Emulsification Properties of Puka Gum – an Exudate of a Native New Zealand Tree (*Meryta sinclairii*)

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Abstract

This study investigated the emulsification properties of puka gum (PG) by determining an optimum gum concentration by evaluating various PG amounts (1-10% w/w), added to stabilise 15% w/w oil-in water emulsions (O/W). The effect of pH (2-8.5) on the emulsification ability of the gum, as well as the thermostability (80 °C for 30 min) of the emulsions were also tested. The emulsification properties of PG were evaluated in terms of mean particle size (d_{32}) and the particle size distribution at the initial day and over a storage period, the zeta potential, the rheological and microstructural properties and the visual phase separation (at 20 °C for one-month storage) of the emulsions. The effect of shear on the rheological properties of a 4% w/w PG in water solution was also carried out.

PG stabilized-oil-in-water emulsions exhibited monomodal size distributions with average d_{32} below 2 µm at 4% w/w PG. Generally, PG emulsions are resistant to a wide range of pH changes and heat treatment (80 ºC for 30 min), especially at extreme acidic conditions (pH 2) and more alkaline conditions (pH 8.5). Acidification seems to improve the emulsification ability of PG by changing the d_{32} from 1.9 µm at native pH (~5) to 0.48 µm at pH 2. Meanwhile, there were no changes in d_{32} of heated emulsions at pH 2 and 8.5. PG-stabilised droplets of unheated and heated emulsions were negatively charged with zeta potential *ca*. -30 – (-45) mV at pH >3.5. Emulsions around native pH (5 and 6.5) were observed to have a mixture of bridging and depletion flocculation, as confirmed by the microstructure images of the emulsion and after the addition of SDS. At pH 6.5, coalescence occurred in heated emulsions.

The apparent viscosity of these emulsions was dependent on gum concentration generally showing shear-thinning behaviour above 100 s^{-1} and a Newtonian plateau at low shear rate. The loss modulus (G'') was higher than the storage modulus (G') at all concentrations indicating that the liquid-like behaviour dominated at all frequencies. The apparent viscosity of PG solutions was found to be shear-thinning and time dependant, with an irreversible loss of viscosity found after shearing at constant shear rate over a period of time. This could have major implications for the emulsion droplet sizes obtained depending on the shear history of the gum solution.

Furthermore, PG-stabilized emulsions remained stable against phase separation for at least 30 days at all studied pHs and after heat treatment. Crude and purified PG exhibited no major differences in terms of their emulsification properties suggesting that a purification process may not be required for food formulations. Overall, PG can be considered as a promising natural emulsifier for emulsion-based foods and beverage products.

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CHAPTER 1 GENERAL INTRODUCTION AND OBJECTIVES

Consumers demand on healthier and sustainable food options, have driven researchers and manufacturers, to create products with natural ingredients, replacing synthetic and animal-based materials. There have been many studies identifying an abundance of natural ingredients, including emulsifiers. Many food products exist as emulsions classified into oil-in-water (O/W) and water-in-oil (W/O) emulsions. An emulsion produced by mixing two immiscible liquid phases such as oil and water is thermodynamically unfavourable and tends to phase-separate over time. Therefore, an emulsifier is considered to be a critical ingredient in food and beverages as many products exist in the form of emulsions. The addition of an emulsifying agent is required to produce the emulsion and to maintain the kinetic stability over time. This provides longer shelf life and better resistance to environmental changes improving the stability of many of these emulsion-based products (McClements, Bai, & Chung, 2017). Plant-based polysaccharides are an emerging source of natural emulsifiers due to their abundance in nature, effectivity, and sustainability.

Therefore, many scientists have paid special attention to studying novel plant-based polysaccharides for a wide range of food applications. Polysaccharides produced by the biotechnology process are more likely to be non-toxic, easy to decompose, natural and environmentally friendly (Prajapati, Jani, Moradiya, & Randeria, 2013). The term "natural emulsifiers" in this study is defined as emulsifiers produced without the use of solvents during the purification process and which are also minimally processed.

Generally, proteins are utilised as effective emulsifying agents in food systems. However, their sensitivity to environmental conditions such as pH, ionic strength and heat becomes an issue for product development (McClements & Gumus, 2016). In order to overcome this issue, several plant polysaccharides have been considered as alternative emulsifying agents. The presence of a protein moiety and/or a hydrophobic component as side chains in some polysaccharides are believed to be responsible for their emulsifying properties (Funami *et al.*, 2007; Osano, Hosseini-Parvar, Matia-Merino, & Golding, 2014; Xu, Wang, Fu, Huang, & Zhang, 2018). In the food and beverage industry, gum arabic (GA) is known as the gold standard for plant biopolymer-based emulsifiers as it has been proven to have good surface activity. Emulsification occurs when protein-rich fractions of GA adsorb onto the oil phase (Atgie, Masbernat, & Roger, 2018). Although GA exhibits excellent emulsification properties, there is still a demand to explore other alternative natural emulsifiers. This is because GA requires high concentrations (10% w/w) in its usage before it produces good emulsification, which in turn affects its cost efficiency and supply availability (Charoen *et al.*, 2011; Nakauma *et al.*, 2008; Qian, Decker, Xiao, & McClements, 2010).

Earlier studies have reported the surface activity of various polysaccharides such as galactomannans (Bai, Huan, Li, & McClements, 2017; Chanamai & McClements, 2002; Chivero, Gohtani, Yoshii, & Nakamura, 2016; Xiang *et al.*, 2015), pectins (Nakauma *et al.*, 2008; Schmidt, Schütz, & Schuchmann, 2017; Verkempinck *et al.*, 2018; Zhang *et al.*, 2015), basil seed gum (Gahruie, Eskandari, Khalesi, Van der Meeren, & Hosseini, 2020; Hosseini-Parvar, Osano, & Matia-Merino, 2016; Naji-Tabasi & Razavi, 2017; Osano, *et al.*, 2014), corn fibre gum (Bai *et al.*, 2017; Yadav, Johnston, Hotchkiss, & Hicks, 2007; Yadav, Moreau, Hotchkiss, & Hicks, 2012), soybean soluble polysaccharide (Jin *et al.*, 2017; Nakauma *et al.*, 2008), fenugreek gum (Mathur, 2012; Sav, Meer, Fule, & Amin, 2013), and octenyl succinic anhydride starch (OSA-starch) (Chivero *et al.*, 2016; Nilsson & Bergenståhl, 2007; Tesch, Gerhards, & Schubert, 2002). Most of the surface-active polysaccharides have shown their ability to adsorb at the oil-water interface and form stable O/W emulsions, and therefore they can be regarded as emulsifiers and stabilisers in these colloidal systems.

Meryta sinclairii, also known as puka in Māori, is a tree that is native to islands off the northern coast of New Zealand and it is often cultivated as a garden plant. The tree can be easily differentiated from other native plants by its large, glossy leaves, clustered fruits and flowers, and tall trunks that are approximately 8 m high (Foster, 2008). The trunk or branches of the puka tree can exude gum when wounded, or as a response to external stress. The composition and structure of puka gum (PG) has been studied and consists of more than 95% w/w carbohydrate and 2% w/w protein, rich in hydroxyproline (Sims and Furneaux, 2003). Thus, this gum is classified as an arabinogalactan-protein (AGP) and shares structural similarity with GA. The polysaccharide portion of PG contains a highly branched backbone of β-D-1,3-linked galactopyranosyl residues with side-chains comprised of arabinofuranose-containing oligosaccharides which are terminated either by rhamnopyranosyl, arabinopyranosyl, galactopyranosyl or glucuronopyranosyl residues. The molecular weight of PG (4.45×10^6 Da) is much larger than GA (6.02×10^5 Da).

A study on the rheological and physicochemical properties of PG showed that it exhibited Newtonian behaviour (at low gum concentration) to shear-thinning behaviour for up to 25% w/w gum level, which is associated with its spherical conformation (Wee, Sims, Goh and Matia-Merino, 2019). The viscosity of PG is higher when compared to GA at similar concentrations, due to its larger molecular weight, hydrodynamic radius and particle size. Furthermore, the zeta-potential of PG is similar to GA (~-30 mV) and therefore, it is sensitive to ions and acidic pH although no viscosity changes are observed at alkaline pH. Due to PG′s negative charges, highly viscous coacervates were formed when PG was complexed with whey protein isolate via electrostatic interactions (Wee *et al.*, 2014). Based on these characterisation studies, PG is believed to be a potential substitute to GA for food applications.

To date, no study has evaluated PG′s ability to form and stabilise O/W emulsions and the effect of environmental conditions, such as pH and heat on the emulsifying properties of PG. Therefore, this study aimed to investigate the characteristic of the emulsification properties of PG by:

1. Determining the effect of gum concentration on the emulsifying properties of PG
2. Determining the optimum concentration of PG to form stable emulsions
3. Determining the effect of varying the pH on the emulsifying properties of PG
4. Determining the thermostability and storage stability of PG-stabilised emulsions

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

The aim of this review is to discuss the role of plant-based polysaccharides in emulsification and stability of emulsions with an emphasis on oil-in-water emulsions stabilised by novel natural emulsifiers. Emulsifying properties of novel plant-based polysaccharides including its emulsifying mechanism, the conformational structure of each polysaccharide, and the stability of the emulsions prepared by each plant-based polysaccharide under several environmental conditions were highlighted. Furthermore, factors affecting emulsifying properties and emulsion instability were also covered.

2.2 Novel Plant-based polysaccharides

Over the past few decades, the rapid development of natural additives, including emulsifiers, is driven by the trend of consumer's concern towards health and 'clean label' foods. The term of natural emulsifiers in this review is defined as emulsifiers that are produced without the use of solvents during the purification process and are minimally processed. Synthetic surfactants have been utilised by the food industry due to their superior emulsifying ability and cost-effectivity (McClements *et al*., 2017; Pereira *et al.*, 2019). Despite these advantages, an increase in the incidence of allergies and autoimmune diseases has been reported as a result of a high usage of chemically- made surfactants in food products (Csáki, 2011; Pereira *et al*., 2019).

Besides health and functionality concerns, environmental factors should also be taken into account. Emulsifiers based on natural polymers are likely to be highly biodegradable, ecologically friendly and readily available due to their abundance in nature (Golkar, Taghavi, & Aghili Dehnavi, 2018; Prajapati *et al*., 2013). They are also a renewable source which can provide consistent supply of raw materials if grown in a sustainable manner. However, there are some challenges that can arise, for instance, sourcing natural emulsifiers such as those from plants will definitely have differences in their compositional chemistry due to different growing locations and seasons. This can pose difficulties and inconsistencies during characterisation of their functionality and application tests for various products

Identification of plant-based polysaccharides as a source of novel emulsifiers has been established by many studies in this field (Appendix). The term of natural emulsifiers in this review is defined as emulsifiers that are produced without the use of solvents during purification process and are minimally processed. Natural emulsifying agents can be categorised into these four groups; namely polysaccharides, proteins, phospholipids, and saponins. All of them, apart from polysaccharides, are

highly susceptible to pH, ionic strength, and temperature. Emulsions stabilised by plant-based polysaccharide emulsifiers are less affected by environmental changes due to steric hindrance. This primary stabilisation occurs when hydrophobic chains (non-polar group) of the polysaccharide-based emulsifier adsorb onto the oil droplet surface while its dangling chain of carbohydrate (polar group) interacts with the water phase of the emulsion. As a consequence, thick and vigorous interfacial films are produced inhibiting droplet aggregation and maintaining emulsion stability (Dickinson, 2009; Ozturk & McClements, 2016; Zhu *et al.*, 2020).

Many studies in the literature cover various plant-based polysaccharides roles, such as stabilising, thickening, and gelling ability. However, this review focuses specifically on the emulsifying capacity functionality of several plant-based polysaccharides. The scope of this review includes the emulsification mechanism of the plant-based polysaccharides, the conformational structure of each polysaccharide, and the stability of the emulsions prepared by each plant-based polysaccharide under several environmental conditions. In addition, this study also provides an update on recent research work done on novel natural emulsifiers.

