11 | 2.91 ± 0.13 |

### 5.4.2 Rheological Properties

The rheograms of the instant emulsion samples formulated with different types and concentrations of stabilisers are presented in Figures 5.2. The results show the viscosity of the instant emulsions as a function of shear rate measured in the range of 5-100 $s^{-1}$. According to these rheograms, all the instant emulsion samples exhibited the non-Newtonian and shear-thinning flow behaviour, in which a decrease in the apparent viscosity of the emulsions was observed when the shear rate was increased. When the shear rate was relatively high, the relatively constant apparent viscosity was observed for all the instant emulsion samples.

It was found that higher viscosity was observed from the instant emulsions containing higher concentrations of stabilisers, and the higher viscosity was observed from the emulsion samples formulated with CMC especially when the addition of CMC exceeded 0.2% w/w (Figure 5.2C). A comparison of the apparent viscosity measured at shear rate of 20 $s^{-1}$ between the different emulsion samples is also shown in Table 5.3. The results confirmed that the apparent viscosity of instant emulsions increased significantly with the increasing concentration of added stabilisers for all four types of hydrocolloids ($p < 0.05$). In addition, when the emulsions were formulated with lower concentration of stabilisers (e.g. 0.05%), there was no significant difference obtained between the viscosity of the emulsion samples formulated with different types of hydrocolloids. However, when the concentration of added stabilisers was above 0.1% w/w, the highest viscosity was observed from the instant emulsions formed with CMC, while the emulsions prepared with λ-

carrageenan had the lowest viscosity ($p < 0.05$). The overall slightly lowerviscosity of the emulsion samples with λ-carrageenan may be partly related to the properties of carrageenan being relatively less stable to the low pH than other hydrocolloids (Blakemore & Harpell, 2009). The low pH of the instant emulsions (less than pH 4.3) can cause polymer degradation of carrageenan which results in a loss in viscosity and gel strength of the carrageenan solution (Pegg, 2012).

The flow behaviour of instant emulsion samples was also described by utilising the Power Law model. The model parameters that characterise the rheological properties of the instant emulsion samples are summarised in Table 5.4. It was noticed that high correlation coefficients ($r^2$) were obtained for all the samples ranging from 0.97 to 0.99, indicating a good fit of the Power Law model to describe the rheological properties of the instant emulsion samples. All the emulsion samples had the flow behaviour index values ($n$) smaller than 1 (0.19-0.48) that are characterised as pseudoplastic fluids, which manifests the shear-thinning behaviour of the samples (Santana et al., 2015). For the emulsions formed with xanthan gum, CMC or carrageenan except for guar gum, the value of the flow behaviour index ($n$) was decreased when the concentration of the added hydrocolloids was increased. This indicates that the addition of these hydrocolloids (xanthan gum, CMC or carrageenan) promoted the pseudoplastic behaviour of the instant emulsions. Furthermore, when the emulsion was formulated with xanthan gum at 0.2% or above, the flow behaviour index ($n$) of the formed emulsions was the smallest among all the samples, indicating that the xanthan gum-stabilised emulsions had the most pseudoplastic flow characteristics. This observation might be related to the semi-flexible structure of xanthan gum molecules, which results in the sharper dropping of the viscosity under the influence of applied shear compared to the other hydrocolloids such as CMC and guar gum (Quintana et al., 2002; Sworn, 2009). On the other hand, the emulsion samples formulated with guar gum showed the higher value of flow behaviour index ($n$) at all the concentrations, which indicates the weaker pseudoplastic behaviour of the guar gum-stabilised emulsions than the other instant emulsion samples prepared with xanthan gum, CMC and carrageenan.

The consistency coefficient ($k$) increased with the increasing concentrations of added hydrocolloids for all the emulsion samples (Table 5.4). This was in accordance with the increasing apparent viscosity of the instant emulsions when the concentration of hydrocolloids was increased. Similar results were reported by Wang et al. (2016), who

found that the consistency coefficient *k* values increased with the increasing gum concentrations in a yellow mustard sauce that was stabilised by the combination of modified starch and xanthan gum or guar gum. In this study, the highest value of *k* was observed from the emulsion samples formed with CMC. This indicates the most pronounced viscosity of the CMC-stabilised instant emulsion among all the samples. On the other hand, the smaller values of *k* were observed from the instant emulsion samples formulated with guar gum or carrageenan, suggesting the intermolecular interaction in these emulsions might be weaker than the samples containing xanthan gum or CMC and thus lower viscosities were resulted.

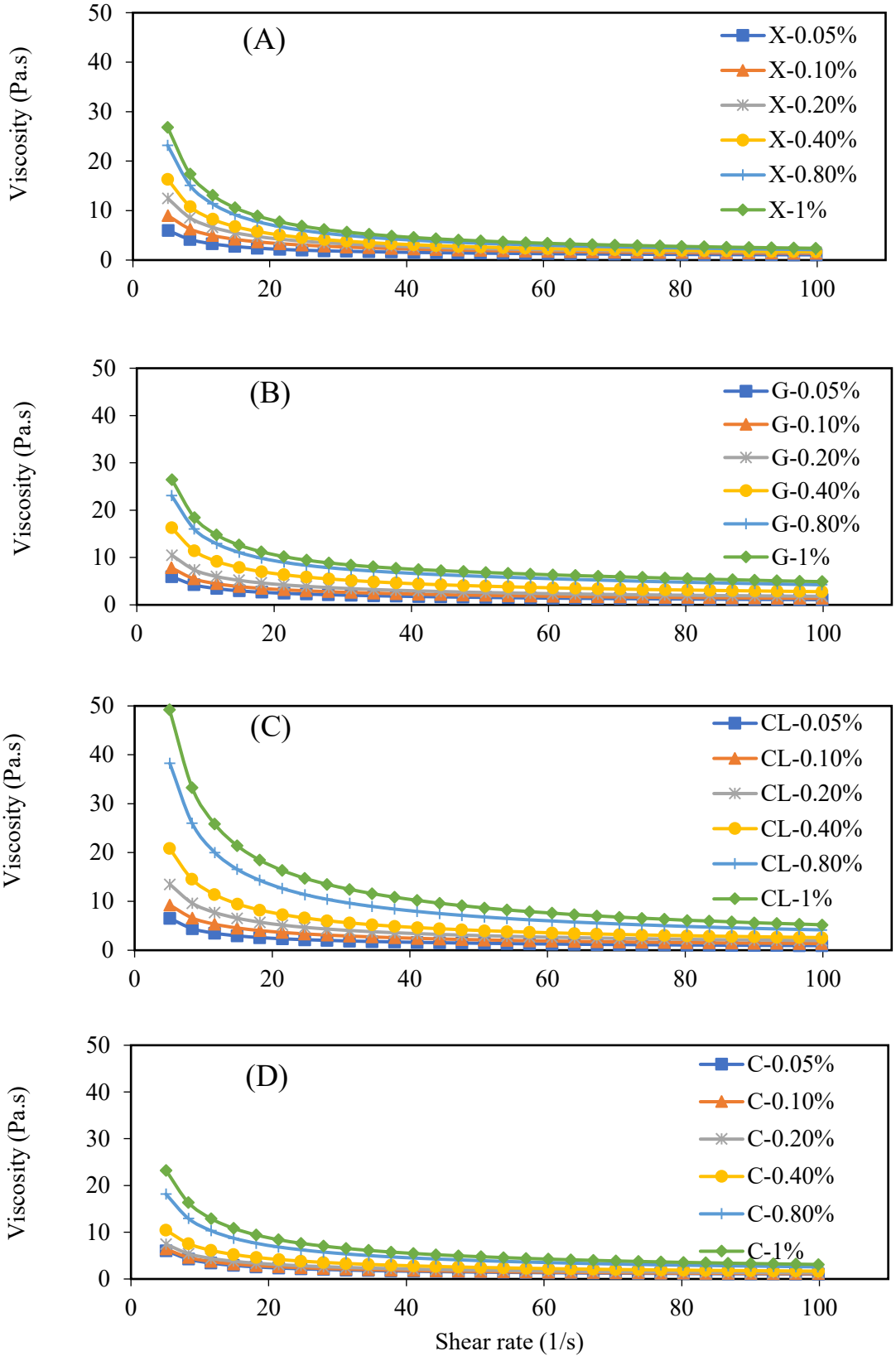



**Figure 5.2 Viscosity versus shear rate rheograms of instant emulsion samples prepared with (A) xanthan gum, (B) guar gum, (C) CMC and (D) λ-carrageenan at different concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w).**

**Table 5.3 The shear viscosity of instant emulsion samples formulated with different hydrocolloids at different concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w) measured at a 20 $s^{-1}$ shear rate.**

| Samples | Shear Viscosity (Pa·s) | | | |
|---|---|---|---|---|
| | Xanthan gum | Guar gum | CMC | λ-Carrageenan |
| 0.05% | $2.17 \pm 0.26^{1f}$ | $2.45 \pm 0.23^{1f}$ | $2.35 \pm 0.19^{1f}$ | $2.35 \pm 0.21^{1f}$ |
| 0.10% | $3.32 \pm 0.15^{2e}$ | $3.19 \pm 0.15^{2e}$ | $3.65 \pm 0.10^{1e}$ | $2.62 \pm 0.08^{3e}$ |
| 0.20% | $4.24 \pm 0.16^{2d}$ | $4.23 \pm 0.07^{2d}$ | $5.15 \pm 0.16^{1d}$ | $3.07 \pm 0.10^{3d}$ |
| 0.40% | $5.08 \pm 0.16^{3c}$ | $6.38 \pm 0.21^{2c}$ | $7.32 \pm 0.14^{1c}$ | $4.17 \pm 0.19^{4c}$ |
| 0.80% | $6.77 \pm 0.25^{3b}$ | $8.99 \pm 0.46^{2b}$ | $12.67 \pm 0.61^{1b}$ | $6.85 \pm 0.09^{3b}$ |
| 1.00% | $7.74 \pm 0.27^{4a}$ | $10.19 \pm 0.36^{2a}$ | $16.34 \pm 0.62^{1a}$ | $8.40 \pm 0.16^{3a}$ |

$^{a, b, c, d}$ Different letters within the same column represent significant differences ($p < 0.05$).
$^{1, 2, 3, 4}$ Different numbers within the same raw represent significant differences ($p < 0.05$).

**Table 5.4 Power law parameter values of instant emulsion samples formulated with different hydrocolloids at different concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w). "X" xanthan gum; "G" guar gum; "CL" CMC; "C" λ-carrageenan.**

| Samples | $k$ (Pa·$s^n$) | | | | $n$ | | | | $r^2$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X | G | CL | C | X | G | CL | C | X | G | CL | C |
| 0.05% | 12.64 | 13.44 | 16.35 | 13.80 | 0.45 | 0.45 | 0.38 | 0.43 | 0.98 | 0.99 | 0.99 | 0.99 |
| 0.10% | 23.10 | 18.29 | 25.98 | 15.28 | 0.37 | 0.44 | 0.36 | 0.43 | 0.99 | 0.99 | 0.99 | 0.99 |
| 0.20% | 36.12 | 24.88 | 37.24 | 18.17 | 0.31 | 0.43 | 0.36 | 0.42 | 0.99 | 0.99 | 0.99 | 0.99 |
| 0.40% | 52.36 | 37.63 | 62.35 | 27.30 | 0.24 | 0.43 | 0.30 | 0.39 | 0.99 | 0.99 | 0.99 | 0.99 |
| 0.80% | 79.29 | 47.97 | 122.77 | 50.63 | 0.20 | 0.47 | 0.26 | 0.35 | 0.98 | 0.99 | 0.99 | 0.99 |
| 1.00% | 93.28 | 53.81 | 162.69 | 66.32 | 0.19 | 0.48 | 0.25 | 0.33 | 0.97 | 0.99 | 0.99 | 0.99 |

$k$: flow consistency coefficient, $n$: flow behaviour index, $r^2$: coefficient of correlation.

### 5.4.3 Particle Size and Particle Size Distribution

The mean particle sizes of oil droplets D[4,3] in the instant emulsions formed with different types and concentrations of hydrocolloids are shown in Table 5.5. It was found that when the concentration of xanthan gum was increased from 0.05 to 0.1% w/w, the volume mean diameter of the emulsion increased whereas when its concentration was continuously increased from 0.1 to 1% w/w, no significant changes in the particle size were observed.

Also, the particle size of the instant emulsions containing xanthan gum was slightly larger than those formulated with other hydrocolloids at the same concentration. As mentioned in the previous section, the pH of the instant emulsions was acidic being in the range of 2.6 to 4.1, which was lower than the isoelectric pH of egg yolk proteins known as pH 6.0 (Kudre et al., 2018). At this low pH level, egg yolk proteins would possess a net positively electrical charge. If some of egg yolk proteins were accumulated at the surface of oil droplets besides Tween 80 during the emulsification process to stabilise the emulsion, the xanthan gum molecules which are anionic might be adsorbed at the interface via attractive electrostatic forces that could consequently result in a thicker protective coating layer around the oil droplets (Rahmati et al., 2015). In addition, the presence of xanthan gum might promote droplet flocculation to increase the mean particle sizes via the bridging mechanism. The anionic xanthan gum molecules might be associated with two or more cationic oil droplets together to form flocculation (McClements, 2016; Rahmati et al., 2015), which may explain a significant increase of d(0.9) when the concentration of xanthan gum was increased from 0.05 to 0.1% w/w (data shown in Appendix 1). On the other hand, when the concentration of xanthan gum was above 0.1% w/w, the surface of the droplets might be completely covered by the xanthan gum and the excess addition of xanthan gum was present in the aqueous phase to increase the viscosity (McClements, 2016). Therefore, no significant difference was observed in all the particle size parameters but resulted in a significant increase of the viscosity of the emulsions with the increasing concentration of xanthan gum. The particle size distribution of the instant emulsion samples prepared with xanthan gum at different concentrations are illustrated in Figure 5.3A. It could be noticed that a bi-modal particle size distribution with two ranges of particle sizes was encountered with the emulsion containing 0.05% w/w xanthan gum. However, with the increasing addition of xanthan gum, it was changed to a mono-modal particle size distribution. This manifests that the emulsion made with higher concentration of xanthan gum contained more uniform oil droplets in size.

In case of the emulsions prepared with guar gum, the particle size of emulsion droplets didn't seem to be affected by the different concentrations of guar gum as no consistent pattern in the particle size changes was observed that could be attributable to the concentration of guar gum (Table 5.5). Guar gum is a non-ionic polysaccharide that could produce viscosity in cold water and remain stable from pH 2 to 10 (Wüstenberg, 2015). However, the mono-modal particle size distribution was shifted to the bi-modal particle

size distribution when the concentration of guar gum was increased from 0.05 to 1% w/w, suggesting the formation of some particles in larger size induced by the addition of a relatively large amount of guar gum (Figure 5.3B. This may be due to an increase in the attractive force between oil droplets caused by an osmotic effect arising from the presence of guar gum in the continuous phase at above certain concentration (McClements, 2016). In another word, oil droplets might have undergone aggregation and the aggregation process could be promoted when the amount of guar gum presence in the continuous phase was increased at above a critical level (McClements, 2000).