2.3 Plant-based polysaccharides acting as emulsifying agents

A hydrocolloid is defined as a high molecular weight water-soluble biopolymer that quickly dissolves and/or disperses to form hydrated colloidal dimension particles via hydrogen bonding (Dickinson, 2003). In the food industry, apart from gelatine, polysaccharides derived from seeds, roots, fruits, seaweed, tree exudates and plant extracts, are classified as hydrocolloids and have been widely recognised and used in an array of food applications as stabilisers, thickeners, viscosifying agents, texturisers and gelling agents. In an aqueous system, the hydrophilic nature of the hydrocolloid will cause it to interact with the surrounding water molecules and other hydrocolloid molecules creating a viscous mixture at comparatively low concentrations. This key ability is responsible for the broad food applications, from bakery products and beverages to dietetic foods (Nussinovitch & Hirashima, 2014). Another noticeable functionality that certain hydrocolloids can exhibit is emulsifying capacity due to some surface activity. This functionality allows the hydrocolloid to be used as an emulsifier or ingredient for stabilising O/W emulsions, hence providing a structural-mechanical barrier to slow down emulsion breakdown (Dickinson, 2018).

2.3.1 Emulsifier Properties

The characteristics of an effective emulsifying agent are associated with its physicochemical properties. Emulsion formation and its stability are the pivotal roles of an emulsifier (Figure 1). As stated by McClements (2016), a desirable characteristic for emulsion formation, is associated with the surface activity of the emulsifier. Surface active components have to (a) be able to adsorb rapidly covering the surfaces of droplets during homogenization and (b) be able to lower the interfacial tension by a sufficient amount to facilitate further droplet disruption. Meanwhile, emulsion stability is described as the ability to form a protective coating around the droplets that prevents their aggregation by generating strong repulsive forces, such as steric or electrostatic repulsion.

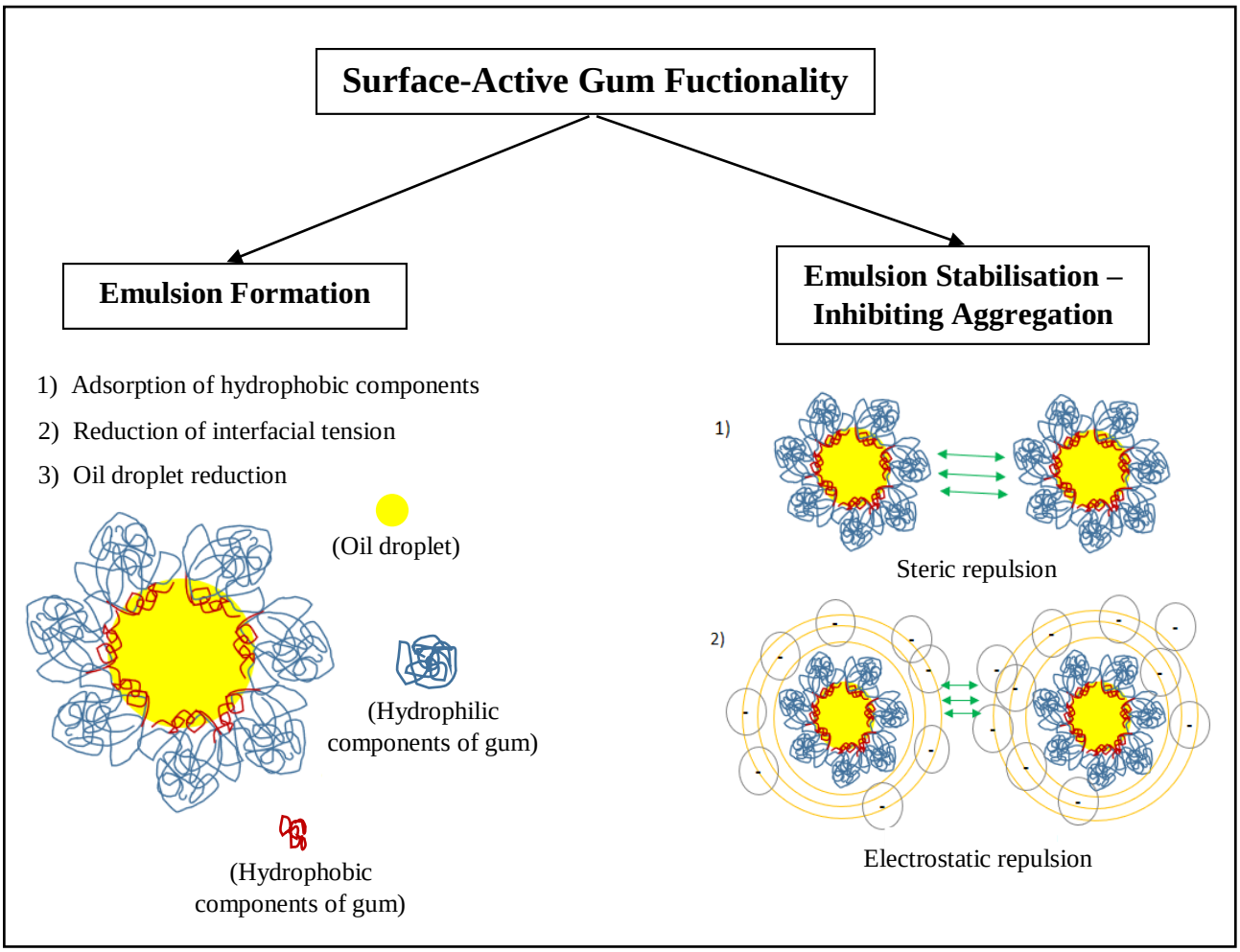


Figure 1. Schematic diagram of the functionality of plant-based polysaccharide emulsifiers in O/W emulsion; yellow: oil phase; blue: surface-active polysaccharides; red: hydrophobic components (protein or acetyl segments) of gums.

2.3.1.1 Emulsion Formation

2.3.1.1.1 Surface Activity

The surface activity of a hydrocolloid is shown when it lowers the interfacial tension at the oil-water interface. Lower interfacial tension can be achieved when an emulsifying agent is able to interact with both phases by adsorbing at the interface, reducing the free energy of the system. Subsequently, this lowers the imbalance of molecular interactions between the two immiscible phases (oil and water), thus the interfacial tension is reduced (McClements, 2016). The surface-active ability of some hydrocolloids has been evaluated as shown in Table 1. This ability is deemed as critical throughout the process of emulsification (Dickinson, 2018).

Table 1. Interfacial tension of different hydrocolloids.

Concentration	Hydrocolloid	Interfacial Tension (mN/m)		References
		Initial	Final	
0.1%	Crude BSG	54	42	(Osano *et al*., 2014)
	TPC-W		41.5	(Han *et al*., 2020)
0.2%	Carrot pectin	25	15	(Neckebroeck *et al*., 2020)
0.3%	Crude BSG	54	35	(Osano *et al*., 2014)
0.50%	SBP	26.5	17.5< SBP <20	(Liu, Guo, & Meng, 2020)
	AP	26.5	20< AP <22.5	(Liu, *et al*., 2020)
	CP	26.5	20< CP <22.5	
	RRSG	35	17	(Chouaibi *et al*., 2019)
	Crude BSG	54	28	(Osano *et al*., 2014)
	GT	20	13	(Abdolmaleki, *et al*., 2019)
	Persian Gum		34	(Golkar, *et al*., 2018)
	TPC-W		20.6	(Han *et al*., 2020)
0.70%	Crude BSG	54	24	(Osano, *et al*., 2014)
0.75%	ASKP	10.6	9.2	(Li *et al*., 2018)
1%	CP	17	14	(Ren, *et al*., 2020)
	Mutamba seed	8	5	(Pereira *et al*., 2019)
	Crude BSG	54	12.6	(Osano, *et al*., 2014)
	PSG		37	(Golkar, *et al*., 2018)
1.5%	PSG		15	(Golkar, *et al*., 2018)
	YM	70	47	(Wu *et al*., 2015)
2%			14	
2.5%			13	
3%			15	

Generally, a good surface activity is exhibited by small-molecule surfactants as their structure is mostly amphiphilic (McClements & Gumus, 2016; Ozturk & McClements, 2016). In an amphiphilic molecule, the hydrophilic tail interacts with the water phase, while the hydrophobic head is exposed and subsequently dominates the interface. Molecules with higher surface activity require lower concentration when forming a stable emulsion. Factors affecting emulsion formation are illustrated in Figure 1.

Despite the claim that hydrocolloids are not true emulsifiers—as they normally act through increasing viscosity and remain in the continuous phase—some hydrocolloids such as GA, SBP and

galactomannans possess surface-activity properties (Garti & Leser, 2001). Generally, polysaccharides have a chemical structure that provides them with water-liking character, which makes them not easily adsorbed on to hydrophobic surfaces; thereby, their surface activity is considered to be negligible. However, in some cases, these polysaccharides carry proteinaceous materials that are covalently bonded or physically conjugated or even exist as contaminants, while in other cases they may have hydrophobic auxiliary groups attached to them such as methyl or acetyl groups. These protein fractions and possible non-polar groups are responsible for the surface activity (Dickinson, 2003; Garti & Leser, 2001). The above explanation is congruent with many studies. For instance, Liu *et al*. (2016) reported that emulsion stability of flaxseed gum was decreased to 60% after protease treatment. In another study, the emulsion activity of sugar beet pectin was found to be enhanced by its protein component (Funami *et al*., 2007). Better emulsifying properties of soybean soluble polysaccharide were ascribed to its protein fraction of around 2.2% (Nakamura, Yoshida, Maeda, Furuta, & Corredig, 2004). Besides the protein component, a sufficient amount of accessible hydrophobic group is also critical to possess a good surface activity. This accessibility is probably related to an extended molecular configuration of some hydrocolloids (Li *et al*., 2018).

2.3.1.1.2 Adsorption Kinetics

Another important characteristic of an effective emulsifying agent lies in its adsorption kinetics. Adsorption kinetics refer to the rate at which the emulsifier is able to cover the oil droplets during the homogenisation process. The more rapid the emulsifier disrupts the droplets and adsorbs to the oil surfaces, the better the emulsion formation. A quick full coverage of the inhibits droplet coalescence (McClements *et al*., 2017). The rate of adsorption during emulsification generally depends on the molecular dimensions of the emulsifier and its surface chemistry. Typically, plant-based polysaccharide emulsifiers adsorb onto the interfaces at a slower rate than small molecules surfactants. The relationship between a reduction in molecular weight of the plant-based polysaccharides with an improvement of the emulsifying properties has been reported by many studies. Wu *et al*. (2015) found that molecular weight, viscosity, and molecular interactions affected the oil droplet size. YM-stabilised emulsions were observed to have larger oil droplets size (>1,500 nm) as compared to two other emulsions that were prepared by GA and CP due to increased viscosity thus slower movement of the molecules occurred. In another study, *Portulaca oleracea* gum was found to form a stable emulsion with oil droplets that were smaller than 2 μm compared to emulsions that were prepared by GG and FG which had oil droplets between 5 and 10 μm due to its lower molecular weight (Garti & Leser, 2001). Pectins with similar degree of esterification were found to have far better emulsifying properties when they have low molecular weight between 50 and 80 kDa (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002; Leroux *et al*., 2003). *Artemisisa sphaerocephala*

Krasch polysaccharide (ASKP) which has medium molecular weight was reported to exhibit poor emulsifying properties even though its surface activity was better than GA (Chen *et al*., 2014; Li, Hu, Li, & Ma, 2016). However, when a xylanase treatment was carried out, high-molecular-weight fractions (60P) in ASKP were found to improve its emulsifying activity. This study concluded that lower or higher molecular weight may lead to partial loss of emulsifying abilities (Li *et al*., 2018).

2.3.1.2 Emulsion Stability

Long-term emulsion stability is achieved by providing steric repulsion to the oil droplets. It is the next essential function of emulsifiers once the dispersed small oil droplets have been prepared. Steric stabilisation is defined as a repulsive interaction between the interfacial layers of two approaching droplets (McClements, 2016). Steric stabilisation is likely to occur when the hydrocolloid exhibits specific characteristics, such as having strong adsorption, full surface coverage and formation of a thick physical stabilising adsorbed layer, also contributing to a supplementary electrostatic stabilising role if charged (Dickinson, 2003, 2018).

The strong adsorption is dependable on the ability of the hydrophobic segments in the biopolymers to permanently adhere to and its durability at the interface. At the same time, sufficient concentration of biopolymers should be present to fully cover and saturate the interface. Furthermore, the dangling hydrophilic polymer chains should be attached to the adsorbed layer to form the thick stabilising layer (Dickinson, 2018).