The particle size of emulsions formulated with CMC was decreased slightly with the increasing CMC concentration from 0.05 to 0.2% w/w. When the CMC concentration was continuously increased from 0.2% to 1%, the values of d(0.1) and d(0.9) were further reduced in the significant level ($p < 0.05$) (data shown in Appendix 1). Similar results have been reported by several studies (Huan et al., 2016; Liu et al., 2012) which found that the mean particle sizes in the protein-stabilised O/W emulsions were significantly reduced with the increasing concentration of CMC when the pH of the emulsion was below or near the isoelectric point of the protein. As mentioned previously, the pH of the instant emulsion (2.6-4.1) was below the isoelectric point of the egg yolk protein as pH 6.0 (Kudre et al., 2018) that was coupled with Tween 80 to emulsify and stabilise the instant emulsion. Thus, a net positive charge is believed to be formed on the egg yolk coated oil droplets. With the addition of CMC, the anionic carboxylate groups on the CMC chain might be adsorbed onto the surface of the cationic egg yolk protein-coated droplets to reduce electrostatic repulsion between the positive protein molecules at the interface and increase the protein packing at the surface of oil droplets (Ganzevles et al., 2006; Huan et al., 2016). The particle size distribution curves of the emulsion samples are presented in Figure 5.3C. The increasing concentration of CMC led to a shift of the particle size distribution curves toward the smaller particle sizes (shift toward left along the x-axis), and the bi-modal particle size distribution was changed to the mono-modal distribution. This observation reveals that at higher concentration of CMC, the instant emulsion was produced with the smaller mean particle size, more uniform particle size distribution and reduced oil droplet aggregation.

In case of the emulsion prepared with λ-carrageenan, no significant impact of the concentration of λ-carrageenan on the particle size characteristics of the instant emulsions was found. Also, as shown in Figure 5.3D, the bi-modal particle size distribution was

observed for all the samples. The use of carrageenan in the application of the acidic instant emulsions may be limited as λ-carrageenan is relatively less acid resistant than other hydrocolloids and hydrolysis may occur in the acidic system with the pH < 4.3 (Pegg, 2012). However, it seemed like this adverse effect did not occur in this study.

Overall, the particle sizes of the instant emulsions prepared with different types of hydrocolloids as stabilisers were not very different although some differences existed to a smaller extent. In addition, the range of their concentrations (0.05-1%) used in this study also seemed to have no pronounced effect on the particle size of oil droplets in the instant emulsions prepared by hand shaking.

**Table 5.5 Mean particle diameter (D[4,3], μm) of instant emulsion samples prepared with xanthan gum, guar gum, CMC and λ-carrageenan at different concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w).**

| Sample | D[4, 3] (μm) | | | |
|---|---|---|---|---|
| | Xanthan gum | Guar Gum | CMC | λ-Carrageenan |
| 0.05% | $55 \pm 3.42^{b}$ | $42 \pm 2.29^{d}$ | $53 \pm 6.60^{a}$ | $52 \pm 4.45^{bc}$ |
| 0.10% | $65 \pm 3.80^{a}$ | $45 \pm 6.84^{bc}$ | $50 \pm 5.08^{b}$ | $53 \pm 4.45^{bc}$ |
| 0.20% | $66 \pm 5.55^{a}$ | $43 \pm 2.34^{cd}$ | $46 \pm 2.73^{c}$ | $58 \pm 6.28^{a}$ |
| 0.40% | $64 \pm 9.26^{a}$ | $43 \pm 2.44^{cd}$ | $44 \pm 1.04^{cd}$ | $48 \pm 2.52^{c}$ |
| 0.80% | $63 \pm 3.01^{a}$ | $47 \pm 2.61^{ab}$ | $41 \pm 0.76^{d}$ | $54 \pm 6.88^{ab}$ |
| 1.00% | $64 \pm 10.18^{a}$ | $50 \pm 3.76^{a}$ | $43 \pm 0.78^{cd}$ | $55 \pm 4.02^{ab}$ |

[a, b, c, d] Different letters within the same column represent significant differences ($p < 0.05$).

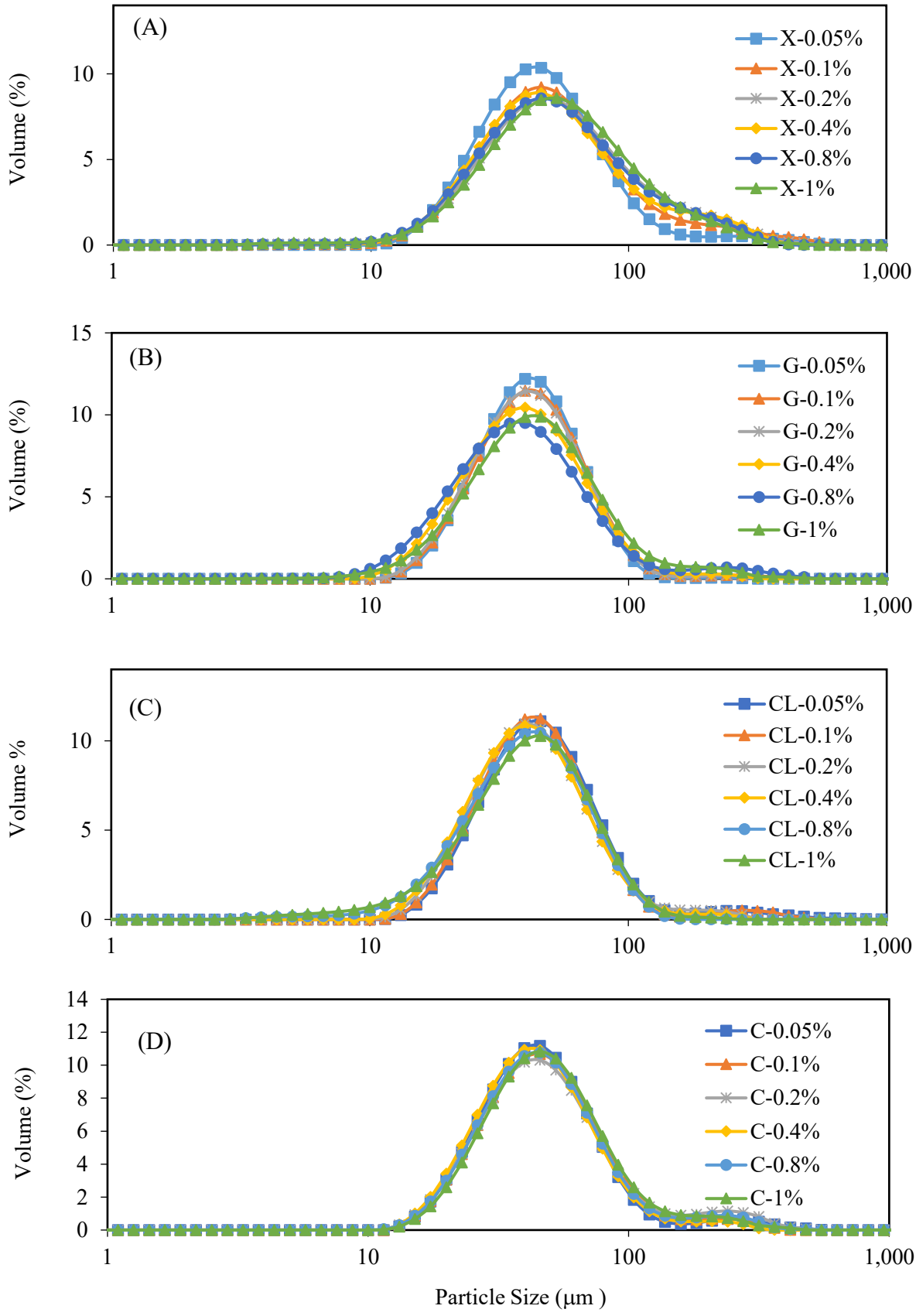



**Figure 5.3 Particle size distribution of oil droplets in instant emulsions with (A) xanthan gum, (B) guar gum, (C) CMC and (D) λ-carrageenan at different concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w)**

### 5.4.4 Microstructure of Emulsions

As revealed by the microscopic images as shown in Figures 5.4 and 5.5, the microstructure of the instant emulsions contained closely packed oil droplets in various sizes were observed. Figure 5.4A shows the microstructure of the xanthan gum-stabilised emulsions. It was observed that the size of oil droplets was reduced with the increasing addition of xanthan gum. Also, the presence of oil droplets in relatively larger sizes was gradually disappeared from the images, indicating the formed emulsions became more uniform under the effect of the increasing concentrations of xanthan gum. The microstructure of guar gum emulsions is presented in Figure 5.4B. Oil droplets in smaller sizes were produced at higher concentration of guar gum. However, it could be noticed that the greater extend of droplet aggregations was observed in the emulsion samples contained higher concentration of guar gum. The droplet aggregation increased the mean particle sizes of the emulsion samples so that larger values of D[4,3] were detected by the particle analyser (Table 5.5). Similar microstructure was observed from the CMC and carrageenan-stabilised emulsions. At concentration of 0.05 to 0.1% w/w, both emulsions had the largest non-uniform oil droplets among all the samples. By increasing the concentration of CMC and carrageenan from 0.2 to 1% w/w, the produced oil droplets became more uniformly distributed with smaller sizes (Figures 5.4C and D). Furthermore, a network-like structure was observed from the emulsion sample formulated with 1% of CMC, which might be formed due to the relatively more viscous continuous phase of the emulsion sample that traps the clusters of oil droplets (Figure 5.4C).

The microstructure of the emulsions contained xanthan gum and CMC were also reviewed by using the CLSM. As shown in Figures 5.5, the red-stained oil droplets in various sizes were distributed in the dark continuous phase, which contained the non-staining convoluted starch polymers and green-stained protein molecules. For the xanthan gum-stabilised emulsions, the micrographs of samples contained 0.2 to 1% w/w of xanthan gum were similar with some droplet flocculation (Figure 5.5A). In addition, as shown in Figure 5.5B, no sign of droplet flocculation was found in the micrographs of emulsions stabilised with 0.05 to 0.8% w/w of CMC. When the CMC concentration was further increased to 1%, a network structure resulted from the droplet aggregation was observed.

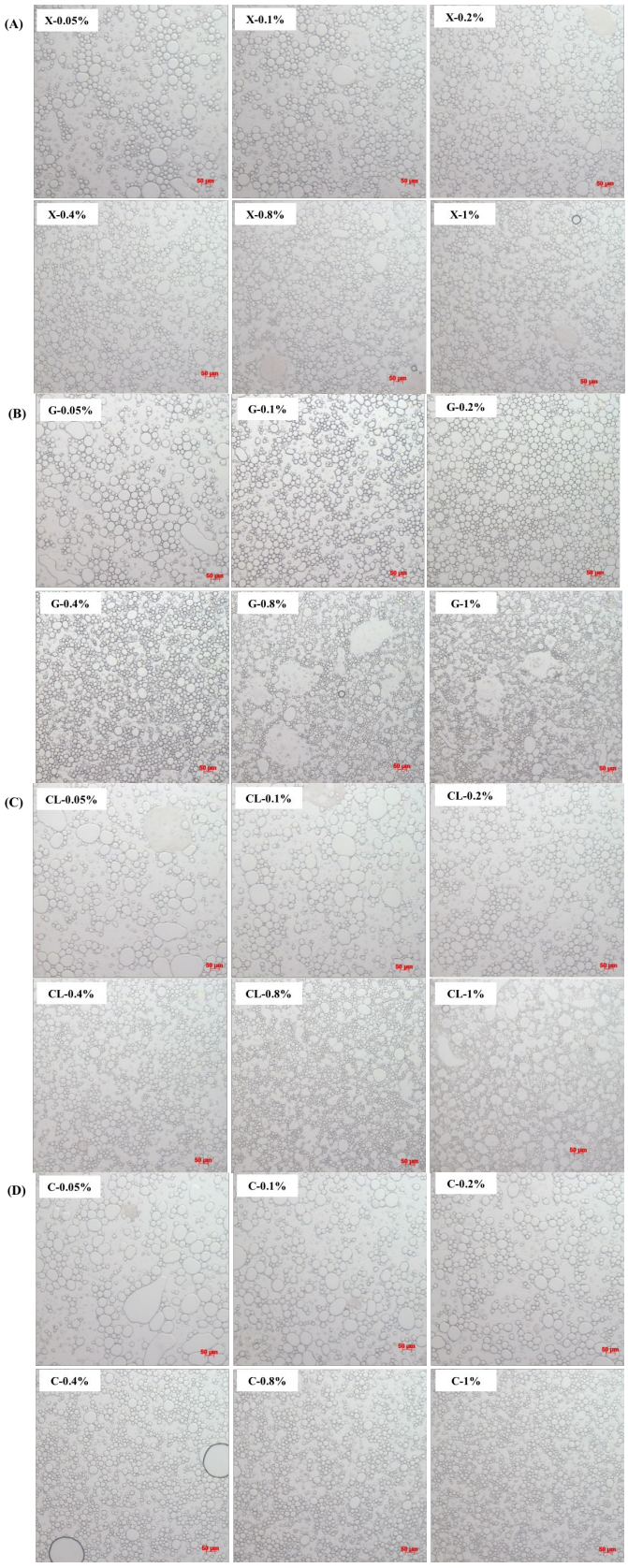



**Figure 5.4 Light microscopic images (scale bar 50 μm) of instant emulsions formulated with different types of hydrocolloids: (A) xanthan gum, (B) guar gum, (C) CMC and (D) λ-carrageenan, at different concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w).**

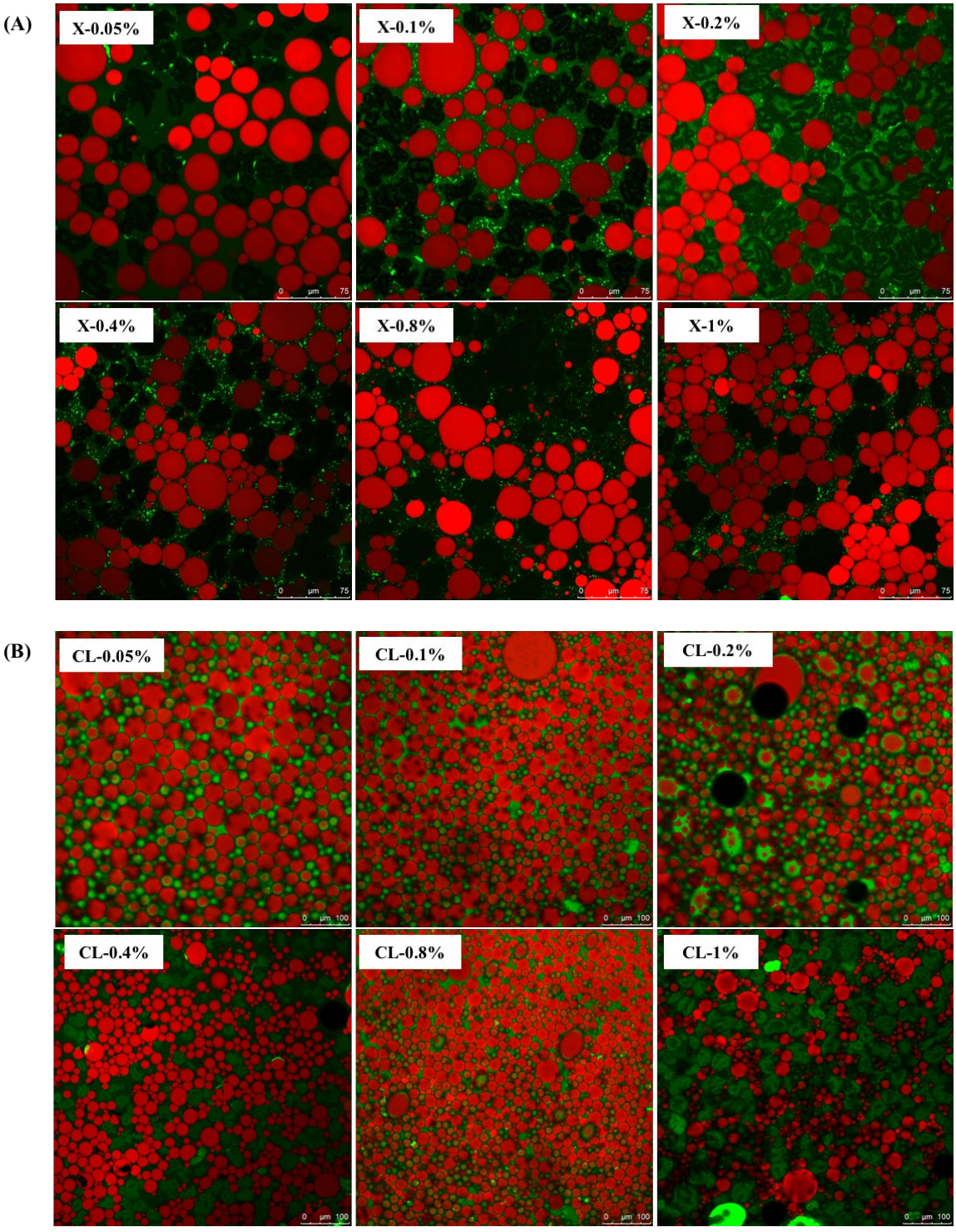



**Figure 5.5 CLSM images of instant emulsions formulated with (A) xanthan gum (scale bar 75 µm) and (B) CMC (scale bar 100 µm) at different concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w).**

### 5.4.5 Stability of Emulsions

The ability of different types and concentrations of non-starch hydrocolloids to stabilise the instant creamy emulsions was assessed by centrifuging the emulsion samples at a relatively low speed (3792 g) for 10 minutes. As shown in Figures 5.6, different degrees of emulsion destabilisation were observed from the emulsion samples after centrifugation.