To additionally stabilise the emulsion, electrostatic stabilisation may be conferred as some polysaccharides carry charged groups. In general, the presence of charged components, such as mineral ions, ionic surfactants, or other polysaccharides in single or multilayer emulsions can considerably affect the stability of emulsion over time by forming a coated charged layer (McClements, 2012; McClements *et al*., 2017). This can lead to the formation of a robust macromolecule interfacial barrier that prevents emulsion instability (flocculation and coalescence) under van der Waals attractive forces, supporting the steric stabilisation. However, the charged compounds may also promote droplet aggregation via ionic bridging between droplets.

2.4 Novel Plant-Based Polysaccharides with Emulsifying Properties

Gum Arabic has received a lot of attention in the food industry as far as its emulsification properties are concerned specially dealing with the production of flavour emulsions for the beverage industry (de Barros Fernandes *et al*., 2016; Esfahani, Jafari, Jafarpour, & Dehnad, 2019; Esmaeelian, 2016; Kim & Morr, 1996; Raikos, Duthie, & Ranawana, 2017; Trubiano & Lacourse, 1988; Williams,

Phillips, Stephen, & Churms, 2006). Therefore, the following sections are dedicated to the remaining polysaccharides which are gaining momentum due to their potential to be used as emulsifiers.

2.4.1 Pectins

Pectins are extracted from plant cell walls and widely used in food applications as gelling and thickening agents. This hydrocolloid group has been steadily investigated as a food emulsifier and/or an emulsion-stabilising agent (Drusch, 2007; Funami *et al*., 2011; Mirhosseini *et al*., 2008; Ngouémazong, Christiaens, Shpigelman, Van Loey, & Hendrickx, 2015). Sugar beet pectin (SBP) is widely known as a surface-active pectin which can readily stabilise O/W emulsions (Dickinson, 2003). A stable emulsion with oil droplet size ranging from 0.1 to 1 μm can be produced by using 2% w/w of sugar beet pectin concentration, which is as effective as GA (Nakauma *et al*., 2008). Besides SBP, some novel pectins have also been reported having marked emulsifying properties, such as citrus pectin (Ren *et al*., 2020; Schmidt, Schütz, & Schuchmann, 2017; Verkempinck *et al*., 2018; Zhao *et al*., 2018), apple pectin (Liu, Guo, & Meng, 2020), cacao pods husk pectin (Trujillo-Ramírez *et al*., 2018), Alperujo pectin (Rubio-Senent, Rodríguez-Gutiérrez, Lama-Muñoz, García, & Fernández-Bolaños, 2015), pumpkin pectin (Cui & Chang, 2014), and pomegranate peel pectin (Yang *et al*., 2018).

The ability of pectin to facilitate smaller oil droplets formation has been associated with the presence of its protein moiety and the hydrophobic character of acetyl and feruloyl esters (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002; Funami *et al*., 2007; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Ngouémazong *et al*., 2015). The pivotal role of the proteinaceous component in emulsification can be proven by removing the protein from SBP. In a freshly prepared emulsion, protein-free SBP has been reported to exhibit a decrease in its interfacial activity resulting in a higher oil droplet size and greater particle size distribution (Funami *et al*., 2011; Funami *et al*., 2007). On the other hand, the importance of acetyl group was studied by Leroux *et al*. (2003) who stated that chemically acetylated citrus pectin showed better emulsifying stability than de-acetylated SBP as acetyl groups were likely to reduce calcium bridging flocculation.

As other polysaccharide-based emulsifiers, stabilisation mechanisms of pectins are achieved through steric stabilisation and/or electrostatic stabilization(s) (Funami *et al*., 2011). The steric stabilisation of pectin-stabilised emulsions is achieved once pectins are fully adsorbed at the interface covering the oil droplets. Meanwhile, any non-adsorbed pectins due to the saturated interface may lead to the viscosity modification of the continuous phase by the carbohydrate chain of pectin. Ngouémazong *et al*. (2015) stated that homogalacturonan (HG) and the neutral sugar side chains of rhamnogalacturonan-I (RGI) of pectin are responsible for the emulsion stability. The neutral sugar side chains provide the steric stabilisation by creating robust hydrated layers on the adsorbed pectin.

On the other hand, electrostatic repulsion is attributed to the HG structure of pectin. The ionized pectin and the charged carboxylic groups in HG form an electrical layer around the droplets surface conferring electrostatic stability to the emulsions (McClements, 2004; Ngouémazong *et al*., 2015).

Owing to the acidic nature of pectin, pectin-stabilised emulsions are typically prepared under acidic conditions. The addition of cations such as calcium (Ca^{2+}) and sodium (Na^{+}) ions can cause a decrease in electrostatic repulsion (Ngouémazong *et al*., 2015) as well as induce bridging flocculation. Larger emulsion droplets were observed as a result of the interaction between Ca^{2+} and citrus pectin (Ren *et al*., 2020) in agreement with the study carried out by Yang *et al*. (2018), which proved that the presence of high Ca^{2+} and Na^{+} in pomegranate peel pectin-stabilised emulsions were unfavourable due to the disruption of repulsions between emulsion droplets inducing aggregation. However, the addition of Ca^{2+} at low concentration, such as below 60 mg/g pectin can improve the emulsion stability by modifying the viscosity of continuous phase through the formation of intermolecular cross-linkages.

Most of pectin-stabilised emulsions are resistant to low pH (Zhao *et al*., 2018) and tend to become more stable at acidic conditions rather than at higher pH's. For instance, carrot pectin is able to form a more stable emulsion at pH 2.5 compared to pH 6 due to better adsorption of pectin and a denser association of adsorbed pectin chains at the interface at lower pH (Neckebroeck *et al*., 2020; Verkempinck *et al*., 2018). On the contrary, pomegranate peel pectin has been found to have wider working pH range; at pH 2 to 6, emulsions prepared with this pectin were observed to be stable (Yang *et al*., 2018). Ren *et al*. (2020) also have found that creating an acidic environment by adding citric acid and ascorbic acid into citrus oil emulsions reduced the oil droplet size from 1.47 to 0.83 µm and improved the emulsion stability over a period of 20-day storage.

2.4.2 Galactomanannans

A galactomannan is a hydrophilic biopolymer with a backbone of polymannose (β-(1→4)-D-mannose) containing more than 5% of galactose (α-D-galactose) unit as side chains (Dickinson, 2003; Sharma *et al*., 2018; Srivastava & Kapoor, 2005). Some galactomannans that have been widely used are guar gum (GG), locust bean gum (LBG), fenugreek gum (FG), and tara gum (TG). The solubility of galactomannans is determined by the ratio of mannose to galactose (M/G ratio) which varies depending on the source of galactomannan. For instance, FG has the lowest M/G ratio (1, 2, 3, and 4) compared to GG, TG, and LBG respectively (Wu, Cui, Eskin, & Goff, 2009). Lower M/G ratio is associated with better solubility of galactomannans as the mannan backbones are inhibited by the galactose side chains to form hydrogen bonded aggregates (Garti, 1997). Emulsifying capacity and stability of galactomannan are also affected by the M/G ratio. Galactomannans with lower M/G ratio, such as FG was found to significantly lower the surface tension of emulsions more than those with

higher M/G ratio (Wu, *et al* 2009). In addition, more gel-like layers covering the oil droplets, are likely to be formed at lower M/G ratio and subsequently, enhance the emulsion capacity and stability.

The stabilising mechanism of galactomannans in O/W emulsions is in many cases associated with the modification of the rheological properties of the continuous phase. The higher viscosity of the continuous phase retards the movement of the dispersed particles; thus, improving long-term physical stability of the emulsion (Chouaibi *et al*., 2019; Dickinson, 2003). However, some galactomannans have been reported to exhibit surface activity at the interfaces and as a consequence, to lower the interfacial tension, providing steric hindrance after adsorption and retarding droplet aggregation (Chouaibi *et al*., 2019; Huang *et al*., 2001; Wu *et al*., 2009). FG has the highest surface activity among the most common galactomannans, namely GG, LBG, and TG (Rashid, Hussain, & Ahmed, 2018; Wu *et al*., 2009).

Chouaibi *et al*. (2019) has recently studied one of the promising novel galactomannans known as the *Retama reatam* galactomannan (RRG) reporting the presence of surface-active material in the gum. The presence of an amphiphilic fraction—mainly protein, in this galactomannan was found to decrease o/w interfacial tension at relatively low concentration. The interfacial activity of this galactomannan was comparable to that obtained by highly flexible proteins such as lysozyme, ovalbumin, and bovine serum albumin at similar O/W interfaces. Also, this investigation has shown that RRG has better surface activity than other surface active hydrocolloids such as GG, SBP, and GA. At similar galactomannan concentration (1% w/w), the mean diameter of RRG-stabilised O/W emulsions was comparably lower (45 nm) than GG-stabilised emulsions (55 nm) (Chouaibi *et al*., 2019). At high temperature up to 80 ˚C, this novel emulsifier was still able to stabilise the emulsions indicating that the RRG is also quite resistant to thermal treatment inhibiting droplet aggregation.

2.4.3 Basil Seed Gum

Basil seed gum (BSG) is extracted from the seeds of the *Ocimum basilicum* L. plant. This seed is commonly used in some Asian countries such as India, Iran, Indonesia, and Malaysia in the beverage industry as a source of dietary fibre (Hosseini-Parvar, Matia-Merino, Goh, Razavi, & Mortazavi, 2010; Mathews, Singhal, & Kulkarni, 1993) and for aesthetic purposes (Naji-Tabasi & Razavi, 2017). BSG is a high molecular weight heteropolysaccharide (2320 kDa), which comprises of two major fractions (43% of glucomannan and 24.29% of (1 → 4) linked xylan) and a minor fraction (2.31% of glucan) (Anjaneyalu & Gowda, 1979). BSG is also characterised as an acidic polysaccharide consisting of 6.51% of uronic acid. A study done on its rheological properties showed that BSG exhibits pseudoplasticity behaviour with a gradual increase in concentration and it is also naturally heat-tolerant (Hosseini-Parvar *et al*., 2010; Razavi & Naji-Tabasi, 2017).

Surface activity and emulsifying properties in BSG have been studied by several researchers (Hosseini-Parvar, Osano, & Matia-Merino, 2016; Naji-Tabasi & Razavi, 2016, 2017; Naji-Tabasi, Razavi, Mohebbi, & Malaekeh-Nikouei, 2016; Osano, Hosseini-Parvar, Matia-Merino, & Golding, 2014; Osano, Matia-Merino, Hosseini-Parvar, Golding, & Goh, 2010). Factors that influence the adsorption properties of BSG include the existence of protein moieties, molecular weight, flexibility of BSG structure, the presence of uronic acid and the presence of possible acetyl groups in the structure which would contribute to the hydrophobic character of the gum (Hosseini-Parvar, Osano, & Matia-Merino, 2016). The role of protein moiety in BSG was investigated by doing a protein depletion in the gum and then characterizing its ability to adsorb on interfaces. Naji-Tabasi *et al*. (2016) found that protein-free-BSG after enzyme-hydrolysis treatment led to a decrease in surface activity regardless of gum concentration. This is in agreement with a study carried out by Osano *et al*. (2010) where the adsorption ability of BSG was significantly decreased for fractions with lower protein content, though it was argued that the methodology used to remove protein could have altered the gum structure affecting its adsorption properties. In general, there is an undeniable pattern that relates a reduction of surface activity with a decrease in the amount of protein moieties present in these polysaccharides, as this has been frequently observed also for other gums such as FG, SBP and tamarind seed gum (Brummer, Cui, & Wang, 2003; Crispín-Isidro *et al*., 2019; Funami *et al*., 2007; Youssef, Wang, Cui, & Barbut, 2009). However, studies like those from Osano *et al*. (2014) stating that with a minimum residual protein as low as 0.60%, BSG could still produce small droplet sizes similar to those sizes obtained with fenugreek containing 3% protein, indicates that protein may only be part of the story for some of these gums.

Besides protein moiety, the surface activity of BSG is also associated with its molecular weight; smaller molecular weight can ease its adsorption on interfacial surfaces. According to a study carried out by Naji-Tabasi *et al*. (2016), SUPER-BSG obtained by fractionation exhibited the best surface activity (57 mN/m at 0.3% w/w) as it has the lowest molecular weight (1045 kDa). It also exhibited the highest chain flexibility (K_H = 0.36) resulting in a stronger surface activity, while its high uronic acid (13.39%) conferred BSG as a polyelectrolyte gum indicating higher solubility.

The concentration level BSG in the emulsion plays an essential role in emulsion stability. The presence of only 0.3% w/w of BSG can provide sufficient coverage resulting in uniform droplet size with the average d_{32} below 1.0 μm and without any creaming observed in the emulsion (Osano *et al*., 2014). There are three possible properties that are related to the impact of gum concentration on BSG-emulsion stability based on the interfacial layer: (1) complete surface coverage (Dickinson, 2003), (2) highly viscoelastic layer at the interface on sufficient gum concentration contributing to steric stabilisation (McClements, 2016; Osano *et al*., 2014), and (3) an elevating viscosity of continuous

phase due to the presence of non-adsorbed BSG. Therefore, a sufficient amount of adsorbed BSG at the interface could provide a strong steric repulsive layer that completely covered the oil droplet surface, this all relate to BSG surface activity. On the other hand, the excess of non-adsorbed BSG is likely to increase the viscosity of the emulsion restricting the movement of oil droplets and therefore delaying any creaming. Osano *et al*. (2014) found that BSG-stabilised emulsions did not show any sign of phase separation for as long as one-month storage, which is in agreement with a study carried out by Naji-Tabasi and Razavi (2016). Phase separation of BSG-stabilised emulsions was only visible on the 5th week of storage.

Regarding the impact of environmental conditions, BSG-emulsions exhibited monomodal particle size distributions and smaller average droplet sizes (d_{32} < 1 µm) under alkaline conditions (Hosseini-Parvar *et al*., 2016). Despite the alteration of particle size due to heat treatment, extreme pH, and addition of salt, BSG-stabilised emulsions remained stable from phase separation due to their strong gel-like behaviour conferred by the non-adsorbed gum (Hosseini-Parvar *et al*., 2016; Naji-Tabasi & Razavi, 2017). Based on its promising emulsifying properties, BSG was studied in food applications such as processed cheese (Hosseini-Parvar, Matia-Merino, & Golding, 2015). The addition of BSG resulted in a more elastic behaviour, lower meltability and greater oil emulsification of the processed cheese, with no significant effect on the final pH.

2.4.4 Persian Gum

Persian gum (PRG) is a natural exudate gum of a wild almond called *Amygdalus scoparia* Spach or *Prunus scoparia* which is mostly found in Iran and other Central Asia regions. This gum is also known as gum zed, zedu, angun, and gharacia (Abbasi & Rahimi, 2015). The gum is partially soluble; therefore, in order to improve the gum effectiveness as a food emulsifier, fractionation is needed to separate the soluble and insoluble parts (Abbasi, 2017). PRG is categorised as an acidic and anionic polysaccharide containing 0.20 to 1.02% w/w protein and 82 to 90% w/w carbohydrate with major monosaccharide components namely galactose, arabinose, and rhamnose. Based on its molecular structure, Abbasi (2017) concluded that PRG is considered as an arabinogalactan with some distinctions compared to GA, such as the adverse ratio of Ara:Gal, lower protein content and the presence of xylose and mannose. Therefore, PRG may be utilised as a substitute for the highly priced GA.

Although protein is observed in PRG, the amount is very low, which would indicate the gum itself may contribute to the emulsification by a possible presence of other hydrophobic groups, such as acetyl groups. Meanwhile, the overall physical stabilization of the emulsion against creaming is likely to be obtained by the increase viscosity of the continuous phase linked to the non-adsorbed gum. Studies on PGR have reported that 0.5% w/w PRG exhibited a good emulsifying capacity up to 5%

w/w oil with a low average droplet size of 1.13 µm (Abbasi & Rahimi, 2015; Rahimi, 2012). Therefore, Abbasi (2017) stated that the optimum ratio of PRG:oil was 2:1; achieving a better emulsion with smaller average droplet size up to 0.8 µm. In other studies, Yosefi, Abbasi, and Ezatpanah (2012) reported that high concentration of soluble PRG (0.75 and 1% w/w) was able to stabilise 5-20% w/w O/W emulsions against creaming by means of an increase in bulk viscosity. The emulsions were found to be more stable at pH 8 and after heat treatment at 80 ˚C for 30 min compared to the emulsions prepared in acidic conditions, which showed phase separation. The particle size of PRG-stabilised emulsions was monomodal with the average droplet size being less than 2 µm.

Some researchers also have attempted to utilise PRG into various food applications. For instance, for the creation of orange peel essential oil nano emulsions for beverages (Hashtjin & Abbasi, 2015), as a stabiliser in milk-orange juice (Abbasi & Mohammadi, 2013) and emulsifier in milk-sour cherry juice mixtures (Teimouri, Abbasi, & Scanlon, 2018), and London rocket seed drinks (a type of refreshing Persian drink) (Behbahani & Abbasi, 2017).

2.4.5 Puka Gum

Puka gum (PG) is an exudate of a native New Zealand tree called *Meryta sinclairii* which grows naturally in the northern coast of New Zealand. Sims and Furneaux (2003) reported that PG consists of more than 95% w/w carbohydrate and 2% w/w protein. Its molecular structure comprises of β-1,3-linked galactopyranosyl (Gal*p*) with a highly branched backbone with arabinofuranosyl (Ara*f*) oligosaccharides as side chains terminated variously by rhamnopyranosyl (Rha*p)*, arabinopyranosyl (Ara*p)*, Gal*p* and glucuronopyranosyl (Glc*p*A) residues (Carnachan, Bell, Hinkley, & Sims, 2019). This gum is identified as an arabinogalactan-protein which shares structural similarity with GA. However, the molecular weight of PG is seven times bigger than GA; 4.45×10^6 Da and 6.02×10^5 Da, respectively (Carnachan *et al*., 2019). Sims and Furneaux (2003) stated that minor structural differences between PG and GA occur in its side chain of oligosaccharides. PG has 5-linked Ara*f*, terminal Ara*p*, and terminal 4-*O*-methyl Glc*p*A while GA has Gal*p* as its terminal.

The structural, physicochemical and rheological properties of PG have been investigated by Wee, Sims, Goh, and Matia-Merino (2019). They found that PG has spherical conformation like GA but is distinctly different from other polysaccharides which have flexible or semi-flexible coil type conformation, such as pectin and GG. This may lead to different functional properties. PG is categorised as an anionic polysaccharide; hence it is sensitive to low pH and ionic strength. Wee *et al*. (2014) reported that the optimum coacervation pH value (pH 3.6) shows maximum structural strength, but there is no difference in terms of protein-polysaccharide ratio.

Recent studies carried out by Breckenridge and Holthaus (2019) have shown the potential of PG as an emulsifying agent in several food applications, such as soft drinks and ice cream. When 10% of PG and 15% of weighted orange oil (WOO) were used in beverage flavour emulsion, it produced very small oil droplets with globule size 0.8 μm. Meanwhile, 15% PG and 10% WOO yielded the best stability against creaming for over 14 days of storage. Despite its promising emulsifying properties, PG application studies are still in their infancy stage; therefore, they need further exploration and development. PG similarity in structure with GA and the presence of small amount of protein may lead to promising properties and application in the food industry, offering a potential alternative for GA. However, there is a lack of research on the emulsification of vegetable oil using this gum in terms of gum concentration and environmental stresses on PG-stabilised O/W emulsion.

2.4.6 Combination of Natural Emulsifiers

Each natural polysaccharide-based emulsifier varies in terms of its effectiveness at forming and/or stabilizing O/W emulsions. Typically, these polysaccharide-based emulsifiers may have only one effective functionality; it may be effective in emulsification but poor in stabilising emulsions whereas others may be effective in providing long-term emulsion stability yet poor in emulsifying ability. In recent studies, some researchers attempted to combine two or more natural emulsifiers, especially those which are new or underexploited, in an effort to improve the functionality of the emulsifying agents. Besides that, these studies also considered factors like production cost efficiency and the development of regional economy.

When studying two or more natural emulsifiers, combination of mechanisms may be involved such as co-adsorption, complexation/conjugation and layer-by-layer deposition (McClements, Bai, & Chung, 2017). Co-adsorption is described as the usage of two or more emulsifying agents in the same system to form a mixed interfacial layer covering the oil droplets (Dickinson, 2011; Pugnaloni *et al*., 2004). For instance, Ma *et al*. (2017) attempted to combine polysaccharide from *Dioscorea opposita thumb* (DOP) and GA in medium chain triglycerides emulsions. It is reported that emulsions stabilised by DOP and GA with a mixture ratio of 1:1:1 were stable, and this mixture produced a relatively smaller droplet size (0.94 μm) as compared to those prepared by using individual emulsifiers. This stabilising mechanism might occur due to the synergetic effects between both polysaccharide-based emulsifiers.

Emulsions containing a mixture of mesquite gum and nopal mucilage (MG-NM) exhibited more stability against thermal processing. The emulsion also had the highest electrostatic stability and the combined polysaccharides, had both functionalities emulsifying and foaming abilities (Cortés-Camargo *et al*., 2018). In another study carried out by Yang *et al*. (2020), sodium alginate mixed with konjac glucomannan at a ratio of 5:5 was found to produce a low-fat mayonnaise-like food with

desirable viscosity, good thermal stability (100°C for 30 min) and freeze-thaw stability (-18 °C for 24 h) by displaying no signs of droplet coalescence.

The mechanism involved in complexation/conjugation is attributed to physical or covalent complexation between emulsifier and other substances before or after homogenisation (Dickinson, 2011). Covalent bonding may occur by promoting natural Maillard reactions or by using enzymes. A formation of multilayer coatings covering oil droplets using electrostatic deposition can be categorised as layer-by-layer deposition (Guzey & McClements, 2006). A study conducted by Hou *et al*. (2010) demonstrated that stable β-carotene emulsions can be achieved by forming multilayer emulsions employing soybean soluble polysaccharide and chitosan as a primary and secondary layer, respectively. A recent investigation by García-Márquez *et al*. (2015) also reported that degradation of carotenoids and aggregation of oleoresin-in-water emulsions can be avoided by using a calcium phosphate-chitosan complex layered by mesquite gum with ratio 1: 10.

CHAPTER 3 MATERIALS AND METHODS

3.1 Introduction

This chapter shows the material and methods used in this study. The materials and preparation for emulsions including the PG chemical compositional analysis of PG are described. All techniques used for characterising the emulsifying properties of PG-stabilised emulsions are also detailed.

3.2 Materials

Crude puka gum (CPG) and purified puka gum (PPG) (Figure 2) were isolated at the Ferrier Research Institute, Victoria University of Wellington, Wellington, New Zealand, as described by Sims and Furneaux (2003). In summary, the trunks of *Meryta sinclairii* trees were cut to obtain gum tears. Once collected, the gum (25 g) was dispersed in hot water (250 ml, 80 ˚C) for 1 h until completely dissolved. CPG was obtained by filtering the hot solution under pressure (Whatman GF-B glass fibre filter) and then freeze-dried. PPG was prepared by dissolving CPG in distilled water then dialysing against distilled water (MWCO 12-14,000 MWCO) for 48 h and freeze-dried. Soybean oil was purchased from AMCO, Goodman Fielder Ltd., New Zealand. Other chemicals including hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium chloride (NaCl) and sodium azide (NaN_3) were used as analytical grade reagents and antimicrobial agents. All experiments were carried out in triplicate unless specified otherwise.

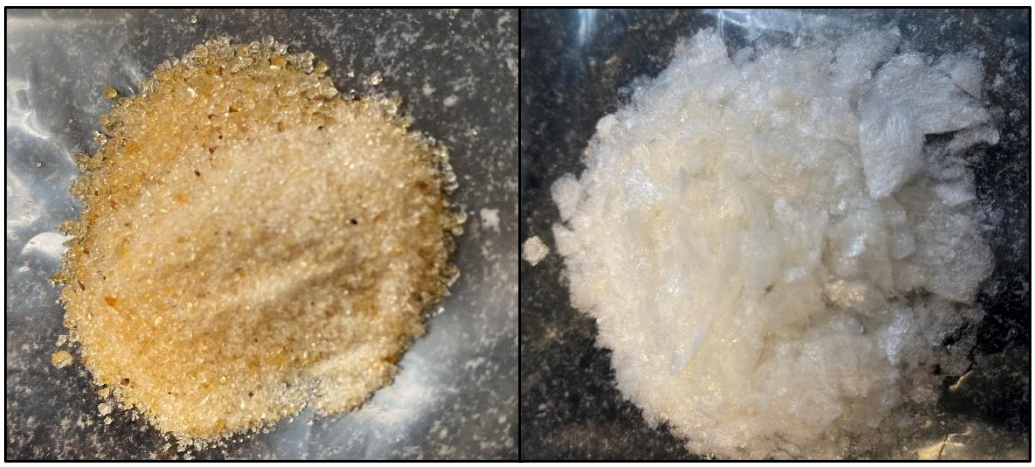


Figure 2. Visual appearance of crude puka gum (left) and purified puka gum (right).