Phase separations were clearly observed from the emulsion samples prepared with low concentrations of hydrocolloids ranging from 0.05 to 0.1% w/w, which was due to the rapid movement of oil droplets under the centrifugal force. The larger oil droplets were moved upwards and then concentrated at the top of the emulsions, thus forming a relatively thicker creaming layer. Also, a separate thin layer of free oil was observed to be present on the surface of the most emulsions. This may be partly resulting from the coalescence of some packed oil droplets. However, with increasing the concentration of hydrocolloids to 0.4% w/w, the best emulsion stability was observed as no creaming was noticed in the emulsions stabilised by all four types of hydrocolloids which was also coupled with no noticeable free oil or a relatively smaller amount of free oil. This might be attributed from the increasing level of added hydrocolloids up to an optimum level that strengthened a network structures of the emulsions against the centrifugation. However, when the concentration of hydrocolloids was continuously increased to above 0.8%, the stability of the emulsions was slightly reduced again as a noticeable layer of free oil was observed on top of the samples containing 0.8% and 1% stabilisers while the body of the samples still remained homogeneous. The possible reason for the loss of emulsion stability (e.g. free oil and creaming) might be due to the presence of the larger oil droplets presented in the systems, which might be formed due to the limited breaking up of oil phase in a highly viscous continuous phase of the emulsions (Rahmati et al., 2015).

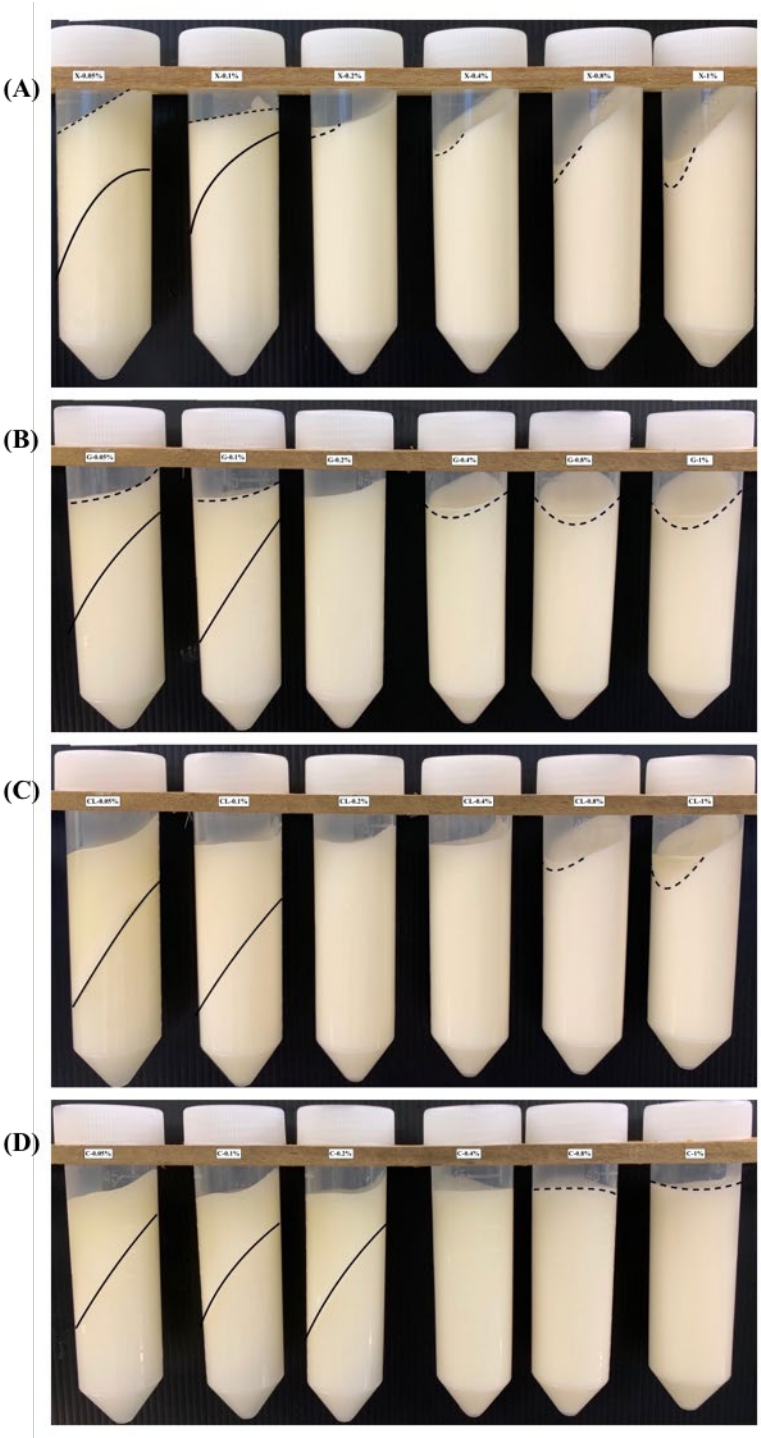



**Figure 5.6 Pictures of instant emulsion samples containing different types of hydrocolloids: (A) xanthan gum, (B) guar gum, (C) CMC and (D) λ-carrageenan at different concentrations (from left to right: 0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w). Pictures taken after centrifugation to observe the stability of emulsions against the formation of creaming, free oil, etc. (----- indicates the boundary of free oil and cream layer; —— indicates the boundary of cream layers).**

### 5.4.6 Textural Properties of Emulsions

The results of texture profile analysis for all the instant emulsions prepared with different non-starch hydrocolloids at various concentrations are shown in Table 5.6 below. It was obvious that the type and concentration of the added hydrocolloids had the significant effects on the hardness, consistency, consistency, cohesiveness and viscosity of the instant creamy emulsions ($p < 0.05$).

All the texture parameters of the emulsions, including the firmness, consistency and cohesiveness as well as the index of viscosity, were increased markedly by increasing the concentration of the added hydrocolloids ($p < 0.05$). This was mainly related to the increasing viscosity of the emulsion samples due to the addition of the hydrocolloid stabilisers to the formulations. In addition, at the concentration of 0.05 to 0.2% w/w, the instant emulsions formulated with xanthan gum showed the largest firmness, cohesiveness, and index of viscosity. While at concentration of 0.4 to 1% w/w, the samples stabilised with CMC has the largest values for all four parameters. This result was probably caused by the molecular interactions between the xanthan gum or CMC with the egg yolk protein and starch, which strengthened the emulsion structures and improved the texture characteristics. On the other hand, the lowest values of all the texture attributes were recorded for the emulsions formulated with carrageenan at all concentrations. This might have been likely due to the relatively low viscosity of the carrageenan-stabilised emulsions compared to the other samples containing xanthan gum, guar gum or CMC (Table 5.3).

**Table 5.6 Firmness, consistency, cohesiveness and index of viscosity of instant emulsions prepared with different concentrations of hydrocolloids (0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w). "X" xanthan gum, "G" guar gum, "CL" CMC and "C" λ-carrageenan.**

| Samples | Firmness (g) | | | |
|---|---|---|---|---|
| | X | G | CL | C |
| 0% | 53.22 ± 6.52$^{f}$ | 53.22 ± 6.52$^{f}$ | 53.22 ± 6.52$^{f}$ | 53.22 ± 6.52$^{f}$ |
| 0.05% | 68.93 ± 12.78$^{ef}$ | 60.93 ± 2.57$^{f}$ | 62.15 ± 1.91$^{ef}$ | 57.20 ± 1.37$^{ef}$ |
| 0.10% | 83.28 ± 9.25$^{e}$ | 71.41 ± 7.05$^{e}$ | 71.38 ± 3.81$^{e}$ | 59.612 ± 0.68$^{e}$ |
| 0.20% | 111.49 ± 13.50$^{d}$ | 82.03 ± 3.65$^{d}$ | 91.78 ± 4.50$^{d}$ | 64.16 ± 1.42$^{d}$ |
| 0.40% | 137.74 ± 13.23$^{c}$ | 127.73 ± 8.85$^{c}$ | 138.00 ± 6.47$^{c}$ | 79.64 ± 2.40$^{c}$ |
| 0.80% | 198.88 ± 23.10$^{b}$ | 185.35 ± 11.00$^{b}$ | 248.62 ± 17.87$^{b}$ | 124.30 ± 5.13$^{b}$ |
| 1.00% | 232.89 ± 29.00$^{a}$ | 197.65 ± 11.52$^{a}$ | 262.56 ± 16.73$^{a}$ | 151.10 ± 2.16$^{a}$ |

| Samples | Consistency (g·sec) | | | |
|---|---|---|---|---|
| 0% | 328.77 ± 24.63$^{g}$ | 328.77 ± 24.63$^{f}$ | 328.77 ± 24.63$^{g}$ | 328.77 ± 24.63$^{f}$ |
| 0.05% | 435.30 ± 67.82$^{f}$ | 370.50 ± 26.71$^{f}$ | 397.69 ± 10.86$^{f}$ | 362.00 ± 11.14$^{e}$ |
| 0.10% | 536.42 ± 45.47$^{e}$ | 460.04 ± 35.90$^{e}$ | 456.64 ± 19.09$^{e}$ | 371.35 ± 5.64$^{e}$ |
| 0.20% | 708.65 ± 60.39$^{d}$ | 539.78 ± 15.01$^{d}$ | 588.68 ± 17.54$^{d}$ | 404.89 ± 11.33$^{d}$ |
| 0.40% | 879.92 ± 61.81$^{c}$ | 812.27 ± 46.54$^{c}$ | 872.43 ± 21.32$^{c}$ | 505.27 ± 12.84$^{c}$ |
| 0.80% | 1264.96 ± 118.70$^{b}$ | 1132.54 ± 77.05$^{b}$ | 1534.11 ± 84.53$^{b}$ | 783.32 ± 15.03$^{b}$ |
| 1.00% | 1458.10 ± 69.00$^{a}$ | 1225.64 ± 65.41$^{a}$ | 1662.30 ± 72.89$^{a}$ | 915.77 ± 32.65$^{a}$ |

| Samples | Cohesiveness (g) | | | |
|---|---|---|---|---|
| 0% | -36.20 ± 4.03$^{a}$ | -36.20 ± 4.03$^{a}$ | -36.20 ± 4.03$^{a}$ | -36.20 ± 4.03$^{a}$ |
| 0.05% | -52.68 ± 9.78$^{b}$ | -46.96 ± 1.22$^{b}$ | -48.58 ± 2.46$^{b}$ | -42.43 ± 1.23$^{b}$ |
| 0.10% | -68.66 ± 5.88$^{c}$ | -58.96 ± 6.00$^{c}$ | -61.71 ± 1.65$^{c}$ | -45.29 ± 1.08$^{b}$ |
| 0.20% | -93.73 ± 7.79$^{d}$ | -77.44 ± 2.56$^{d}$ | -85.72 ± 2.74$^{d}$ | -51.22 ± 1.80$^{c}$ |
| 0.40% | -116.87 ± 6.57$^{e}$ | -121.05 ± 6.87$^{e}$ | -135.39 ± 1.44$^{e}$ | -72.65 ± 0.91$^{d}$ |
| 0.80% | -167.86 ± 12.39$^{f}$ | -181.23 ± 9.84$^{f}$ | -244.23 ± 14.37$^{f}$ | -121.55 ± 2.34$^{e}$ |
| 1.00% | -192.35 ± 7.11$^{g}$ | -195.37 ± 11.71$^{g}$ | -263.95 ± 11.05$^{g}$ | -148.33 ± 6.07$^{f}$ |

| Samples | Index of Viscosity (g·sec) | | | |
|---|---|---|---|---|
| 0% | -118.30 ± 12.24$^{a}$ | -118.30 ± 12.24$^{a}$ | -118.30 ± 12.24$^{a}$ | -118.30 ± 12.24$^{a}$ |
| 0.05% | -167.79 ± 28.66$^{b}$ | -155.71 ± 11.32$^{b}$ | -157.77 ± 9.40$^{b}$ | -137.78 ± 4.69$^{b}$ |
| 0.10% | -219.35 ± 19.63$^{c}$ | -187.92 ± 17.15$^{c}$ | -196.18 ± 3.94$^{c}$ | -146.11 ± 3.07$^{b}$ |
| 0.20% | -292.73 ± 27.23$^{d}$ | -244.03 ± 12.45$^{d}$ | -264.99 ± 5.66$^{d}$ | -164.28 ± 6.74$^{c}$ |
| 0.40% | -361.51 ± 22.65$^{e}$ | -362.80 ± 23.22$^{e}$ | -421.34 ± 8.98$^{e}$ | -226.35 ± 7.73$^{d}$ |
| 0.80% | -522.35 ± 41.28$^{f}$ | -524.55 ± 34.26$^{f}$ | -743.69 ± 42.50$^{f}$ | -369.87 ± 11.02$^{e}$ |
| 1.00% | -595.65 ± 25.23$^{g}$ | -569.65 ± 23.89$^{g}$ | -810.40 ± 42.01$^{g}$ | -449.57 ± 15.58$^{f}$ |

$^{a, b, c, d, e, f, g}$ Different letters within the same column represent significant differences ($p < 0.05$).