3.3 Chemical Composition Analysis

The composition of CPG and PPG was determined by AOAC methods. Moisture was determined following oven-drying (AOAC 925.10) at 130 °C for 1 hour. Ash was determined after combustion (AOAC 942.05). Crude protein was determined by measuring total nitrogen and converted to protein content by multiplying by 6.25 (AOAC 968.06). Fat was measured by acid hydrolysis method described in AOAC 922.06. Samples were hydrolysed by adding ethanol and HCl and were heated at 70 – 80 °C for 30 - 40 min. A Mojonnier flask was used to extract fat with combination of ethyl and petroleum ether. The extracts were evaporated on a steam bath, re-dissolved, and filtered over a column of sodium sulphate. The filtered extracts were then dried in a 100 °C oven to obtain constant weight. Total carbohydrate was estimated by difference. Total dietary fibre was determined using AOAC 991.43 (Product number of kit used; Megazyme Ltd., Ireland). Protein and starch of the samples were removed by sequential enzymatic digestion using heat stable enzyme of α-amylase, protease and amyloglicosidase. Soluble dietary fibre containing in the digested samples were then precipitate with alcohol. The residue was filtered, washed with 78% ethanol, 95% ethanol and acetone; dried and weighed. Uronic acid content was estimated by the *m*-hydroxydiphenyl method using glucuronic acid as the standard (Blumenkrantz and Asboe-Hansen, 1973). Mineral profile was determined by and Inductively Coupled Plasma method. The samples were given plasma energy by ionizing argon gas in a torch tube. The samples were fully decomposed into exited constituent elements and released emission rays. The content of each element was determined based on the intensity of the rays while the position of the photon rays determine the element type (Cherevko & Mayrhofer, 2018).

3.3.1 Constituent sugar composition

Constituent sugar compositions of CPG and PPG were determined by high-performance anion-exchange chromatography (HPAEC) after hydrolysis of the polysaccharides to their component monosaccharides (Wee, Matia-Merino, Carnachan, Sims, & Goh, 2014). Briefly, samples were hydrolysed in duplicate with methanolic HCl (3 N, 80 °C, 18 h) followed by trifluoroacetic acid (TFA, 2.5 M, 120 °C, 1 h) to their component monosaccharides. The resulting hydrolysates were analysed on a CarboPac PA-1 (4 x 250 mm) column equilibrated in 20 mM NaOH and eluted with a simultaneous gradient of NaOH and NaOAc at a flow rate of 1 mL min^{-1}. The sugars were identified from their elution times relative to a standard sugar mix, quantified from response calibration curves of each sugar and converted to relative mol% anhydrosugar.

3.4 Preparation of emulsions

PG solutions were prepared by hydrating freeze-dried gum using an overhead mixer for about 5 minutes. For a complete hydration, the solution was left overnight at room temperature under continuous stirring at 2,000 rpm, as shown in Figure 3. Emulsions containing a known amount of PG were prepared by heating soybean oil (15% w/w) and the PG solution in a water bath for 20 minutes at 60 ˚C. The mixture was then pre-homogenised using a Silverson mixer at 9,500 rpm for 1 minute. This coarse emulsion was then immediately homogenised in a two-stage high-pressure homogeniser with four passes at 350/80 bar. The final fresh emulsion was collected, sodium azide (0.02% v/v) added as a preservative and analysed by the methods described in following sections.

In order to determine the effect of gum concentration, formulations of emulsions containing a series of PG concentrations (1-10% w/w), as shown in Table 2 were used following the same method indicated above. In order to evaluate the effect of pH, 4% w/w PG-stabilised emulsions were prepared and the pH was adjusted between pH 1 to pH 9 on the emulsions by using a solution of 0.1-1 M NaOH or 0.1-1 M HCl. The pH adjustment was done before the emulsification process, following a method by Hosseini-Parvar, Osano, and Matia-Merino (2016).

Figure 3. Overnight hydration of freeze-dried PG with overhead mixer under continuous stirring.

2% v/v Sodium dodecyl sulfate (SDS) was added to the emulsions in order to confirm the origin of the big particle sizes after one-month stored emulsions at 20 ºC. In terms of the thermostability test, freshly prepared emulsions were heated in a water bath at 80 ˚C for 30 minutes under four different pH conditions: at native (pH 5), a slightly higher than native (pH 6.5), at extremely acidic conditions (pH 2), and at alkaline conditions (pH 8.5) to investigate the resistance of the emulsions towards thermal treatment. All the emulsions with different treatments were stored at 20 ˚C for one month in order to evaluate emulsion stability.

Table 2. Emulsion formulation.

Type of PG Emulsion	Concentration of PG Solution in the Final Emulsion (% w/w)	Oil Concentration in the Final Emulsion (% w/w)
CPG emulsions	1	15
	2	
	3	
	4	
	6	
	8	
	10	
PPG emulsions	1	15
	2	
	3	
	4	
	6	
	8	
	10	

3.5 Droplet size measurements

Droplet size and particle size distribution measurements of the PG-stabilised O/W emulsions were determined using the Mastersizer 2000 Hydro MU (Malvern Instruments Ltd., Worcestershire, UK) based on laser light scattering technique, as shown in Figure 4. In this technique, two different wavelengths (λ) of monochromatic lights are passed through the measurement cell; then the light is scattered by the emulsion droplets in the low and high range of particle sizes, ranging from 0.02-2,000 μm (McClements, 2016). The intensity of the scattered light is measured as a function of the scattering angle. The particle size distribution is then calculated to find the optimum size between experimental measurements and the predictions using light scattering theory (Mie theory). The refractive index of water as a dispersant and soybean oil used were 1.33 and 1.456, respectively. The

particle size distribution was measured on fresh emulsions and on the following days for 30 days on the emulsions stored at 20 ˚C.

The measurements were expressed as surface area mean diameter or Sauter-average diameter (d_{32}) and volume mean diameter (d_{43}) at a laser obscuration between 7-9%. The equations are expressed as follows:

$$d_{32} = \sum \frac{n_i d_i^3}{n_i d_i^2} \qquad \text{Equation 1. Surface area mean diameter}$$

$$d_{43} = \sum \frac{n_i d_i^4}{n_i d_i^3} \qquad \text{Equation 2. Volume mean diameter}$$

where n_i is the number of droplets or particles and d_i is diameter of the droplets or particles. The d_{32} value refers to the sphere of the equivalent area to the droplets, which is used for observing the changes in the average area of the droplet surfaces. Meanwhile, the d_{43} value refers to the sphere of equivalent volume and is sensitive to the appearance of large droplets or aggregates and therefore is normally used to talk about coalescence.

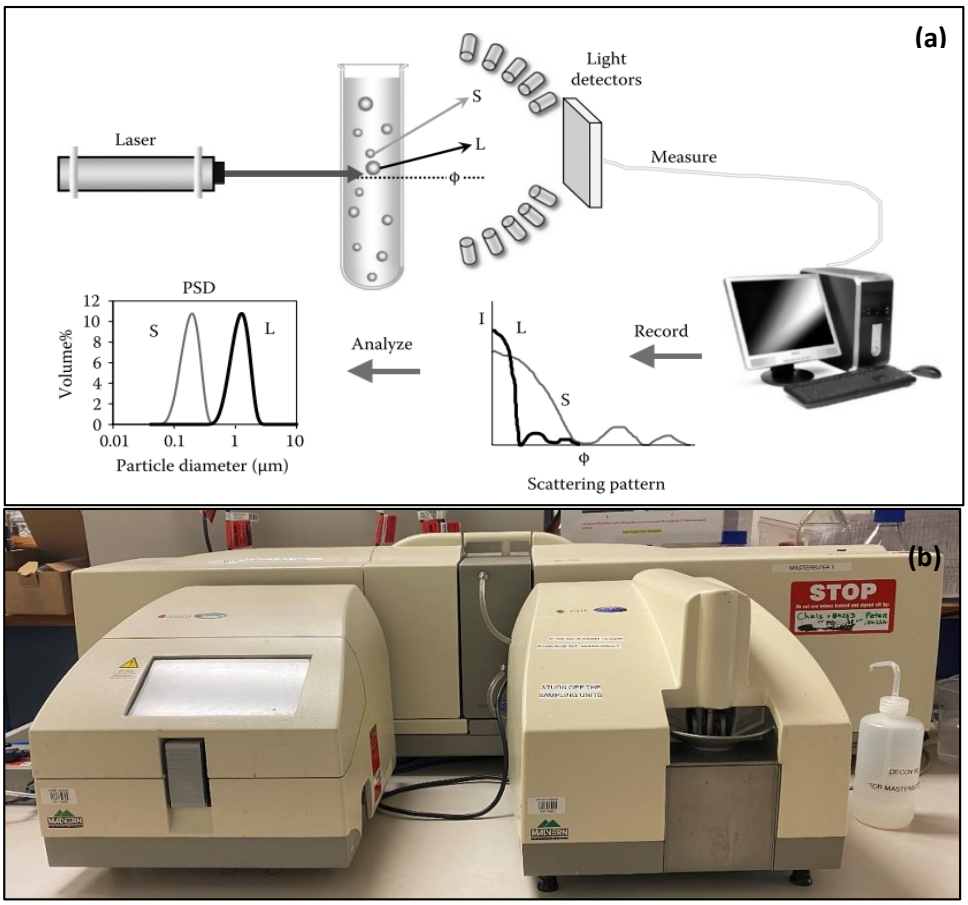


Figure 4. (a) Illustration of light scattering technique (McClements, 2004) and (b) The Mastersizer 2000 Hydro MU.

3.6 Zeta Potential Measurement

The zeta-potential of the samples was measured using the Malvern Zetasizer Nano ZS instrument (Malvern Instruments Ltd., Worcestershire, UK), as shown in Figure 5. The pH of emulsions was first determined and then diluted 75 times prior to measurements. The samples were injected into an electrophoresis cell (DTS 1060C, Malvern Instrument Ltd., UK) and any air bubbles were carefully removed before inserting a stopper. The refractive index of water used was 1.33.

The zeta potential value is converted using the Henry equation before the measurement of the ratio between velocity and electrophoretic mobility (μ_e), as follows (ISO 13099-2, 2012):

$$\mu_e = \frac{2\varepsilon\zeta f(k\alpha)}{3\eta} \quad \text{Equation 3. Henry equation}$$

ε is the dielectric constant, η is the viscosity of the medium, and f(kα) is "the Henry function", where α is the radius of the particle and k is known as the Debye parameter (ASTM, 2018).

In order to investigate the effect of pH and heat on the properties of the emulsions, a series of pH's were adjusted in the prepared emulsions and were measured by the Zetasizer. The values were reported as the average of two sample measurements with at least three readings for each sample.

Figure 5. Zetasizer Nano ZS, (Malvern Instruments, UK).

3.7 Rheological Measurement

Rheological measurements of PG-stabilised emulsions were carried out on an MCR 301 Rheometer (Anton Paar Physica, Germany) equipped with a cylindrical geometry (CC 27), as shown in

Figure *6*. The range of shear rates (0.01-1000 s^{-1}) and temperature (20 ˚C) were chosen to measure apparent viscosity of the emulsions, while viscoelastic properties expressed by storage modulus (G') and loss modulus (G'') were determined at 0.5% strain and frequencies from 0.01-10 Hz. Data were recorded using the Rheoplus software (Anton Paar, Germany).

Rheology properties, including viscosity and gel-like properties, perform a significant role in a variety of food products. Resistance to flow describes the viscosity of a liquid, expressed as the following equation:

$$\eta = \frac{shear\ rate}{shear\ stress}\ (Pa.s) \text{ ———————} \text{ Equation 4. Viscosity}$$

The flow behaviour of liquids can be classified as Newtonian and non-Newtonian. In a Newtonian type, viscosity is independent of shear rate, while the viscosity of non-Newtonian fluids shows a high dependency on the amount of applied shear rates. McClements (2016) stated that apparent viscosity refers to the viscosity at a definite applied shear rate. Furthermore, non-Newtonian fluid types generally exhibit shear-thinning behaviour.

Figure 6. MCR 301 rheometer (Paar Physica, Germany).

In terms of the food emulsion systems, a stable fluid emulsion generally performs as a Newtonian fluid, but if it slightly shows flocculation, the emulsion tends to exhibit a shear-thinning behaviour. This behaviour is also likely to happen because of the rheological properties provided by the non-absorbed gum at a particular range of gum concentrations.

3.8 Visual Phase Separation

Phase separation was investigated by visually observing the emulsion through a 15 mL tightly sealed clear glass tube. The tubes were stored at room temperature for a month without any disturbance. The physical separation of emulsions was monitored, and a photo was captured on day 0 and 30^{th}. The separated layers of emulsion were measured to determine the emulsion stability over time. All samples were freshly prepared and made at least twice before carrying out measurements.

3.9 Light Microscopy

The microstructure of emulsions was visualized by a compound light microscopy (CHA, Olympus, Japan) that is equipped with a camera. A drop of undyed emulsion sample was placed on the microscope slide and visualized at 10, 40, and 100x magnification.

CHAPTER 4 EMULSIFYING PROPERTIES OF PUKA GUM: EFFECT OF SHEAR RATE AND GUM CONCENTRATION

4.1 Introduction

In food emulsion systems, most polysaccharides are known to be used as texture and viscosity modifiers. However, the presence of small protein fractions in a number of plant polysaccharides are believed to be responsible for their emulsifying properties, which makes them effective surface-active agents (Funami *et al.*, 2007; Osano, Hosseini-Parvar, Matia-Merino, & Golding, 2014; Xu, Wang, Fu, Huang, & Zhang, 2018). Besides gum arabic, many scientists have paid attentions to the surface-active properties of other hydrocolloids, which showed the ability to form stable O/W emulsions. Those hydrocolloids that have been reported on earlier studies are galactomannans (Bai, Huan, Li, & McClements, 2017; Chanamai & McClements, 2002; Chivero, Gohtani, Yoshii, & Nakamura, 2016; Xiang *et al.*, 2015), pectins (Nakauma *et al.*, 2008; Schmidt, Schütz, & Schuchmann, 2017; Verkempinck *et al.*, 2018; Zhang *et al.*, 2015), basil seed gum (Gahruie, Eskandari, Khalesi, Van der Meeren, & Hosseini, 2020; Hosseini-Parvar, Osano, & Matia-Merino, 2016; Naji-Tabasi & Razavi, 2017; Osano, *et al.*, 2014), corn fibre gum (Bai *et al.*, 2017; Yadav, Johnston, Hotchkiss, & Hicks, 2007; Yadav, Moreau, Hotchkiss, & Hicks, 2012), soybean soluble polysaccharide (Jin *et al.*, 2017; Nakauma *et al.*, 2008), fenugreek gum (Mathur, 2012; Sav, Meer, Fule, & Amin, 2013), and octenyl succinic anhydride starch (OSA-starch) (Chivero *et al.*, 2016; Nilsson & Bergenståhl, 2007; Tesch, Gerhards, & Schubert, 2002).

Puka gum, which was exudated from the wounded trunks of *Meryta sinclairii* tree, has the potential of having similar emulsification properties with gum arabic due to their similarity of molecular structure and physicochemical properties (Wee *et al*., 2019). Therefore, this study aimed to evaluate the effect of puka gum (PG) (crude and purified) concentration on its emulsification properties in terms of droplet size distribution, rheological properties, zeta potential, microstructure of emulsions and visual phase separation. Different concentrations of PG (1-10% w/w) were used to stabilize 15% soybean O/W emulsions. Due to the importance of rheological properties of gums to determine the desired viscosity in the final product and its performance (pouring and swallowing), the effect of constant shear rate and time on the apparent viscosity of PG solutions was also tested.

4.2 Results and Discussion

4.2.1 Chemical composition of PG

Table 3 shows the chemical composition of CPG and PPG. The protein contents of CPG and PPG (5.02% and 2.65%, respectively) were in general agreement with that obtained previously by quantitative amino-acid analysis where PG contained 2% w/w protein (Sims and Furneaux, 2003). The higher apparent protein content of CPG may indicate the presence of low molecular weight protein that was removed by dialysis or may reflect the presence of non-proteinaceaous nitrogenous material detected by the total nitrogen assay. The monosaccharide compositions of CPG and PPG were similar to that reported previously, and differences in the molar proportions of the sugars are probably due to differences in the methods used (high-performance anion exchange chromatography in the current study vs. gas chromatography of carboxyl-reduced gum; Sims and Furneaux, 2003).

Table 3. Chemical composition of PG.

Composition*	Unit	Puka Gum	
		Crude	Pure
Ash	%	4.15	3.55
Protein	%	5.02	2.65
Fat	%	1.40	1.21
Total Carbohydrate	%	72.61	82.34
Minerals			
Calcium	g/100g	2.03	2.01
Magnesium	g/100g	0.09	0.09
Potassium	g/100g	0.55	0.39
Sodium	g/100g	0.08	0.06
Phosphorus	g/100g	0.01	<0.002
Chloride	g/100g	<3.3	7.72
Iron	mg/kg	0.47	1.76
Copper	mg/kg	0.02	0.52
Iodine	mg/kg	92.87	90.96
Manganese	mg/kg	<0.02	<0.02
Selenium	mg/kg	59.00	56.78
Zinc	mg/kg	0.02	<0.010
Molar proportion of monosaccharide			
Rhamnose		8.8	6.4
Arabinose		30.6	28.4
Galactose	mol%	41.9	46.3
4-O-Me-GlcA		9.3	6.1
Glucuronic Acid		9.5	12.9

*Calculated on a dry weight basis (%db).

4.2.2 Effect of Shear Rate on PG Solution Rheology

At 4% gum concentration, PG solutions exhibited shear-thinning flow behaviour, as shown in Figure 7 with the viscosity decreasing with shear, especially above $10s^{-1}$. Shear-thinning behaviour is frequently exhibited by many other gum solutions above a critical concentration, such as GA (Gómez-Díaz, Navaza, & Quintáns-Riveiro, 2008; Mothé & Rao, 1999), basil seed gum (Hosseini-Parvar, Matia-Merino, Goh, Razavi, & Mortazavi, 2010), *Dioscorea opposita Thunb* (Ma *et al.*, 2020) and cashew gum (Mothé & Rao, 1999) and it is related to the stretching and alignment of the polymer

chains with the flow. However, the thinning behaviour that occurred in PG was not as pronounced as other polysaccharides, such as corn fiber gum, gum arabic and soy soluble polysaccharide (Jin *et al*., 2017), brea gum (Castel, Rubiolo, & Carrara, 2017), gum tragacanth (Farzi, Emam-Djomeh, & Mohammadifar, 2013) and persian gum (Golkar *et al*., 2018). This may be because the gum itself showed a more-like Newtonian behaviour up to shear rate 50 s^{-1}. Furthermore, shear-thinning behaviour of PG solution appeared when the shear rates were above 100 s^{-1}.

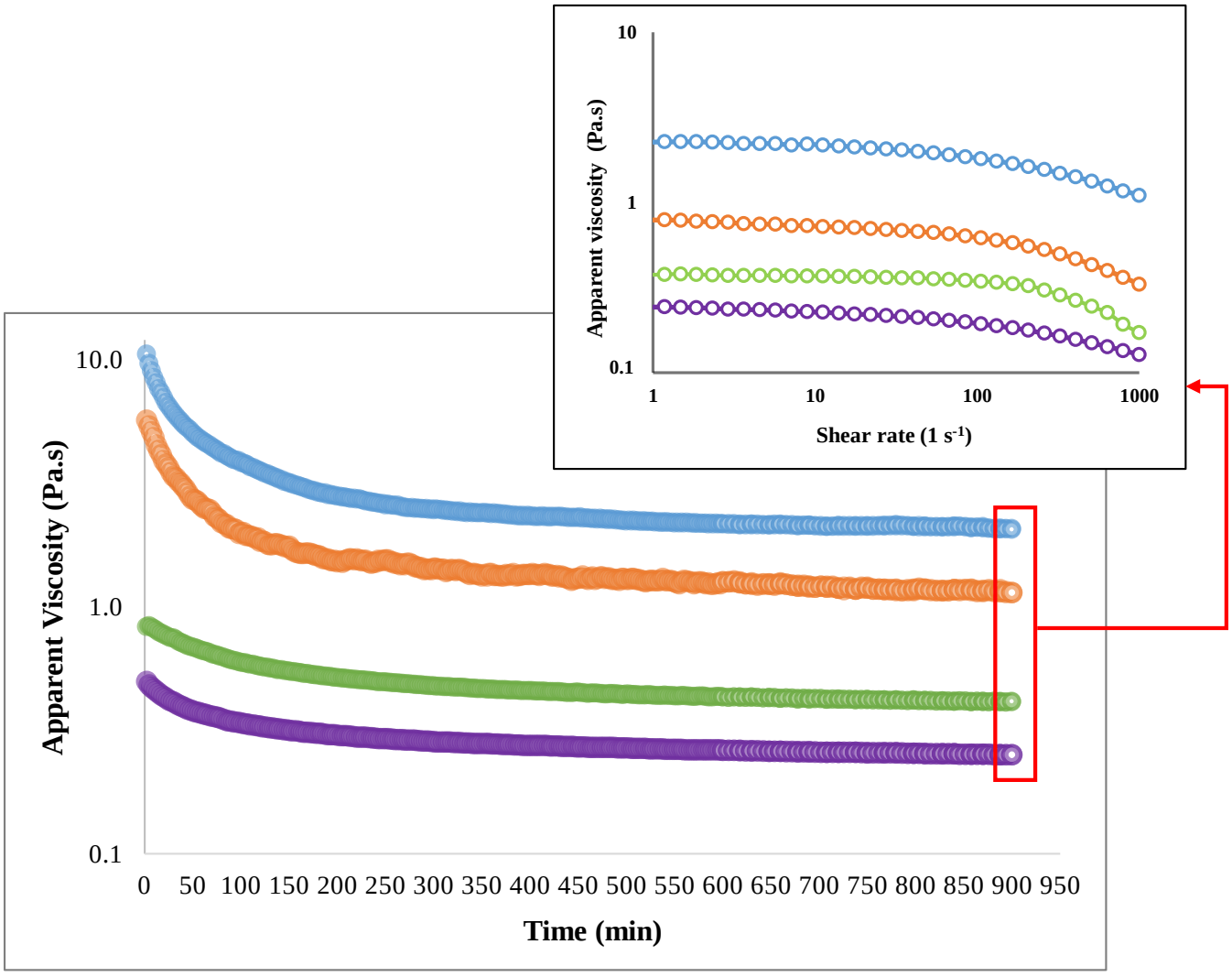


Figure 7. Effect of time at various steady shear rates on the viscosity of 4% PG solution (= 1s^{-1} : = 10 s^{-1} ; = *100* s^{-1} ; = *500* s^{-1}). After stirring overnight (900 min), the PG solutions were subjected to various shear rates (1-1000 s^{-1}).

The effect of time and shear on the viscosity of the 4% w/w PG solutions was investigated in a control manner as shown in Figure 7. When constant shear rates were applied over a period of time, the PG solution exhibited shear-thinning behaviour with a tendency to reach almost Newtonian plateau after a given time, indicating the sensitivity of the gum to shear. Shear-thinning time dependent behaviour dominated the apparent viscosity of PG solutions during the first 400 min at low shear rate (1 s^{-1} and 10 s^{-1}). A decrease in viscosity was also confirmed after the PG solutions were stirred overnight for complete hydration before measurements. It was noticed that the visual viscosity of the solution had decreased considerably after the overnight stirring (as opposed to the usual viscosity enhancement with the hydration of many polysaccharides), as shown in Figure 8.