## 5.5 Conclusions

The instant emulsions formulated with different types of hydrocolloids as stabilisers, such as xanthan gum, guar gum, CMC and λ-carrageenan, at different concentrations were able to be produced successfully by the relatively low shear generated by simply hand shaking. The white and creamy emulsions were observed for all the samples with a similar appearance to commercial mayonnaise. All the samples showed the pseudoplastic flow behaviour and the increasing concentration of hydrocolloids (xanthan gum, CMC and carrageenan) promoted the shear thinning behaviours of the emulsions except for the samples formulated with guar gum which was non-ionic. It was also found that the type and concentration of the hydrocolloids had the significant effects on the texture characteristics of the instant emulsions. All the texture parameters, including the firmness, consistency, cohesiveness and index of viscosity, were increased markedly by increasing the concentration of added hydrocolloids. At concentration of 0.05 to 0.4% w/w, the highest values of all texture attributes were obtained in the emulsion containing xanthan gum, whereas, at concentration of 0.4 to 1% w/w, the highest results were obtained from the emulsions formed with CMC. All the emulsion samples showed the better emulsion stability against creaming when they were formulated with 0.2 to 0.4% w/w of hydrocolloids.

It was noticed that the sample formulated with CMC showed some outstanding physiochemical properties than the other samples. The highest viscosity was obtained from the CMC stabilised emulsions. At the same time, with the increasing concentration of added CMC, the mean particle diameter of the oil droplets in the emulsions was decreased significantly with the more uniform particle size distribution. These properties revealed the potential of CMC to be utilised in the application of the instant creamy emulsions.

# Chapter 6 Effects of Types and Concentrations of Emulsifiers on the Formation and Properties of Instant Creamy Emulsions

## 6.1 Abstract

Emulsifier is one of the key ingredients in O/W emulsions to improve the formation and stability of the emulsions. In Chapter 5, the study investigated the effects of different types and concentrations of hydrocolloids as stabilisers. In this chapter, the effects of emulsifiers on the formation and properties of instant emulsions containing 40% w/w oil was explored. Therefore, the instant O/W emulsions were prepared with four different types of emulsifiers, such as egg yolk powder, Tween 80, alpha-cyclodextrin and OSA-starch, at different concentrations (0.1, 0.5, 1, 2% w/w). The emulsions prepared were then characterised for their visual appearance, pH, viscosity, particle size, microstructure, textural properties and emulsion stability. The results revealed that the instant emulsions formulated with different emulsifiers at different concentrations had significantly different properties in all aspects. Egg yolk was found to be not suitable to be used as a sole emulsifier in making the instant creamy emulsions produced by hand shaking, due to the presence of a substantial amount of free oil which was detected visually and microscopically. A stable instant emulsion could be formed with Tween 80 at a relatively low concentration of 0.5% w/w in which small oil droplets with the relatively uniform particle size distribution were observed. However, the instant emulsion formed with Tween 80 had a significant decrease in its viscosity with increasing concentration of Tween 80, which also lowered the values of textural parameters measured for firmness, consistency and cohesiveness. The results of this research project also showed the potential of alpha-cyclodextrin and OSA-starch to aid the formation and stabilisation of the instant emulsions. The emulsions prepared with alpha-cyclodextrin showed the relatively higher viscosity then the other emulsion samples. However, the results showed that the concentration of alpha-cyclodextrin should be less than 2% w/w, as a different microstructure was observed from the instant emulsion sample containing 2% of alpha-cyclodextrin which might be formed due to the extremely thick and viscous texture of the sample. OSA-starch was identified to be the most appropriate emulsifier to be incorporated in making the instant emulsions as it aided the formation of oil droplets in a more uniform particle distribution with the smallest particle size. In addition, its rheological and textural properties as well as its emulsion stability were also observed to be more suitable.

## 6.2 Introduction

The O/W emulsions (e.g. mayonnaise, salad dressing) are formed by disrupting the oil into small droplets and dispersing them in the continuous aqueous phase. However, the formation of small droplets results in a massive increase in the interfacial area between the oil and water phases, which is thermodynamically unfavourable and the emulsion system thus tends to separate into two bulk layers of oil and water (McClements, 2016; Robins & Wilde, 2003). In order to maintain the droplet structure of emulsions, surface active ingredients, also known as emulsifiers, are added into the emulsions to adsorb at the oil-water interface to reduce its interfacial tension. This is a process of emulsification that prevents the oil droplets from flocculation and coalescence (Robins & Wilde, 2003). The commonly used emulsifiers in the food industry can be either small molecular surfactants (e.g. lecithin, Tween 80) or macromolecular polymers (e.g. some proteins and polysaccharides) (McClements, 2016; Robins & Wilde, 2003). To form and stabilise an O/W emulsion, emulsifiers should be water soluble with a high value (10-18) of hydrophile-lipophile balance (HLB) (McClements, 2016). The appropriate emulsifiers should also be able to rapidly adsorb on the surface of oil droplets formed during emulsification and to reduce the interfacial tension between the water and oil phases to promote the formation of emulsions. Also, the emulsifiers should be resistant to some environmental conditions (e.g. pH, ionic strength, temperature) and stabilise the oil droplets against aggregation during manufacture, transportation and storage (Chung et al., 2017; McClements, 2016).

In this research project, the instant creamy O/W emulsions were produced by vigorously shaking the oil and aqueous phases together in a sealed plastic container by hand with short, quick movements up and down. However, some emulsifiers that have been commonly used in the food emulsions produced by using the high-pressure homogenisation process may not be suitable for this application due to the relatively low shear emulsification process involved in making the instant creamy emulsions. Therefore, based on the preliminary experimental trials and recommendations from ingredient suppliers, four different emulsifiers, such as egg yolk powder, Tween 80, cyclodextrin and octenyl succinic anhydride (OSA) starch, were chosen and used to investigate their functionalities as emulsifiers in producing the instant creamy emulsions.

These four emulsifiers have different chemical structures and functional properties. Egg yolk is widely used as an emulsifier to form and stabilise food emulsions such as mayonnaise and salad dressings (Anton, 2013). The addition of egg yolk also imparts the desirable sensory properties of mayonnaise such as colour, flavour and texture (Yang & Lai, 2003a). It is believed that the emulsifying functionality of egg yolk was mainly attributed from the plasma of egg yolk that contains low-density lipoproteins (LDLs) and soluble proteins (Anton, 2013; McClements, 2016). However, egg yolk can impose some negative constrains, such as a source of microbial contamination with microorganisms (e.g. *Salmonella*), price and a high level of cholesterol (Mirzanajafi-Zanjani et al., 2019). Tween 80 is a powerful small molecular surfactant as a small amount of its use can result in a dramatic reduction in the interfacial tension between oil and water phases (Cottrell & Peij, 2015). Thus, Tweens are recommended to be used in the instant emulsions as an emulsifier (Farrer et al., 2003). However, due to the increasing demand for natural ingredients, Tween 80 which is chemically synthesised can raise some concerns (Campbell, 2019; Chung et al., 2017). OSA starch is a modified starch with amphiphilic properties as an emulsifier which is produced by substituting the hydroxyl groups of starch molecules by octenyl succinic anhydride (OSA) moieties which are hydrophobic (Sweedman et al., 2013). The use of OSA-starch applied in the reduced fat mayonnaise has been studied by some researchers (Bortnowska & Tokarczyk, 2009; Chivero et al., 2016; Thaiudom & Khantarat, 2011). However, all the studies were based on the emulsions prepared by using the mechanical equipment (e.g. mixer, homogeniser) and no study had been conducted on the application of OSA-starch in the instant emulsions prepared by hand shaking. Alpha-cyclodextrin is a water soluble dietary fibre but has surface activity that can stabilise the O/W emulsions effectively (Moreira da Silva, 2009). Although alpha-cyclodextrin has been approved as a novel food ingredient by New Zealand Food Standards and recommended by an ingredient supplier to be suitable for used in dressings and mayonnaise-like applications (Food Standards Australia New Zealand, 2017; Wacker Chemie AG, n.d.). There is no published data in the literature on the application of alpha-cyclodextrin in the instant emulsions.

Taking these considerations into account, the objective of this research was to investigate the effects of egg yolk powder, Tween 80, alpha-cyclodextrin and OSA-starch at different concentrations on the formation and properties of the instant creamy O/W emulsions. The properties of the instant emulsions characterised in this research included their visual

appearance, pH, viscosity, particle size and particle size distribution, microstructure, textural properties and emulsion stability.

## 6.3 Materials and Methods

### 6.3.1 Materials

A preliminary test was initially carried out with seven different emulsifier ingredients as listed in Table 6.1, including egg yolk powder, egg white powder, Tween 80, sodium caseinate, OSA-starch, sucrose ester and alpha-cyclodextrin. These ingredients were selected based on a literature review and/or some recommendations from ingredient suppliers. The other ingredients (e.g. canola oil, citric acid, xanthan gum, etc.) which were also used in preparing the instant emulsions were described in Chapter 3 (Section 3.1).

**Table 6.1 Seven different emulsifiers initially used in the preliminary test to investigate their effect on the formation of instant creamy O/W emulsions.**

| Ingredients | Product Code | Manufacturer |
|---|---|---|
| Egg yolk | Egg yolk powder | EPS S.p.A (Occhiobello, Italy) |
| Egg white | Egg white powder | EPS S.p.A (Occhiobello, Italy) |
| Tween 80 | TW0080 | Scharlau (Barcelona, Spain) |
| Caseinate | Sodium caseinate 167 | Fonterra (Auckland, New Zealand) |
| Octenyl succinate starch | N-Creamer 46 | Ingredion (Auckland, New Zealand) |
| Sucrose esters of fatty acids | DK Ester F-160 | Dai-ichi Kogyo Seiyaku Co. Ltd. (Karawang, Indonesia) |
| Alpha-cyclodextrin | CAVAMAX W6 Food | Wacker Chemie AG (Munich, Germany) |

### 6.3.2 Preliminary Test

Seven instant emulsion samples were prepared with the preselected emulsifier ingredients by following the formulation and methods as described in Chapter 3 (Section 3.2). After the preparation of instant emulsions, the emulsion samples were observed visually to examine the presence of phase separation, free oil, lumps, etc. Then the emulsion samples were centrifuged by following the methods as described in Chapter 3 (Section 3.5) to evaluate the emulsion stability of the samples. Based on the results of the emulsion stability

conducted by the centrifugation method, the four emulsifier ingredients, such as egg yolk powder, Tween 80, alpha-cyclodextrin (CAVAMAX W6 Food) and OSA-starch (N-CREAMER 46), which formed a relatively stable instant emulsion without a pronounced phase separation were selected to further investigate their effects on the characteristics of the instant creamy emulsions.

### 6.3.3 Preparation of Instant Emulsions with Different Types and Concentrations of Emulsifiers

Four emulsifier ingredients were screened out from the preliminary test as described above that were able to form the stable instant creamy emulsions without a pronounced phase separation. Thus, instant emulsion samples were prepared with each of those emulsifier ingredients at various concentrations (0.1, 0.5, 1 and 2% w/w) as shown in Table 6.2 to investigate their effects on the formation and physicochemical properties of instant creamy emulsions. The methods used for sample preparation was described in Chapter 3 (Section 3.2).

**Table 6.2 Formulations of instant emulsions prepared with four different emulsifiers (egg yolk powder, Tween 80, alpha-cyclodextrin and OSA-starch) at different concentrations (0.1, 0.5, 1 and 2% w/w).**

| Ingredients | Concentrations (% w/w) | | | |
|---|---|---|---|---|
| Canola oil | 40 | 40 | 40 | 40 |
| Starch | 4 | 4 | 4 | 4 |
| Xanthan gum | 0.2 | 0.2 | 0.2 | 0.2 |
| Citric acid | 0.3 | 0.3 | 0.3 | 0.3 |
| **Emulsifiers** | **0.1** | **0.5** | **1** | **2** |
| Water | 55.4 | 55 | 54.5 | 53.5 |
| Total | 100 | 100 | 100 | 100 |

### 6.3.4 Analysis of Samples

The instant creamy emulsion samples prepared with four different emulsifier ingredients at different concentrations were analysed for their visual appearance, pH, rheological and textural properties, particle size and particle size distribution, microstructures and emulsion stability using the methods as described in Chapter 3 (Section 3.3 to 3.9).

### 6.3.5 Data Analysis

All the measurements were carried out at least twice for each sample from duplicate experiments. The results were statistically analysed using the IBM SPSS Statistics Program (version 25, IBM Corporation, USA). The one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test were used to examine differences between the mean values at a significance level of $p < 0.05$.

## 6.4 Results and Discussions

### 6.4.1 Appearance and pH

Instant emulsions containing 40% oil were prepared with four different emulsifiers at a series of concentrations (0.1, 0.5, 1 and 2% w/w). These emulsifier ingredients were egg yolk powder (EY), Tween 80 (T80), alpha-cyclodextrin (CD) and OSA-starch (OSA). As shown in Figure 6.1, the instant emulsion samples with some different appearance were observed. Among all the emulsion samples prepared with different types of emulsifiers, the samples formed with egg yolk powder were more yellowish in colour. The yellowish appearance of the emulsion samples was attributed to the natural pigments (carotenoids) present in the egg yolk and more intense yellowish colour was observed from the emulsion samples formulated with higher concentrations of egg yolk powder (e.g. 1 and 2%). However, it was observed that the capacity of the egg yolk to emulsify the same amount of oil was reduced when the emulsion was formulated with higher concentration of egg yolk. As shown in the image of sample with 2% w/w egg yolk powder (EY-2%), a pronounced amount of free oil was observed as the oil phase was not completely emulsified in the sample. On the other hand, all the other emulsion samples were produced efficiently by hand shaking with the other emulsifiers. The semi-solid appearance with a white coloured, creamy texture was observed from all these samples. It was also noticed that the emulsion sample formed with 2% w/w of alpha-cyclodextrin (CD-2%) exhibited a more viscous and thicker texture than the other emulsion samples, which might indicate its more pronounced thickening effect when its concentration exceeds a certain level in the formulations.

The pH values of the instant emulsion samples measured are summarised in Table 6.3. The instant emulsions formed with four different emulsifiers at different concentrations were all acidic with a pH ranged from 2.5 to 3.1. It was observed that the emulsion samples formulated with Tween 80 had the highest pH values. With increasing concentration of

Tween 80 from 0.1 to 2%, the pH increased from 2.6 to 3.1. On the other hand, no marked difference was observed between the pH values of the emulsion samples formulated with egg yolk, alpha-cyclodextrin and OSA-starch. With increasing the concentrations of these three types of emulsifiers in the formulation, no significant impact was also observed on the pH values of the instant emulsion samples.

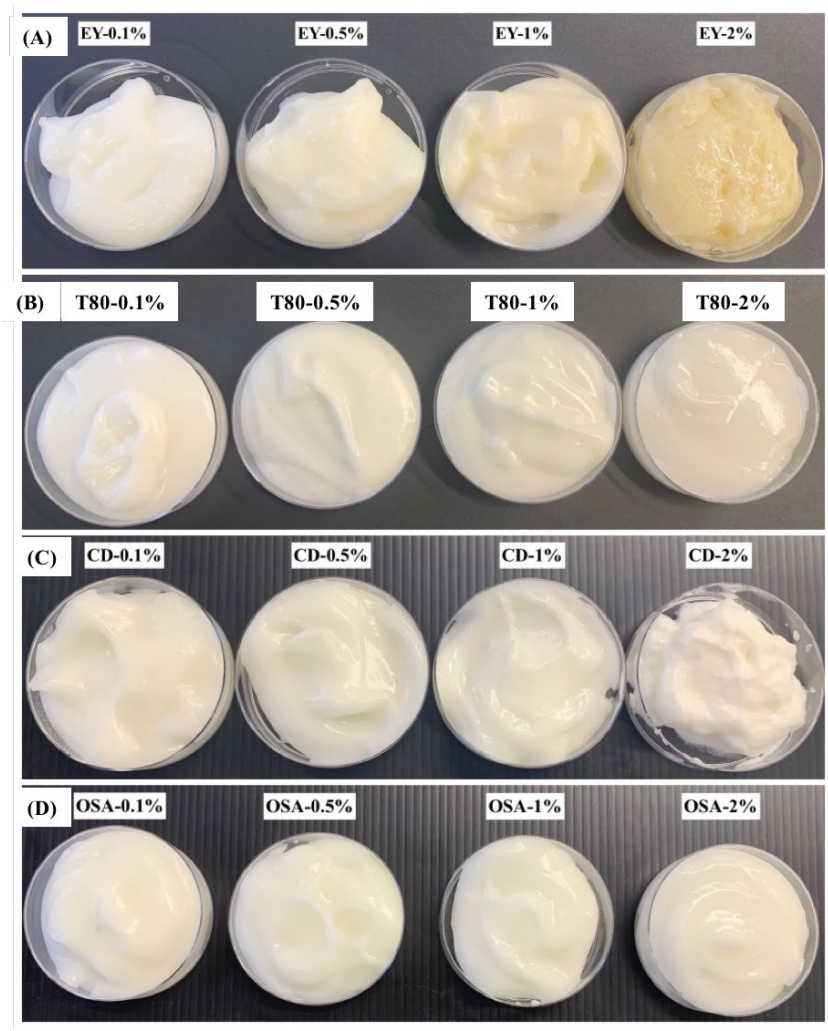



**Figure 6.1 Instant emulsion samples containing 40% w/w oil prepared with (A) egg yolk powder, (B) Tween 80, (C) alpha-cyclodextrin, and (D) OSA-starch at different concentrations (0.1, 0.5, 1 and 2% w/w).**

**Table 6.3 pH values of the instant emulsion samples prepared with different emulsifier ingredients, such as egg yolk powder (EY), Tween 80 (T80), alpha-cyclodextrin (CD) and OSA-starch (OSA), at different concentrations (0.1, 0.5, 1 and 2% w/w).**

| Emulsifier Concentrations | pH | | | |
|---|---|---|---|---|
| | EY | T80 | CD | OSA |
| 0.1% | 2.60 ± 0.06 | 2.65 ± 0.07 | 2.50 ± 0.03 | 2.55 ± 0.02 |
| 0.5% | 2.59 ± 0.03 | 2.72 ± 0.04 | 2.50 ± 0.05 | 2.56 ± 0.02 |
| 1% | 2.58 ± 0.01 | 2.84 ± 0.02 | 2.56 ± 0.02 | 2.56 ± 0.01 |
| 2% | 2.60 ± 0.01 | 3.05 ± 0.02 | 2.56 ± 0.02 | 2.58 ± 0.01 |

### 6.4.2 Rheological Properties

The rheological properties of the instant emulsion samples formulated with four different types of emulsifiers at different concentrations were characterised by measuring the apparent viscosity of the samples at the shear rate ranging from 5 to 100 $s^{-1}$. As shown in Figure 6.2, the apparent viscosity of the instant emulsion samples decreased markedly from the relatively high values to the low constant values when the shear rate increased from 5 to 100 $s^{-1}$. This observation allowed to conclude that all the emulsion samples were non-Newtonian and pseudoplastic fluids. The reduction of viscosity of the instant emulsions under the applied shear could be due to several possible reasons, such as the orientation of random distributed oil droplets to be aligned when a shear applied, or the deformation and disruption of flocculated oil droplets under the shear (McClements, 2016).

The apparent viscosity of the emulsions measured at the shear rate of 20 $s^{-1}$ is also summarised in Table 6.4. The highest viscosity was obtained from the sample prepared with 2% w/w of alpha-cyclodextrin (CD-2%), which was in accordance with the visual observations from the appearance assessment as described previously (Figure 6.1). On the other hand, the lowest viscosity was seen from the samples prepared with Tween 80. In addition, it was found that the viscosity of the instant emulsions increased significantly with increasing concentrations of egg yolk, alpha-cyclodextrin and OSA-starch ($p < 0.05$). However, interestingly, the viscosity of the instant emulsions formed with Tween 80 was observed to decreased significantly when the concentrations of Tween 80 was increased ($p < 0.05$).

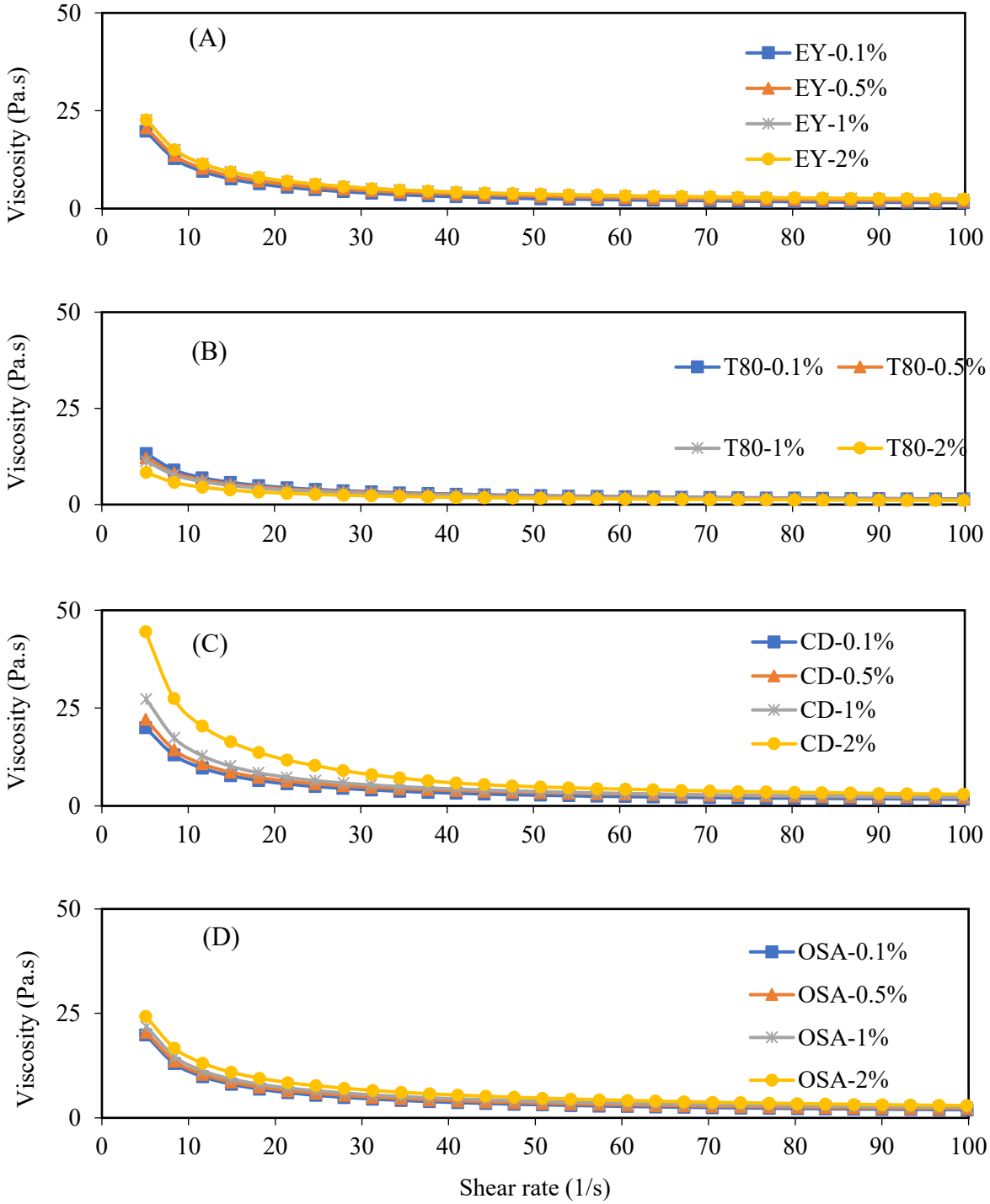



**Figure 6.2 Viscosity versus shear rate rheogram of instant emulsion samples prepared with (A) egg yolk powder, (B) Tween 80, (C) alpha-cyclodextrin and (D) OSA-starch at different concentrations (0.1, 0.5, 1 and 2% w/w).**

**Table 6.4 The shear viscosity of instant emulsion samples formulated with different emulsifiers, such as egg yolk powder (EY), Tween 80 (T80), alpha-cyclodextrin (CD) and OSA-starch (OSA) at different concentrations (0.1, 0.5, 1 and 2% w/w) which were measured at a 20 $s^{-1}$ shear rate.**

| Emulsifier Concentration | Shear Viscosity (Pa·s) | | | |
|---|---|---|---|---|
| | EY | T80 | CD | OSA |
| 0.1% | $5.46 \pm 0.67^{d}$ | $4.37 \pm 0.13^{a}$ | $5.62 \pm 0.21^{d}$ | $6.04 \pm 0.14^{d}$ |
| 0.5% | $6.14 \pm 0.09^{c}$ | $4.07 \pm 0.12^{b}$ | $6.33 \pm 0.58^{c}$ | $6.54 \pm 0.28^{c}$ |
| 1% | $6.91 \pm 0.15^{b}$ | $3.81 \pm 0.20^{c}$ | $7.32 \pm 0.64^{b}$ | $7.16 \pm 0.30^{b}$ |
| 2% | $7.01 \pm 0.70^{a}$ | $2.92 \pm 0.22^{d}$ | $11.72 \pm 0.64^{a}$ | $8.46 \pm 0.20^{a}$ |

$^{a, b, c, d}$ Different letters within the same column represent significant differences ($p < 0.05$).

The rheological data for all the emulsion samples were then fitted to the Power Law model and the model parameters are summarised in Table 6.5. It was observed that the sample prepared with 2% w/w of alpha-cyclodextrin showed a relatively poor correlation coefficient ($r^2$) as 0.68. This indicates the inapposite fitting of this sample's rheological data to the Power Law model which might be due to the very viscous and thicken texture of the emulsion sample. All the other emulsion samples showed the relatively high values of $r^2$ which were all over 0.9, indicating the rheological properties of these emulsion samples except for CD-2% could be well described by the Power Law model. Thus, the Power Law model parameters of the emulsion sample CD-2% was excluded from the comparison between the different samples. On the other hand, the flow behaviour index ($n$) of all the emulsion samples was lower than 1, which confirms the pseudoplastic flow behaviour of the instant emulsion samples (Santana et al., 2015). In addition, the most pseudoplastic behaviour was observed from the sample with 0.1% w/w of egg yolk (EY-0.1%), which showed the smallest value of flow behaviour index. Also, the flow behaviour index increased with increasing the emulsifier concentration for all four emulsifiers, showing that the pseudoplastic flow behaviour of the instant emulsions was inhibited by the addition of more emulsifiers (e.g. egg yolk, Tween 80, alpha-cyclodextrin and OSA-starch).

In terms of the consistency index ($k$), it was believed that the value of $k$ was related to the viscous nature of the emulsion samples (Chivero et al., 2016). With the increasing concentration of alpha-cyclodextrin and OSA-starch in the formulation, their $k$ values increased, which was in accordance with the increasing viscosity of these emulsion samples.

On the other hand, as described above, the viscosity of the instant emulsion formed with Tween 80 decreased with the increasing concentration of Tween 80 in the formulation. Thus, the *k* values of the Tween 80 stabilised emulsions decreased markedly due to the viscosity reduction. However, the *k* values of the instant emulsions formulated with egg yolk decreased with the increasing concentration of egg yolk, even though the viscosity of the emulsion samples increased (Table 6.4). This might be due to some phase separation with a large amount of free oil in the emulsion samples that were visually observed from the appearance assessment as described previously. Thus, the emulsion structure might be weakened to result in the lower consistency index (*k*).

**Table 6.5 Power law parameter values of instant emulsion samples formulated with different emulsifiers, such as egg yolk powder (EY), Tween 80 (T80), alpha-cyclodextrin (CD) and OSA-starch (OSA), at different concentrations (0.1, 0.5, 1 and 2% w/w).**

| Samples | *k* (Pa·s$^n$) | | | | *n* | | | | $r^2$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EY | T80 | CD | OSA | EY | T80 | CD | OSA | EY | T80 | CD | OSA |
| 0.1% | 78.21 | 42.03 | 72.12 | 65.64 | 0.13 | 0.27 | 0.17 | 0.23 | 0.95 | 0.99 | 0.95 | 0.99 |
| 0.5% | 70.72 | 36.14 | 68.26 | 63.36 | 0.21 | 0.29 | 0.24 | 0.27 | 0.98 | 0.99 | 0.96 | 0.99 |
| 1% | 72.69 | 33.49 | 96.16 | 65.41 | 0.24 | 0.30 | 0.17 | 0.29 | 0.97 | 0.99 | 0.94 | 0.99 |
| 2% | 68.65 | 24.34 | 190.39 | 73.20 | 0.26 | 0.31 | 0.08 | 0.30 | 0.96 | 0.99 | 0.68 | 0.99 |

*k:* consistency coefficient, *n*: flow behaviour index, $r^2$: coefficient of correlation.

### 6.4.3 Particle Size and Particle Size Distribution

The mean particle size and particle size distribution of all the emulsion samples containing 40% oil which were prepared with four different types of emulsifiers at different concentrations were characterised. The volume mean diameter (D[4,3]) and the width of the particle size distribution (span) of the emulsions, as well as the values of d(0.1), d(0.5) and d(0.9) are summarised in Table 6.6.