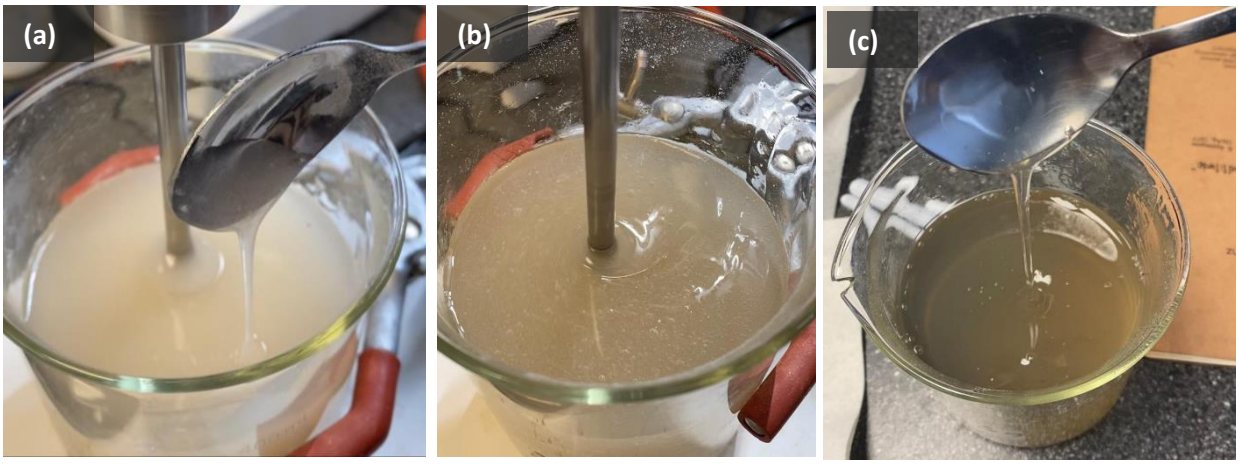


Figure 8. Visual appearances of 10% PPG solution after (a) 2 min, (b) 40 min and (c) overnight stirring at 2,000 rpm using head mixer.

Unlike many polysaccharides, which have semi-flexible or flexible random coil type conformation, such as carrageenan and galactomannans like guar gum (Wee *et al.*, 2019), PG is highly branched, giving a compact, more spherical conformation, closer to the structure of GA (Nie, Wang, Cui, Wang, Xie, & Phillips, 2013) and has a less distinct shear-thinning behaviour (Tirtaatmadja, Dunstan, & Boger, 2001). Furthermore, it is interesting to note that the viscosity loss that happened under shear was observed to be irreversible as shown after applying high shear rate (800 s^{-1}) for 300 min and a rest time of 5 hours (Figure 9). The viscosity reduction may be associated to a change in the chain length, occurring under shear as shown in Figure 10. This is as opposed to a typical shear-thinning behaviour which is categorised as a temporary viscosity loss as elongated polymers will return to the original configuration once shear forces are removed.

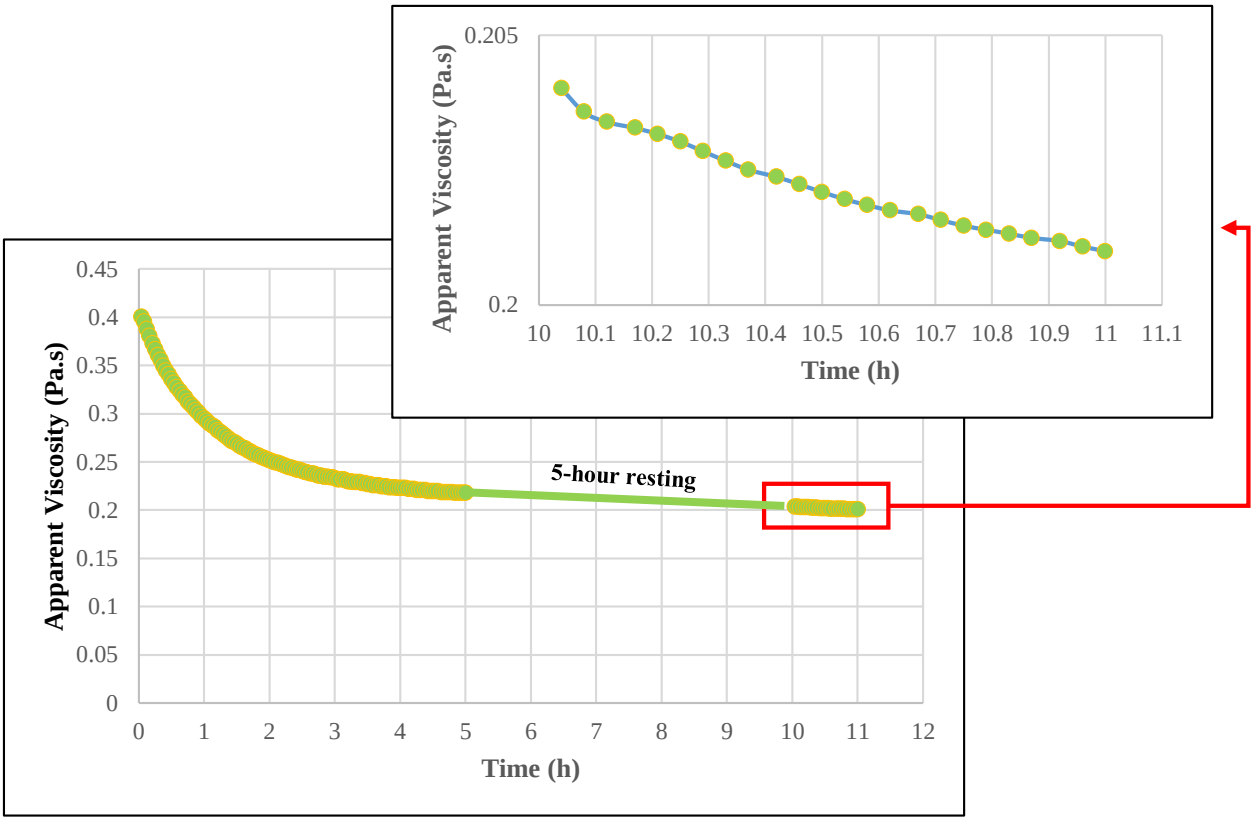


Figure 9. Effect of high steady shear rate (800 s-1) and 5-hour resting on the apparent viscosity of 4% w/w PG solution.

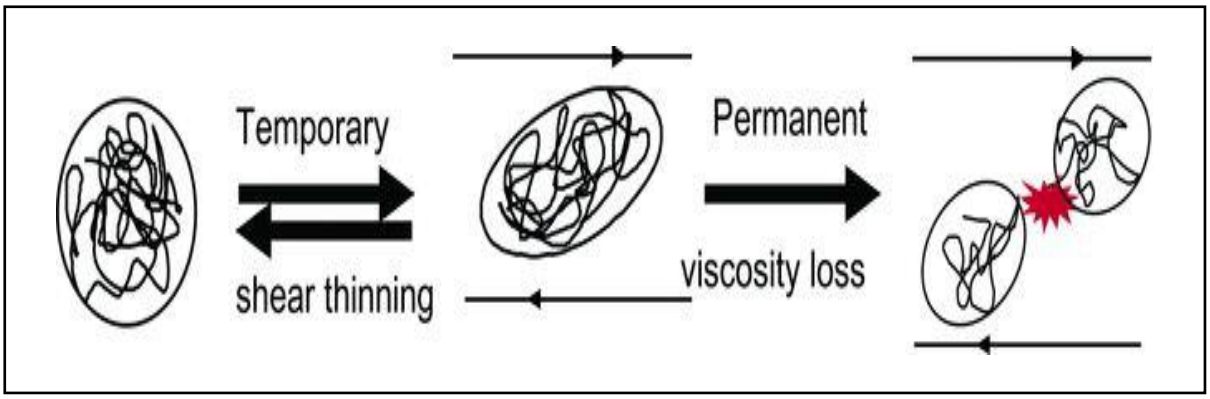


Figure 10. Illustration of shear-thinning and permanent viscosity loss of polymer solution at high shear rate (Holtzinger, Green, Lamb, Atkinson, & Spikes, 2012).

In fact, the mechanical scission of polymer chains, in this case polysaccharides, leads to the irreversible loss of viscosity (Holtzinger *et al.*, 2012; Michael *et al.*, 2018). Some of the important factors affecting the permanent viscosity loss include an intense and prolonged high shear rate, molecular weight reduction, entanglement of polymer chains at high polymer concentration, which

can lead to chain breakage and length of the polymer backbone (Bueche, 1960; Holtzinger *et al*., 2012; Wang, Ye, & Zhu, 2007).

Studies on the effect of shear rate on these solutions are key to determine the desired viscosity in the final product and its performance when pouring or swallowing. High shear-thinning behaviour of polysaccharides has been associated with a low degree of sliminess in the mouth, since early sixties (Szczesniak & Farkas, 1962). It is now clear that a high degree of shear-thinning behaviour also provides thinner consistency during swallowing and ease of pumping of liquid foods (Vardhanabhuti and Ikeda, 2006). Irreversible viscosity loss occurring in hydrocolloids should be taken into account as this may affect the food processing and its final products. Furthermore, this rheological property may generally be considered undesirable for some types of food and beverage products as it may result in fluid systems falling below its designated viscosity. Unfortunately, references regarding the loss of viscosity of gums due to shear is very limited. Therefore, more studies about this topic should be conducted in the future.

4.2.3 Emulsifying Properties of PG

4.2.3.1 Particle Size Distribution of PG

The particle size distribution of PG-stabilised emulsions, as a function of gum concentration, is shown in Figure 11 and the mean average sizes d_{43} and d_{32} are shown in Figure 12. The average droplet size of the emulsions decreased with increasing gum concentration (from 1% to 10% w/w). At 1% w/w gum concentration, a bimodal particle size distribution was observed where one of the peaks fell in the range of the small droplet size range (0.1-10 µm). However, monomodal particle size distributions were observed for both CPG and PPG at ≥4% w/w gum concentration with average droplet sizes (d_{32}) below 2 µm for PPG at 4% w/w PG. Whereas the d_{43} values did not show significant differences between the two gum powders above 2% w/w, the d_{32} values—representing how small the droplets could get—were significantly smaller for CPG below 4% w/w gum, but significantly bigger above 4% w/w gum compared to the means obtained with PPG (Figure 12).

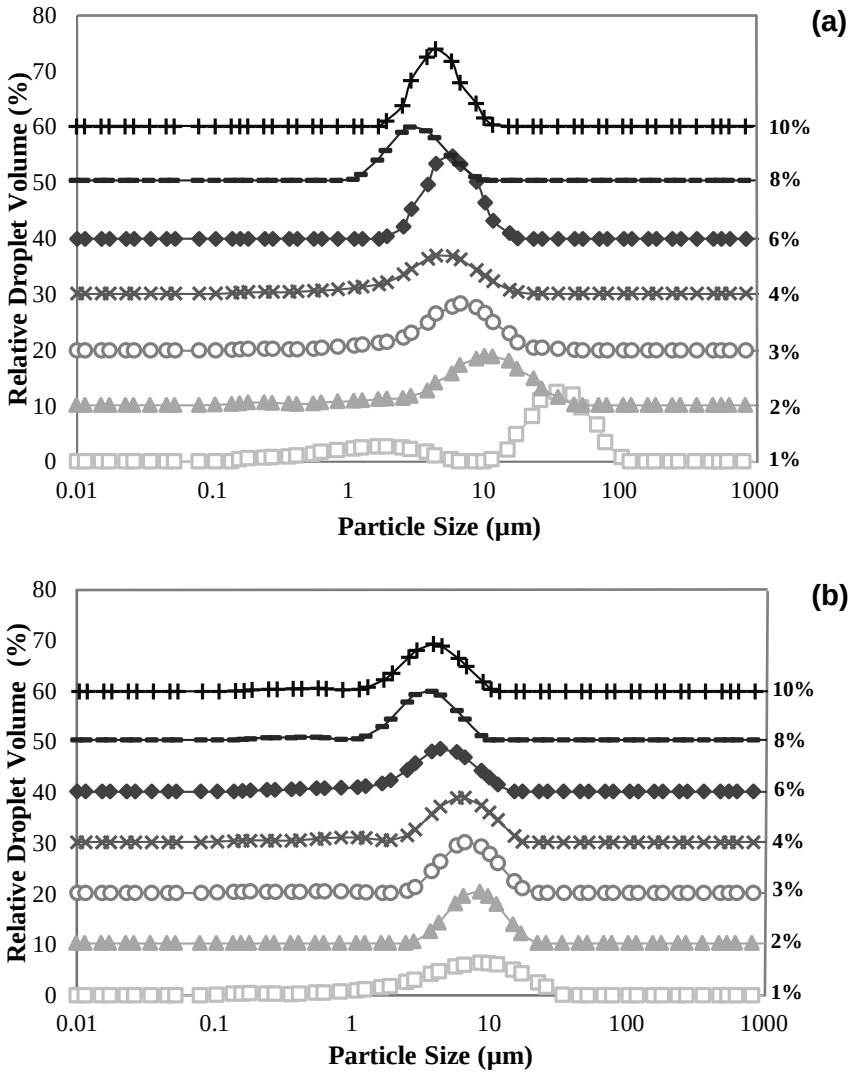


Figure 11. Effect of (a) CPG and (b) PPG concentration on the particle size distribution of 15% soybean oil-in-water emulsions (□ = 1% PG, ▲ = 2% PG, o = 3% PG, x = 4% PG, ♦ = 6% PG, ▬ = 8% PG, ✚ = 10% PG ; light grey to dark black colour = 1 to 10% PG).