**Table 6.6 Particle size of instant emulsion samples prepared with egg yolk powder (EY), Tween 80 (T80), alpha-cyclodextrin (CD) and OSA-starch (OSA) at different concentrations (0.1, 0.5, 1 and 2% w/w).**

| EY | D[4, 3] (μm) | d(0.1) (μm) | d(0.5) (μm) | d(0.9) (μm) | Span |
|---|---|---|---|---|---|
| 0.1% | 60 ± 8.60$^{c}$ | 22 ± 0.75$^{c}$ | 45 ± 4.43$^{a}$ | 107 ± 22.64$^{c}$ | 1.87 ± 0.31$^{c}$ |
| 0.5% | 61 ± 5.71$^{c}$ | 23 ± 0.63$^{ab}$ | 44 ± 2.40$^{a}$ | 109 ± 22.24$^{c}$ | 1.92 ± 0.44$^{c}$ |
| 1% | 67 ± 5.46$^{b}$ | 24 ± 0.59$^{a}$ | 47 ± 2.27$^{a}$ | 138 ± 16.75$^{b}$ | 2.43 ± 0.26$^{b}$ |
| 2% | 78 ± 6.28$^{a}$ | 22 ± 0.95$^{bc}$ | 47 ± 4.32$^{a}$ | 195 ± 30.20$^{a}$ | 3.73 ± 0.82$^{a}$ |
| T80 | | | | | |
| 0.1% | 85 ± 4.20$^{a}$ | 22 ± 0.95$^{a}$ | 51 ± 1.41$^{a}$ | 209 ± 26.50$^{a}$ | 3.66 ± 0.46$^{a}$ |
| 0.5% | 59 ± 1.18$^{bc}$ | 22 ± 0.11$^{a}$ | 45 ± 0.29$^{b}$ | 115 ± 4.42$^{b}$ | 2.09 ± 0.08$^{b}$ |
| 1% | 60 ± 1.35$^{b}$ | 20 ± 0.15$^{b}$ | 44 ± 0.51$^{b}$ | 126 ± 4.71$^{b}$ | 2.38 ± 0.08$^{b}$ |
| 2% | 56 ± 1.53$^{c}$ | 20 ± 0.54$^{b}$ | 42 ± 0.26$^{c}$ | 109 ± 4.88$^{b}$ | 2.09 ± 0.11$^{b}$ |
| CD | | | | | |
| 0.1% | 62 ± 1.70$^{a}$ | 22 ± 0.66$^{c}$ | 40 ± 0.27$^{c}$ | 96 ± 6.81$^{b}$ | 1.83 ± 0.19$^{ab}$ |
| 0.5% | 58 ± 1.73$^{b}$ | 23 ± 0.53$^{b}$ | 43 ± 0.29$^{b}$ | 102 ± 14.28$^{b}$ | 1.86 ± 0.33$^{a}$ |
| 1% | 56 ± 4.44$^{b}$ | 24 ± 1.00$^{a}$ | 46 ± 2.38$^{a}$ | 98 ± 11.01$^{b}$ | 1.60 ± 0.24$^{b}$ |
| 2% | 50 ± 3.50$^{c}$ | 24 ± 0.51$^{a}$ | 43 ± 1.88$^{b}$ | 81 ± 8.78$^{a}$ | 1.32 ± 0.15$^{c}$ |
| OSA | | | | | |
| 0.1% | 51 ± 5.08$^{a}$ | 23 ± 0.55$^{a}$ | 42 ± 0.86$^{a}$ | 82 ± 6.45$^{a}$ | 1.41 ± 0.16$^{a}$ |
| 0.5% | 47 ± 3.36$^{ab}$ | 23 ± 0.64$^{a}$ | 41 ± 0.53$^{a}$ | 76 ± 5.93$^{a}$ | 1.28 ± 0.14$^{ab}$ |
| 1% | 50 ± 4.08$^{a}$ | 23 ± 0.41$^{a}$ | 41 ± 0.71$^{a}$ | 79 ± 4.60$^{a}$ | 1.33 ± 0.17$^{a}$ |
| 2% | 45 ± 5.01$^{b}$ | 23 ± 0.57$^{a}$ | 40 ± 0.58$^{b}$ | 70 ± 6.18$^{b}$ | 1.17 ± 0.16$^{b}$ |

$^{a, b, c}$ Different letters within the same column represent significant differences ($p < 0.05$). D[4,3]: volume-weighted mean diameter; d(0.1), d(0.5) and d(0.9): the diameters where 10%, 50% and 90% of the particles are smaller than the values.

Theoretically, it is normally believed that a smaller particle size could be produced with an increase in the concentration of emulsifiers (McClements, 2016). This is because a greater amount of emulsifiers present in the emulsion system could cover the surface of oil droplets more rapidly and completely, thus preventing the oil droplets from aggregation during the emulsification process. In this research study, instant emulsions with smaller mean particle diameters were produced when preparing with increasing concentration of Tween 80,

alpha-cyclodextrin and OSA-starch. However, the opposite trend was observed from the emulsions formed with egg yolk powder as their mean particle diameter was increased when prepared with increasing concentration of egg yolk powder. As shown in Table 6.6, increasing concentration of egg yolk powder induced the formation of oil particles in larger sizes so that the significant larger value of d(0.9) was observed when the concentration of egg yolk powder was increased from 0.1 to 2% w/w. More specifically, when the emulsion was formulated with 0.1 - 0.5% w/w of egg yolk powder, 90% of the produced oil droplets had the mean particle sizes d(0.9) smaller than 110 μm, which gave the emulsion a mean particle diameter at around 60 μm and a span value smaller than 2. However, when the concentration of egg yolk powder was increased to 1% and 2% w/w, 90% of the oil droplets in the produced emulsions had a significantly larger mean particle sizes of around 140 μm and 195 μm, respectively. The values of D[4,3] of the formed emulsions were also significantly increased to 67 μm and 78 μm with the span values of 2.4 and 3.7, respectively. The changes in the particle size characterisation of the instant emulsions could be also observed from the particle size distribution curves of the samples as shown in Figure 6.3A. The unimodal particle size distribution was slightly changed to the bimodal distribution when the egg yolk concentration was increased from 0.1% to 0.5% w/w, and the volume of the second peak increased continuously as its concentration was increased to 2% w/w. The increased volume of the larger size group might have been due to the droplet flocculation and coalescence as well as the presence of some egg yolk powder lumps. During the emulsification process, the limited very low shear generated by hand shaking might be not strong enough to make egg yolk powder dispersed and dissolved in the aqueous phase of the emulsions effectively. Thus, egg yolk proteins and phospholipids present in the egg yolk powder could not coat the freshly formed oil droplets effectively to protect them from rapid flocculation and coalescence during the emulsification.

The particle size of the instant emulsions formed with different concentrations of Tween 80 is also shown in Table 6.6. At 0.1% Tween 80, the volume mean diameter (D[4,3]) was 85 μm and it was decreased significantly to about 60 μm when the concentration of Tween 80 was increased to 0.5% w/w ($p < 0.05$). However, no significant further changes were observed when the concentration of Tween 80 was continuously increased to 1 or 2% w/w. This result indicates that 0.5% w/w of Tween 80 was sufficient to stabilise the instant creamy emulsion containing 40% oil and prevent the oil droplets in the emulsion system from aggregation. This was in accordance with the observation from the particle size

distribution curves as shown in Figure 6.3B. A bimodal distribution with two clear peaks was observed from the emulsion with 0.1% w/w of Tween 80. However, as the concentration of Tween 80 increased to above 0.5% w/w, the second peak of the bimodal distribution which stands for the large particle size group disappeared. As a result, the unimodal distribution curves were observed for the emulsion samples containing 0.5, 1 and 2% w/w of Tween 80 (T80-0.5%, T80-1% and T80-2%). This change indicates that an instant creamy emulsion with more uniform oil droplets was produced when the emulsion was formulated with at least 0.5% w/w of Tween 80. The ability of Tween 80 to emulsify and stabilise the instant emulsion efficiently even at a relatively low concentration (0.5% w/w) could be related to its low molecular weight. Thus, the diffusion of Tween 80 molecules along the continuous phase of the O/W emulsion to adsorb at the oil-water interface became more favourable than other high molecular weight molecules such as egg yolk proteins (Pugnaloni et al., 2004; Riscardo et al., 2003).

For the instant emulsions formulated with alpha-cyclodextrin (CD), the increasing concentration of the emulsifier led to a significant decrease in the volume mean diameter of the emulsion system. As presented in Table 6.6, the D[4,3] of the instant emulsions reduced from 62 μm to 50 μm when the emulsion samples were prepared with 0.1% and 2% w/w of alpha-cyclodextrin, respectively. The reduction in particle mean diameters could be attributed to the decreasing values of d(0.9) of the emulsions. When the concentrations of alpha-cyclodextrin increased from 0.1 to 2% w/w, the particle sizes of 90% of the oil droplets in the instant emulsions were decreased from 96 μm to 81μm with the reduced span from 1.8 to 1.3. The increasing concentration of alpha-cyclodextrin also caused a slight change in the particle size distribution of the formed emulsions (Figure 6.3C), which was similar to the results observed for the emulsions prepared with egg yolk powder and Tween 80 (Figures 6.3A and 6.3B). As the concentration of the emulsifier increased from 0.1% to 2% w/w, the multi-modal distributions with two peaks (CD-0.1% and CD-0.5%) were changed to the mono-modal distributions with one single peak (CD-1% and CD-2%). Alpha-cyclodextrin could be used as a cholesterol-free, vegetarian alternative for replacing egg yolk or other emulsifiers in mayonnaise and dressing applications (Wacker Chemie AG, n.d.). The results from this research study verified the potential of alpha-cyclodextrin to aid the formation of instant emulsions with small oil droplets in a relatively uniform particle size distribution.

As shown in Table 6.6, among all the instant emulsion samples prepared with different types of emulsifiers, the smallest mean diameters of oil droplets were obtained from the instant emulsions formed with OSA-starch at all concentrations studied. However, no significant particle size difference was observed between the instant emulsion samples at different concentrations of OSA-starch.

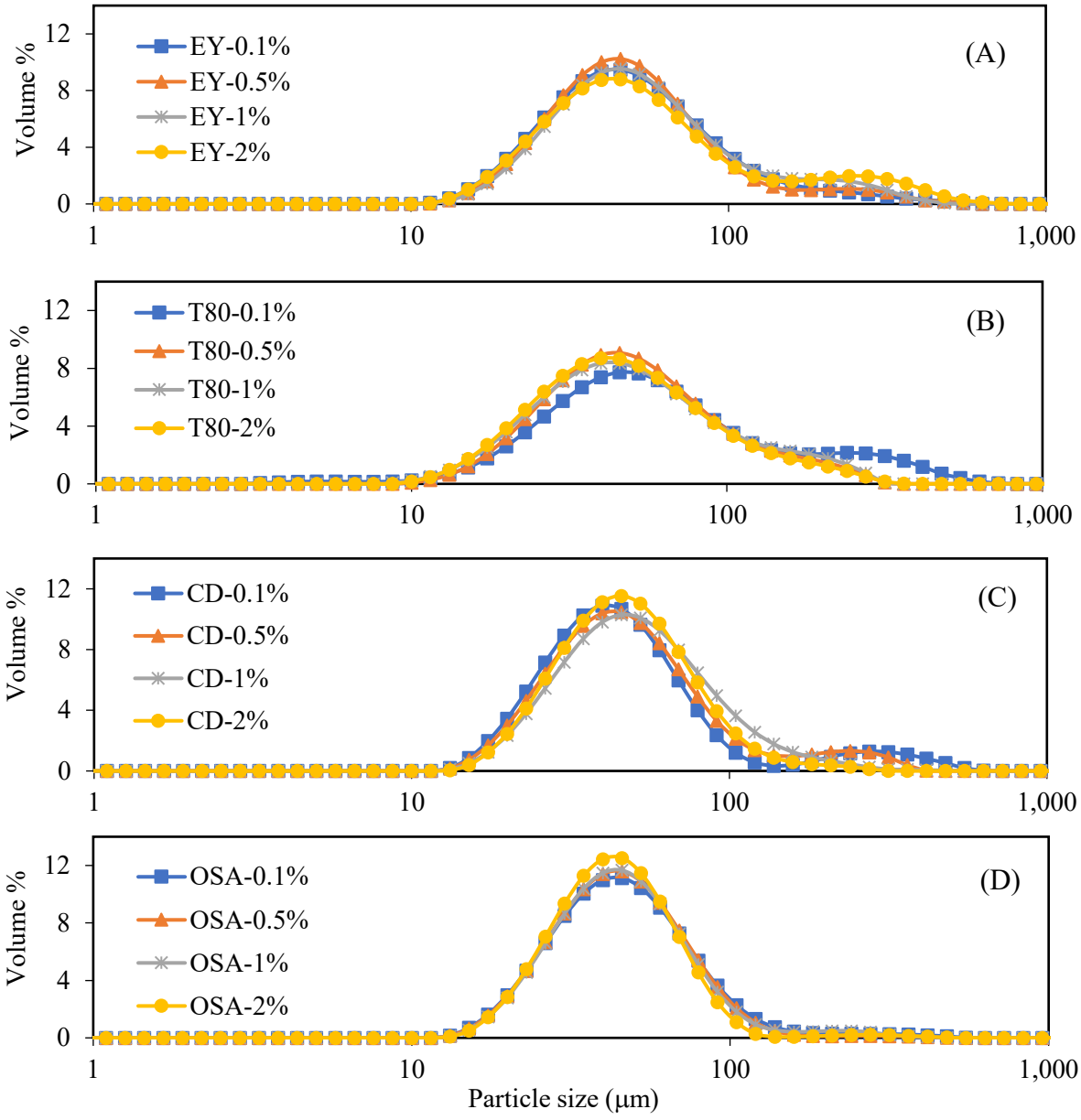



**Figure 6.3 Particle size distribution of oil droplets in instant emulsions formulated with (A) egg yolk powder, (B) Tween 80, (C) alpha-cyclodextrin and (D) OSA-starch at different concentrations (0.1, 0.5, 1 and 2% w/w).**

### 6.4.4 Microstructure of Emulsions

The microstructure of the instant emulsion samples formulated with four different emulsifiers at various concentrations was captured under the light microscope and CLSM. All the emulsion samples were polydisperse emulsions with various droplet sizes. Differences in the extent of droplet aggregation, droplet sizes and polydispersity were observed between the emulsion samples prepared with different types and concentrations of emulsifiers used.

As shown in Figures 6.4A and 6.5A, large oil droplets were observed from the emulsions containing egg yolk powder. Also, it was obvious that the polydispersity of the emulsion systems increased as the concentration of egg yolk increased. This observation was in good accordance with the results of the particle size measurements as already described above. The width of the particle size distribution increased significantly when the concentration of egg yolk increased from 0.1 to 2% w/w (Table 6.6). Some lumps of undissolved egg yolk powder could be seen in the images, which indicates the egg yolk powder was not rapidly dissolved in the aqueous phase of instant emulsion samples prepared by the shear generated through hand shaking which was too mild to break down the egg yolk lumps efficiently. Thus, there might not be sufficient egg yolk proteins and phospholipids present in the emulsions to fully cover the surface of the produced oil droplets and prevent the oil droplets from coalescing with each other.

The most uniform particle size distribution with the finest particle size was observed from the emulsion samples with Tween 80 and OSA-starch (Figures 6.4B and 6.4D). For both emulsions, the formation of large particles was largely eliminated by increasing the concentrations of Tween 80 or OSA-starch to above 0.5% w/w. When comparing the CLSM images between these two emulsions (Figures 6.5B and 6.5D), a higher extent of droplet flocculation was observed from the Tween 80 stabilised emulsions than OSA-starch stabilised emulsions. This could be the reason why the mean particle diameters from the OSA-starch emulsions was smaller than the means from the Tween 80 stabilised emulsions as described in Section 6.4.3, as particle aggregates might be detected by the particle sizer when analysing the particle size of the instant emulsion samples formed with Tween 80.

The microstructure of the instant emulsions formulated with alpha-cyclodextrin at different concentrations of 0.1-1% w/w as shown in Figure 6.4C was in agreement with the results

of particle size characterisation as described in Section 6.4.3. However, the clusters of oil droplets in non-spherical shapes were observed from the image of emulsion sample containing 2% of alpha-cyclodextrin. The formation of these oil droplet clusters might be the reason for the relatively viscous and thicken texture of the emulsion sample, and the observed change in the droplets structure of the sample may indicate that the concentration of 2% w/w alpha-cyclodextrin was excessive for the instant creamy emulsion preparation.

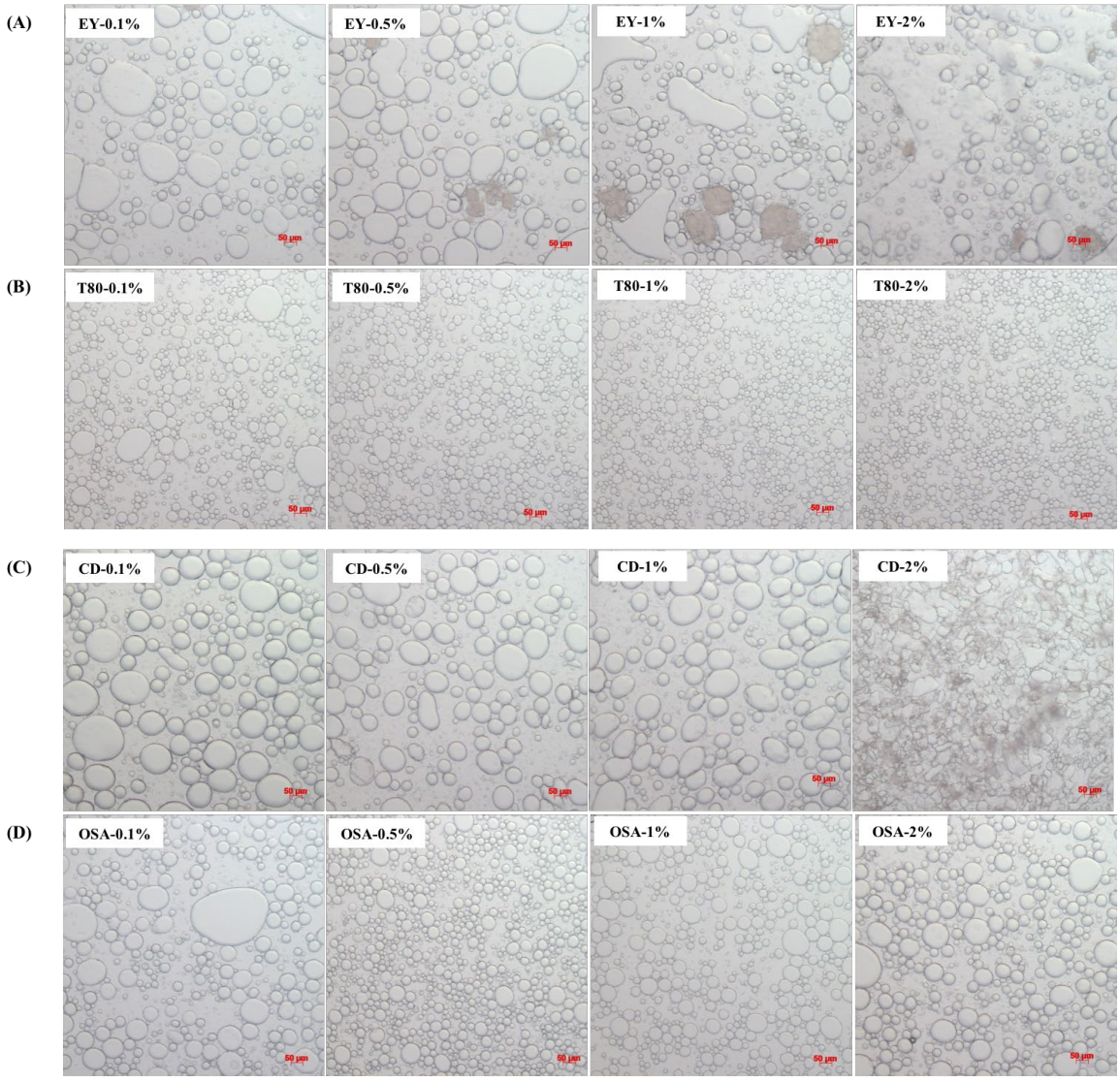



**Figure 6.4 Light microscopic images of instant emulsions prepared with different emulsifiers: (A) egg yolk powder, (B) Tween 80, (C) alpha-cyclodextrin and (D) OSA-starch, at different concentrations (0.1, 0.5, 1 and 2% w/w). scale bar 50 μm**

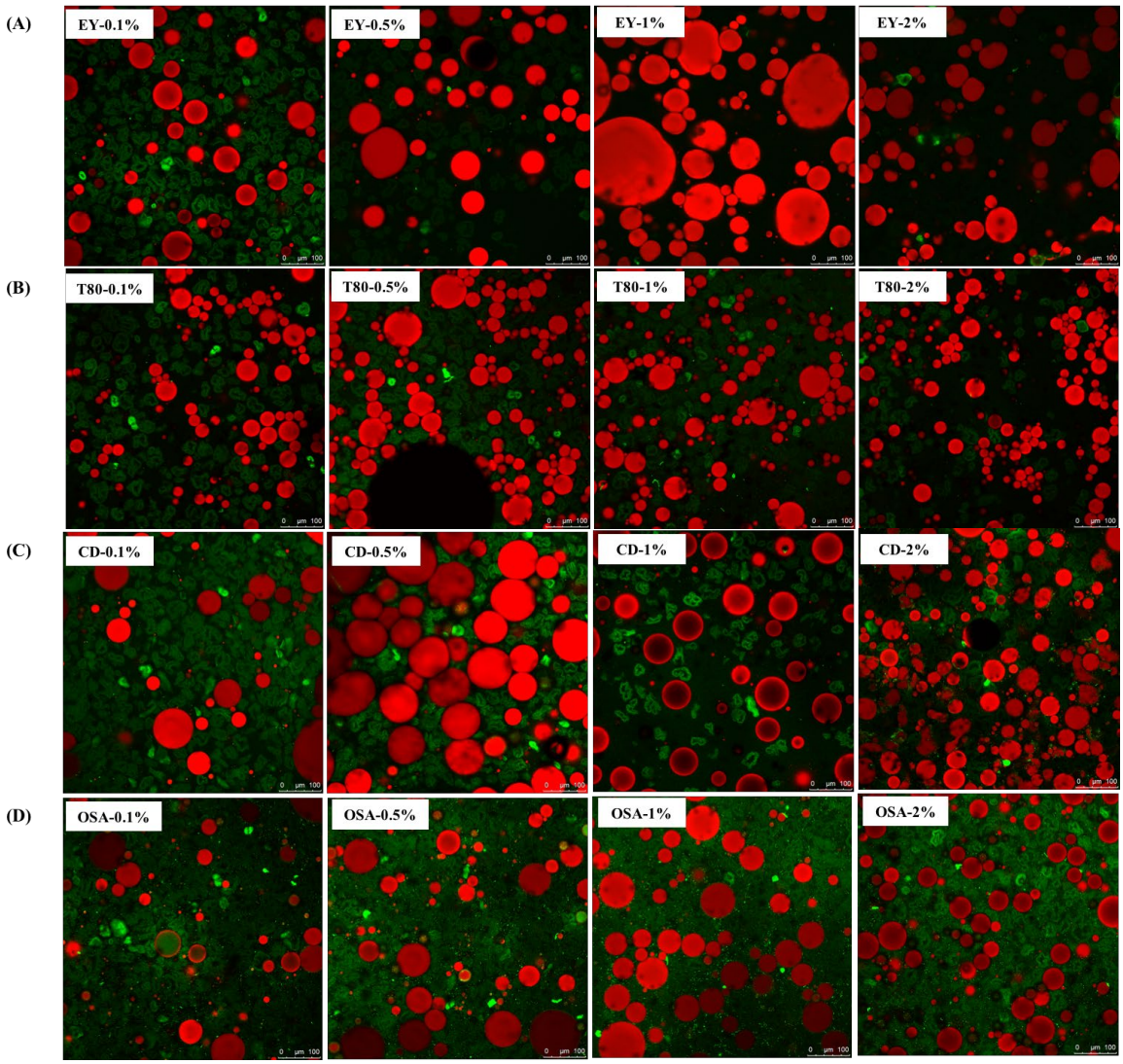



**Figure 6.5 CLSM images of instant emulsions prepared with different emulsifiers: (A) egg yolk powder, (B) Tween 80, (C) alpha-cyclodextrin and (D) OSA-starch, at different concentrations (0.1, 0.5, 1 and 2% w/w). scale bar 100 μm**

### 6.4.5 Stability of Emulsions

The stability of the instant emulsion samples was measured by the accelerated creaming test, in which the emulsions were centrifuged at a relatively low speed (3792 g for 10 minutes 20°C) to simulate the droplet movement under the gravitational forces. The pictures of emulsions taken after centrifugation are shown in Figure 6.6 and some differences in the extent of phase separation were observed between the samples.

The instant emulsions prepared with egg yolk powder were less stable than the other emulsion samples prepared with different emulsifiers as a larger amount of free oil was

observed on top of the emulsions after centrifugation. In addition, the amount of free oil separated from the egg yolk-stabilised emulsions increased with increasing concentration of egg yolk powder, indicating the decreased stability of emulsion samples (Figure 6.6A). The relatively low stability of the egg yolk-stabilised emulsions could also be related to the larger particle sizes of oil droplets in these emulsion systems as described previously (Table 6.6). On the other hand, the stability of the instant emulsions formed with Tween 80, alpha-cyclodextrin and OSA-starch was improved with increasing concentration of these emulsifiers. As shown in Figures 6.6, a thin layer of free oil was observed from the samples formulated with low concentrations of emulsifiers and no phase separation was visually observed when the concentrations of emulsifiers increased.

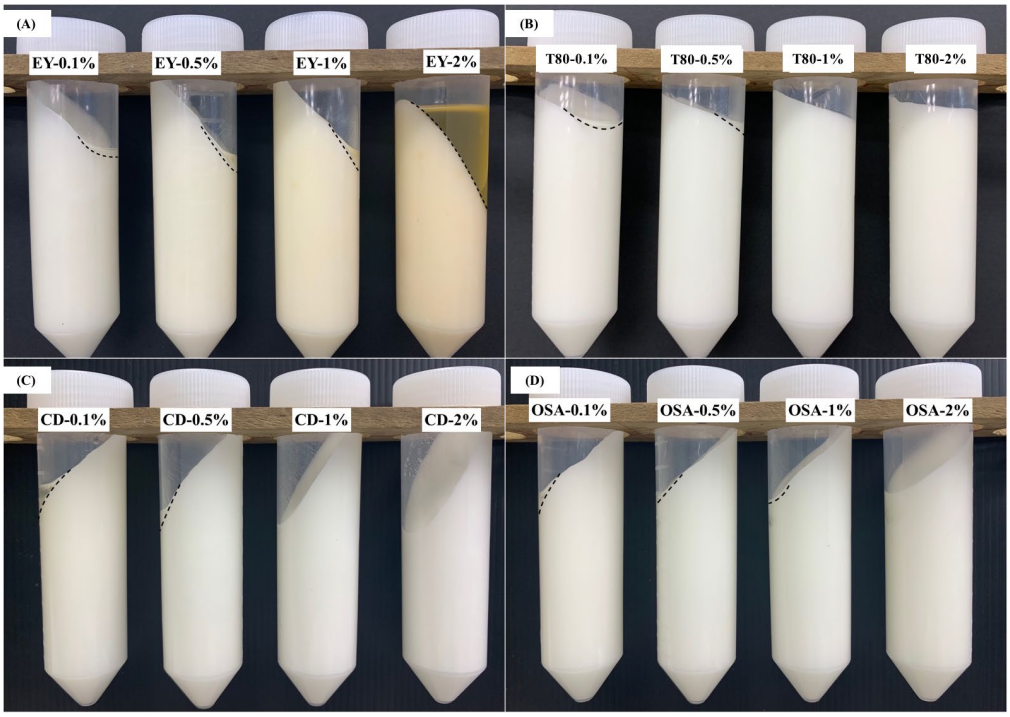



**Figure 6.6 A picture of instant emulsion samples prepared with different types of emulsifiers: (A) egg yolk powder, (B) Tween 80, (C) alpha-cyclodextrin and (D) OSA-starch, at different concentrations (0.1, 0.5, 1 and 2% w/w). Pictures taken after centrifugation to observe the stability of emulsions against the formation of phase separation, free oil, etc. (----- indicates the boundary of free oil and cream layer).**

### 6.4.6 Textural Properties of Emulsions

Table 6.7 presents the texture parameters of the instant emulsions formed with different emulsifiers at different concentrations. It was observed that the types and concentrations of the emulsifiers had a significant impact on the values of all the texture parameters (firmness, consistency, cohesiveness and index of viscosity) of the instant creamy emulsions ($p < 0.05$).

The lowest values for all the texture attributes (firmness, consistency, cohesiveness and index of viscosity) were observed from the instant emulsions prepared with Tween 80 emulsions at all the concentrations. In addition, with the increasing concentration of Tween 80, the values of the texture attributes of the emulsions decreased significantly ($p < 0.05$). This result was likely caused by the decreasing viscosity of the samples due to the addition of Tween 80 as described in Section 6.4.2 (Table 6.4), as the texture attributes can be reflected by the viscosity of the emulsions (Liu et al., 2007). In contrast to the Tween 80 stabilised-instant emulsions, the emulsion samples formed with the other three emulsifiers showed the higher values of firmness, consistency and cohesiveness with increasing concentration of egg yolk powder, alpha-cyclodextrin and OSA-starch. For the sample prepared with 2% w/w egg yolk powder, the results showed the reduced values of all the texture attributes when compared with the samples prepared with lower concentrations of egg yolk powder. This might have been related to the large amount of free oil presented in the emulsion system, which weakens the emulsion structure and had a negative impact on the texture properties of the instant emulsion. The highest values for all texture attributes were obtained from the emulsions formed with 2% of alpha-cyclodextrin, which seemed to be attributed to the pronounced viscosity of this sample. In addition, the emulsions prepared with OSA-starch showed the relatively higher values for all the texture attributes than the emulsions prepared with egg yolk powder and Tween 80, especially at higher concentrations.

**Table 6.7 Firmness, consistency, cohesiveness and index of viscosity of instant emulsions prepared with different concentrations of emulsifiers (0.1, 0.5, 1 and 2% w/w). "EY" egg yolk powder; "T80" Tween 80; "CD" alpha-cyclodextrin; "OSA" OSA-starch.**

| Samples | Firmness (g) | | | |
|---|---|---|---|---|
| | EY | T80 | CD | OSA |
| 0.1% | 156.44 ± 8.13$^{b}$ | 107.57 ± 9.42$^{a}$ | 154.02 ± 17.96$^{c}$ | 163.55 ± 7.70$^{b}$ |
| 0.5% | 166.93 ± 9.64$^{ab}$ | 106.20 ± 11.56$^{a}$ | 169.48 ± 20.96$^{c}$ | 169.67 ± 12.69$^{b}$ |
| 1% | 172.42 ± 8.19$^{a}$ | 97.48 ± 8.10$^{a}$ | 201.07 ± 21.30$^{b}$ | 174.53 ± 3.10$^{b}$ |
| 2% | 143.05 ± 6.34$^{c}$ | 80.27 ± 10.58$^{b}$ | 389.58 ± 22.61$^{a}$ | 187.18 ± 10.58$^{a}$ |

| Samples | Consistency (g·sec) | | | |
|---|---|---|---|---|
| 0.1% | 1037.19 ± 30.96$^{c}$ | 710.93 ± 47.35$^{a}$ | 986.90 ± 89.99$^{c}$ | 1054.47 ± 38.29$^{c}$ |
| 0.5% | 1110.45 ± 33.63$^{b}$ | 671.65 ± 39.72$^{ab}$ | 1090.36 ± 86.21$^{c}$ | 1098.42 ± 64.07$^{bc}$ |
| 1% | 1187.49 ± 64.48$^{a}$ | 638.52 ± 41.91$^{b}$ | 1362.07 ± 147.02$^{b}$ | 1134.28 ± 33.10$^{ab}$ |
| 2% | 957.05 ± 29.22$^{d}$ | 509.39 ± 47.48$^{c}$ | 2682.79 ± 145.25$^{a}$ | 1168.60 ± 22.18$^{a}$ |

| Samples | Cohesiveness (g) | | | |
|---|---|---|---|---|
| 0.1% | -137.31 ± 2.89$^{b}$ | -93.64 ± 6.89$^{c}$ | -130.04 ± 9.84$^{a}$ | -142.92 ± 5.07$^{a}$ |
| 0.5% | -147.53 ± 2.56$^{c}$ | -85.25 ± 4.95$^{b}$ | -143.06 ± 12.37$^{a}$ | -150.51 ± 9.14$^{b}$ |
| 1% | -157.68 ± 8.05$^{d}$ | -79.40 ± 5.71$^{b}$ | -176.99 ± 20.62$^{b}$ | -158.78 ± 4.72$^{c}$ |
| 2% | -117.74 ± 2.93$^{a}$ | -59.34 ± 6.23$^{a}$ | -352.08 ± 23.39$^{c}$ | -169.91 ± 3.17$^{d}$ |

| Samples | Index of Viscosity (g·sec) | | | |
|---|---|---|---|---|
| 0.1% | -427.28 ± 10.86$^{b}$ | -294.70 ± 20.10$^{c}$ | -403.33 ± 32.91$^{a}$ | -442.45 ± 17.03$^{a}$ |
| 0.5% | -452.87 ± 12.26$^{c}$ | -272.92 ± 17.57$^{bc}$ | -445.75 ± 39.40$^{a}$ | -465.60 ± 28.49$^{b}$ |
| 1% | -488.03 ± 30.51$^{d}$ | -254.54 ± 17.91$^{b}$ | -530.32 ± 57.44$^{b}$ | -491.79 ± 12.63$^{c}$ |
| 2% | -381.93 ± 12.06$^{a}$ | -194.58 ± 21.56$^{a}$ | -1061.03 ± 61.54$^{c}$ | -522.08 ± 7.16$^{d}$ |

$^{a, b, c, d}$ Different letters within the same column represent significant differences ($p < 0.05$).

## 6.5 Conclusions

From the results of the present work, it could be concluded that the types and concentrations of the emulsifier had the significant impacts on the formation and physiochemical properties of the instant creamy emulsions containing 40% oil prepared by hand shaking. The results showed that egg yolk powder was not suitable to be used as the sole emulsifying

agent in the application of instant emulsions. The notable amount of free oil was observed from the instant emulsions formed with egg yolk powder as some egg yolk powder seemed to form lumps in aqueous phase. The egg yolk lumps were not readily broken up by simple hand shaking to make them disperse and dissolve, thus decreasing its efficiency as an emulsifier to form and stabilise oil droplets. The instant emulsions formulated with egg yolk powder contained oil droplets in larger particle sizes with a broader particle size distribution and weaker emulsion stability.

However, the instant emulsions containing 40% oil could be effectively produced and stabilised by using Tween 80, alpha-cyclodextrin and OSA-starch. Especially, instant emulsion could be formed and stabilised by a relatively low concentration of Tween 80 (0.5% w/w), which contained uniformly distributed oil droplets in finer particle sizes with high emulsion stability. However, the Tween 80 stabilised-instant emulsions had the relatively lower viscosity than the emulsions formed with the other emulsifiers. The results of this research study also confirmed the considerable potential of alpha-cyclodextrin and OSA-starch to emulsify and stabilise the instant creamy O/W emulsions. The emulsions prepared with alpha-cyclodextrin and OSA-starch showed better physicochemical properties than the emulsions with Tween 80, including higher values of viscosity and texture parameters, and smaller particle diameters with narrower particle size distributions.

# Chapter 7 Overall Conclusions & Recommendations

## 7.1 Research Outcomes

This research project was carried out to investigate the effects of main ingredients (e.g. hydrocolloids and emulsifiers) on the formation, properties and stability of instant creamy emulsion containing 40% oil. This could build a fundamental for the development of a low-fat mayonnaise-like dressing that could be simply obtained within a few minutes by dispersing dry powdered ingredients (e.g. thickening agents, modified starch, egg yolk powder) in oil and then mixing the oil slurry with cold water/emulsifier (Tween 80) by shaking. Three different groups of ingredients were studied for their effects on the formation and properties of instant creamy emulsions, including 1) starch ingredients, 2) non-starch hydrocolloid ingredients and 3) surface-active emulsifiers. Based on a series of experiments, it was found that the types and concentrations of different ingredients had the significant impacts on the appearance, particle size and particle size distribution, microstructure, rheological and textural properties, and stability of instant creamy emulsions.

Due to the reduced fat content (40% oil) and its preparation method of instant emulsions which involves simple hand shaking, it is necessary to use thickening agents (e.g. starch and non-starch hydrocolloids) in order to be able to form and stabilise the instant creamy emulsions. Based on the results of this research work, it was found that among different types of starch ingredients used, a modified waxy maize starch known as "Ultra-Tex 4$^{TM}$" was the most suitable thickener and texturizer for this application. As expected, with increasing the concentration of modified starch, the viscosity and texture attributes of the instant emulsions increased significantly ($p < 0.05$). In addition, when the concentration of the modified starch (Ultra-Tex 4$^{TM}$) was increased from 0 to 4% w/w, the mean particle diameter D[4,3] of instant emulsions decreased significantly from 98 to 47 μm. Also, the most stable emulsion was formed when the concentration of starch of was 4% w/w. However, when the concentration of starch was continuously increased to 6% w/w, no significant changes of the D[4,3] was observed while the emulsion stability was reduced. Thus, it was concluded that the optimal concentration of the modified starch "Ultra-Tex 4$^{TM}$" was 4% w/w in the application of instant creamy emulsions containing 40% w/w oil. This resulted in a creamy and thickened emulsion with desired textural and rheological

properties, as well as the high stability of the emulsion containing uniformly dispersed oil droplets.

The functional properties of starch could be extended by the co-addition of other hydrocolloids, such as xanthan gum and carboxymethyl cellulose (CMC). The results showed that the addition of these hydrocolloids influenced the emulsion characteristics significantly. With the increasing concentration of gums, the viscosities of instant emulsions were increased significantly exhibiting more pronounced pseudoplastic flow behaviour. Also, their mean particle diameter of oil droplets was reduced to a significant level, which also led to a shift of the particle size distribution of emulsion oil droplets from bi-modal to mono-modal particle size distribution. At the same time, better texture attributes were obtained from the emulsion samples containing higher concentration of gums. In addition, the use of CMC in instant emulsions was observed to have more advantages compared to xanthan gum. The emulsion formulated with CMC showed higher viscosity and smaller size of oil droplets. The stability of instant emulsions was also found to increase when using CMC with the preferred concentration of 0.2 to 0.4% w/w. Thus, CMC could be used in conjunction with the modified starch to thicken and stabilise the instant creamy emulsions.

In terms of emulsifiers, the results showed that the type and concentration of emulsifiers had a significant impact on the physicochemical properties of instant emulsions. Firstly, it was found that egg yolk powder was not suitable to be used as a sole surfactant in formulating the instant emulsions. The instant emulsions formed with egg yolk powder showed several drawbacks, such as the oily appearance, incompletely emulsification, large oil droplets and poor emulsion stability. Tween 80 was considered as an effective emulsifier used in the preparation of instant emulsions which reduces the interfacial tension between the oil-water interface and forms a stable O/W emulsion with small droplet size and uniform droplet distribution. However, in this study, it was observed interestingly that the increasing concentration of Tween 80 reduced the viscosity of instant emulsion significantly, resulting in the relatively poor texture characteristics of the instant emulsion. In addition, the potential of the hydrocolloid emulsifier “OSA modified starch” and alpha-cyclodextrin to produce stable instant emulsions was also investigated in this research. Based on the results, it could be concluded that an instant emulsion with an oil content of 40% w/w could be successfully formed with the aid of OSA modified starch or alpha-cyclodextrin. The emulsions prepared with alpha-cyclodextrin and OSA-starch showed better

physicochemical properties than the emulsions with Tween 80, including higher values of viscosity and texture parameters, and smaller particle diameters with narrower particle size distributions.

In summary, a stable creamy instant emulsion containing 40% oil, which was similar in visual appearance to the conventional mayonnaise, was able to be prepared using the base formulation initially used in this project which consisted of 4% starch, 0.2% xanthan gum, 0.3% citric acid, 0.5% egg yolk powder, 0.5% Tween 80 and 54.5% water. The base formulation was further investigated by making some changes in the type and concentration of three different groups of ingredients (e.g. starch, non-starch hydrocolloids and emulsifiers) which were found to have some significant influence.

## 7.2 Recommendations

The research was to investigate the effects of different ingredients on the physicochemical properties of instant emulsions. The experiment of this research was designed to focus on one single ingredient with various concentrations at one time. Based on the results from this study, the use of several ingredients together, such as the modified starch "Ultra-Tex 4$^{TM}$", hydrocolloid CMC and the emulsifier OSA modified starch, is recommended in the preparation and application of instant creamy emulsions. This is because of the possible synergistic interactions between these ingredients and their effects on the formation and physicochemical properties of instant creamy emulsions. This has not been studied and reported yet in the literature.

The physicochemical properties of instant emulsions, including their appearance, emulsion stability, particle size characteristics, microstructure, rheological and textural properties, were analysed and evaluated in this research. However, sensory evaluation was not conducted in this study. Therefore, it is recommended as a future work to design and conduct sensory evaluation on the instant creamy emulsions. Some important sensory characteristics of instant creamy emulsions produced in this study, such as the taste, colour, odour and texture, need to be analysed for any further improvement in the quality of instant creamy emulsions as a consumer product.

Moreover, the instant emulsions were prepared by an instantaneous emulsification process which involves simply hand shaking. In this project, the shaking of the instant emulsions by hand was tried to be consistent and minimize variations via preparing all the instant

emulsion samples by the same person and following the same conditions for sample preparation procedures. However, the shear speed and shear force applied to prepare the instant emulsions by shaking in this project was not quantified. The way and force of hand shaking can vary considerably between individuals. Thus, the stability and properties of instant emulsions can be different to some extent from the results of this study if the shear force generated by hand shaking is different. Thus, it is recommended to quantify the shear speed and shear force generated by hand shaking in the future studies for higher accuracy and repeatability.

Also, this study was mainly focused on investigating how the physicochemical properties of instant emulsions vary when formulated with different types and concentrations of ingredients (e.g. starch, non-starch hydrocolloids and emulsifiers). The molecular interaction between the different components used and present in instant emulsions was not reviewed comprehensively in this study. It is recommended to further study the effects of the chemical structures and interactions of different ingredients on the instant emulsions in the future.

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# Appendix

**Appendix 1 Particle size and span of instant emulsion samples prepared with xanthan gum (X), guar gum (G), CMC (CL) and λ-carrageenan (C) at different concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1% w/w).**

| **X** | d (0.1) μm) | d(0.5) (μm) | d(0.9) (μm) | Span |
|---|---|---|---|---|
| 0.05% | 21 ± 1.06$^{a}$ | 41 ± 2.77$^{b}$ | 86 ± 8.77$^{b}$ | 1.56 ± 0.13$^{c}$ |
| 0.10% | 22 ± 0.36$^{a}$ | 46 ± 3.51$^{ab}$ | 117 ± 15.04$^{a}$ | 2.07 ± 0.24$^{ab}$ |
| 0.20% | 22 ± 0.76$^{a}$ | 49 ± 4.79$^{a}$ | 129 ± 23.39$^{a}$ | 2.15 ± 0.27$^{ab}$ |
| 0.40% | 22 ± 0.81$^{a}$ | 46 ± 4.37$^{ab}$ | 130 ± 31.89$^{a}$ | 2.32 ± 0.47$^{a}$ |
| 0.80% | 22 ± 0.46$^{a}$ | 47 ± 4.35$^{a}$ | 127 ± 10.06$^{a}$ | 2.23 ± 0.27$^{a}$ |
| 1.00% | 23 ± 2.20$^{a}$ | 50 ± 7.07$^{a}$ | 121 ± 23.76$^{a}$ | 1.93 ± 0.19$^{b}$ |

| **G** | d (0.1) μm) | d(0.5) (μm) | d(0.9) (μm) | Span |
|---|---|---|---|---|
| 0.05% | 22 ± 0.89$^{a}$ | 38 ± 1.46$^{ab}$ | 67 ± 4.24$^{c}$ | 1.17 ± 0.08$^{d}$ |
| 0.10% | 21 ± 1.37$^{ab}$ | 39 ± 2.90$^{a}$ | 72 ± 9.43$^{c}$ | 1.29 ± 0.12$^{c}$ |
| 0.20% | 21 ± 0.79$^{ab}$ | 38 ± 1.20$^{ab}$ | 70 ± 3.28$^{c}$ | 1.28 ± 0.06$^{c}$ |
| 0.40% | 19 ± 1.54$^{c}$ | 36 ± 1.78$^{bc}$ | 71 ± 2.42$^{c}$ | 1.44 ± 0.07$^{b}$ |
| 0.80% | 17 ± 0.98$^{d}$ | 35 ± 1.06$^{c}$ | 78 ± 8.2$^{b}$ | 1.73 ± 0.21$^{a}$ |
| 1.00% | 20 ± 3.94$^{bc}$ | 40 ± 4.55$^{a}$ | 84 ± 6.11$^{a}$ | 1.62 ± 0.18$^{a}$ |

| **CL** | d (0.1) μm) | d(0.5) (μm) | d(0.9) (μm) | Span |
|---|---|---|---|---|
| 0.05% | 23 ± 0.67$^{a}$ | 42 ± 1.77$^{a}$ | 82 ± 9.22$^{a}$ | 1.42 ± 0.20$^{a}$ |
| 0.10% | 22 ± 0.54$^{b}$ | 40 ± 1.12$^{b}$ | 77 ± 7.89$^{b}$ | 1.35 ± 0.16$^{a}$ |
| 0.20% | 21 ± 0.49$^{c}$ | 39 ± 0.80$^{cd}$ | 76 ± 6.32$^{b}$ | 1.42 ± 0.15$^{a}$ |
| 0.40% | 20 ± 0.23$^{d}$ | 38 ± 0.94$^{d}$ | 72 ± 3.20$^{bc}$ | 1.38 ± 0.05$^{a}$ |
| 0.80% | 18 ± 0.60$^{e}$ | 38 ± 0.73$^{d}$ | 70 ± 1.74$^{c}$ | 1.37 ± 0.05$^{a}$ |
| 1.00% | 18 ± 0.91$^{f}$ | 39 ± 0.87$^{c}$ | 73 ± 1.60$^{bc}$ | 1.44 ± 0.07$^{a}$ |

| **C** | d (0.1) μm) | d(0.5) (μm) | d(0.9) (μm) | Span |
|---|---|---|---|---|
| 0.05% | 23 ± 0.47$^{b}$ | 41 ± 0.85$^{cd}$ | 81 ± 5.19$^{bc}$ | 1.41 ± 0.10$^{b}$ |
| 0.10% | 23 ± 0.38$^{b}$ | 43 ± 0.88$^{bc}$ | 88 ± 10.83$^{bc}$ | 1.53 ± 0.23$^{b}$ |
| 0.20% | 22 ± 0.89$^{bc}$ | 43 ± 1.19$^{b}$ | 106 ± 22.19$^{a}$ | 1.94 ± 0.47$^{a}$ |
| 0.40% | 22 ± 0.48$^{c}$ | 40 ± 1.26$^{d}$ | 79 ± 5.27$^{c}$ | 1.41 ± 0.07$^{b}$ |
| 0.80% | 23 ± 0.99$^{b}$ | 42 ± 2.17$^{bc}$ | 92 ± 17.84$^{bc}$ | 1.62 ± 0.31$^{b}$ |
| 1.00% | 24 ± 0.33$^{a}$ | 44 ± 0.59$^{a}$ | 93 ± 8.78$^{b}$ | 1.58 ± 0.21$^{b}$ |

$^{a, b, c, d}$ Different letters within the same column represent significant differences ($p < 0.05$).