Bimodal particle size distributions observed in emulsions at low gum concentration (1% w/w) can be explained by two possible phenomena: (i) an incomplete coverage of the oil-water interface due to an insufficient amount of PG present during the emulsification process leading to re-coalescence and therefore large particle size (Jafari, Assadpoor, He, & Bhandari, 2008; Floury, Legrand, & Desrumaux, 2004; Perrier-Cornet, Marie, & Gervais, 2005) and/or (ii) bridging flocculation, which can occur when a polymeric emulsifier is used below its critical concentration as polymer chains are shared between oil droplets forming an elastic bridge and producing flocs (McClements, 2015). At 4% w/w concentration, PG (both crude and pure) is likely to provide sufficient coverage for the emulsification of 15% w/w soybean oil, leading to emulsions with relatively small and uniform size

droplets, that were stable against flocculation and creaming during the observation period (Section 4.2.6).

At higher gum concentrations (> 4% w/w), the particle size distribution of especially CPG-stabilised emulsions appeared to have shifted towards the bigger size range compared to emulsions stabilized by PPG. An increase in the average particle size of emulsions is likely caused by: (i) the presence of insoluble materials in the crude gum (CPG) which could limit the surface-active components in the gum powder inhibiting their adsorption (protein and polysaccharides). The detrimental effect of increasing concentration of a crude extract in the droplet size achieved when making emulsions has also been shown by James (2020), who produced emulsions with lower average droplet size (d_{32} =~1.5 µm) when using 0.50% w/w basil seed gum flour as compared to higher gum concentrations. Also when comparing crude versus purified polysaccharides, a monomodal size distribution and lower average particle sizes were achieved when emulsions were prepared with purified soy soluble polysaccharides as compared to the crude soy polysaccharide (Nakamura, Yoshida, Maeda, Furuta, & Corredig, 2004) in agreement with the present case; (ii) another possibility that can cause the average droplet size to increase is the excess of the non-adsorbed polysaccharide which can increase the viscosity of the aqueous phase, decreasing the efficiency of the homogenization process, thus, hindering the formation of smaller droplets. This is in agreement with previous results observed by Osano *et al*. (2014), who showed that the particle distribution of emulsions prepared with more than 0.30% w/w basil seed gum resulted in bigger average droplet size. However, in contrast to all the findings above, Castel, Rubiolo, and Carrara (2017) have reported that the smaller average droplet size (529.6 nm) was achieved by the highest concentration of brea gum at 20% w/w. These differences could be due to the various factors; (1) the oil percentage used in emulsions, (2) the concentration of emulsifier used to produce a saturation coverage of the fresh interface formed and (3) the rate of droplet coalescence. The effect of having an excess of unadsorbed polysaccharide on emulsion stabilization is discussed in Section 4.2.6.

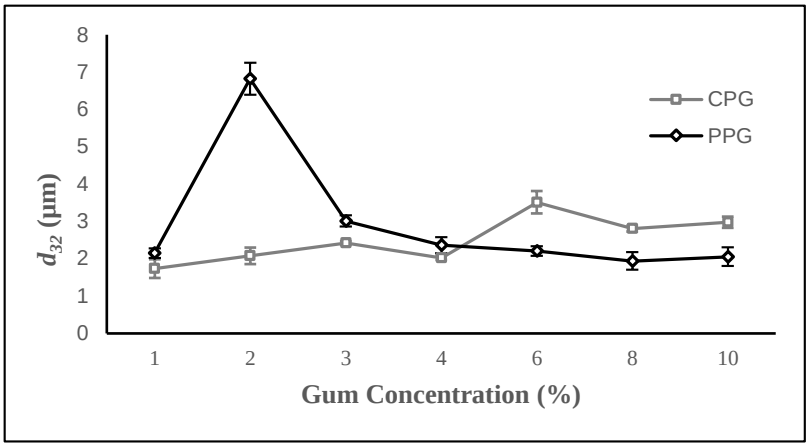


Figure 12. Effect of CPG and PPG concentration on the average particle size (d_{32}) of 15% soybean oil-in-water emulsions (□ = CPG and ♦ = PPG).

4.2.4 Zeta Potential of PG Emulsions

The zeta potential of PG-stabilised emulsions as a function of concentration is shown in Figure 13. In general, both PPG- and CPG-stabilised emulsions were negatively charged with values ranging between -32 mV and -45 mV with no significant changes detected between the crude or the purified gum. In its native condition (~pH 5), PG-stabilised emulsions appeared to be negatively charged regardless of the gum concentration. The highest values *ca.* -45 mV at low gum concentration (1% w/w) compared to an average of *ca.* -35 mV for emulsions at greater gum content, can be related to the ionic strength of the medium which is likely to increase with further addition of the gum with the subsequent increase in mineral content. The fact that both gum powders crude and purified yielded similar zeta potential values may be a reflection of the mineral content being quite similar (Table 3). It has been shown that the zeta potential of gums, *i.e.* tamarind gum, become more negative with increasing purity (Crispín-Isidro *et al.*, 2019) when minerals are in fact removed during the processing, which is not necessary our case.

Physicochemical, organoleptic and nutritional properties of food emulsions are affected by the positive or negative charges of the droplets (McClements, 2015). Droplets having sufficiently large zeta potential values (≥30 mV) are prone to repel each other leading to emulsion stability against flocculation by electrostatic forces. Low zeta potential values have been shown to lead to aggregation and flocculation for emulsions stabilized by gums such as mesquite (Acedo-Carrillo *et al.*, 2006). In our case, stable emulsions (no physical phase-separation observed) were created by either PPG or CPG having the emulsions, zeta potential values within the range of -30 to -35 mV– high enough for electrostatic stabilization. These values agree with those found by Wee *et al.* (2019) where zeta potential of PG itself was stated to be comparable to GA; ~ -35 mV and ~ -30 mV, respectively.

In general, polysaccharides with zeta potential values of approximately -35 mV are considered as relatively weak polyelectrolytes as compared to more acidic polysaccharides such as alginate and pectin which are even more negatively charged, with values of *ca.* -50 mV (Bengoechea, Jones, Guerrero, & McClements, 2011). Overall, the charges on the droplet surface here will be derived not only from the ionizable functional groups on the polysaccharide backbone (hydroxyl, methyl, sulfate, sulphuric ester, phosphate, carbonyl, carboxyl group) but from the proteins present in the gum, which charges strongly dependent on pH (adjusted to ~5.0 here) and their isoelectric point. Finally, mineral ions and other charged substances present in the extract may alter the overall electrical charge of the emulsion by adsorbing onto the oil-water interface.

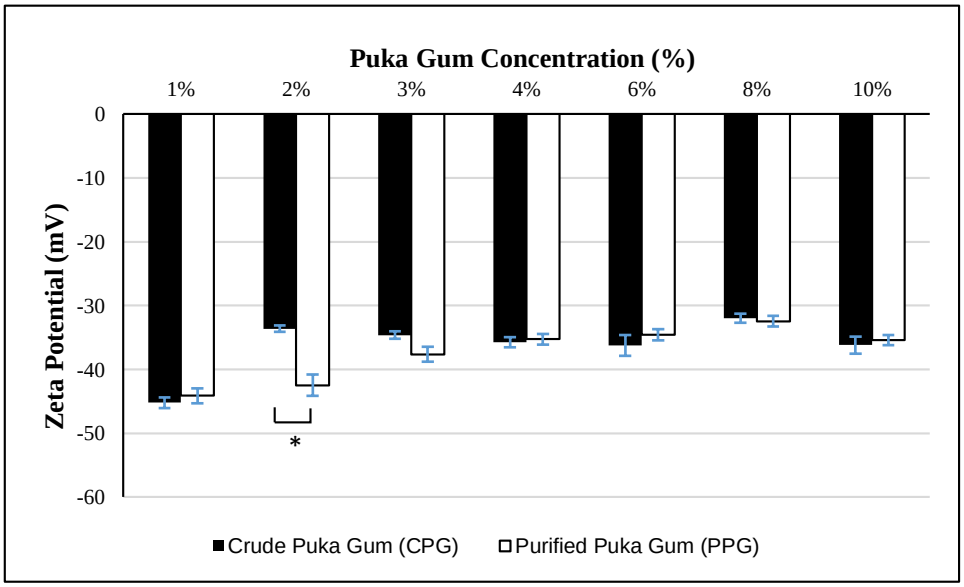


Figure 13. Effect of CPG (black bars) and PPG (white bars) concentration on the zeta potential value of 15% soybean O/W emulsions. The graph represents the means ± SEMs. Tukey HSD test was used for statistical comparison between CPG and PPG at the same gum con concentration, where significantly different (P-values < 0.05) are denoted by an asterisk (*).

4.2.5 Rheological Properties of Puka Gum Emulsions

The effect of CPG and PPG concentration on the apparent viscosity of the emulsions is illustrated in Figure 14. The emulsions showed a gradual increase in viscosity with increasing gum concentration, achieving the highest values for both CPG and PPG at 10% w/w gum producing emulsions of viscosities ~366 and ~217 mPa.s, respectively at 1-10 s^{-1} shear rate. Based on Stoke's law, high viscosity of the continuous phase is likely to provide emulsion physical stabilization against phase separation as confirmed by the absence of creaming during storage for emulsions prepared with PG above 4% w/w concentration (> 30 mPas at 1 s^{-1}).

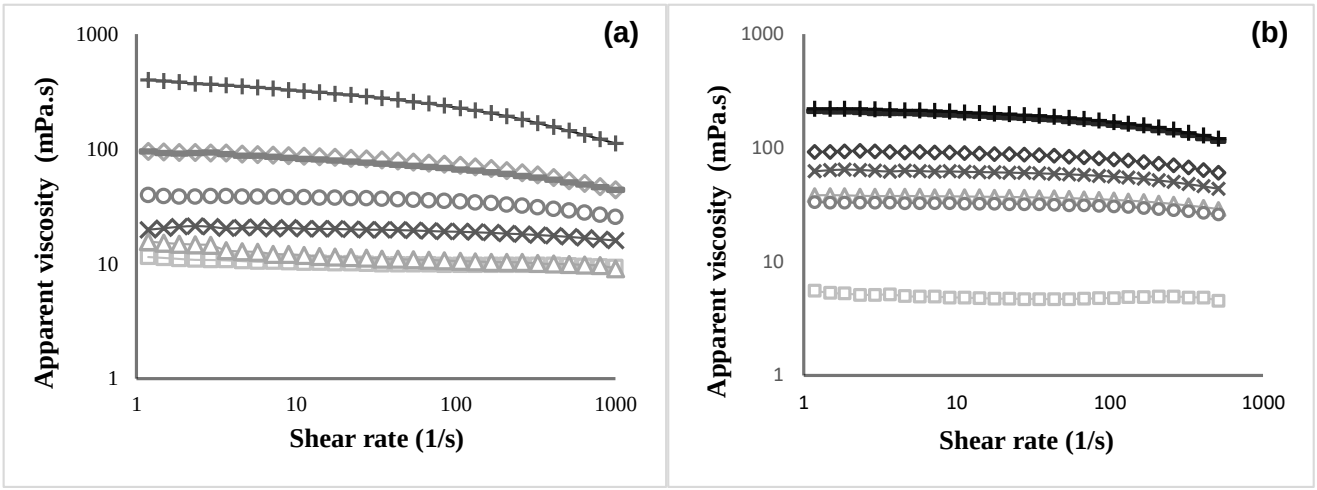
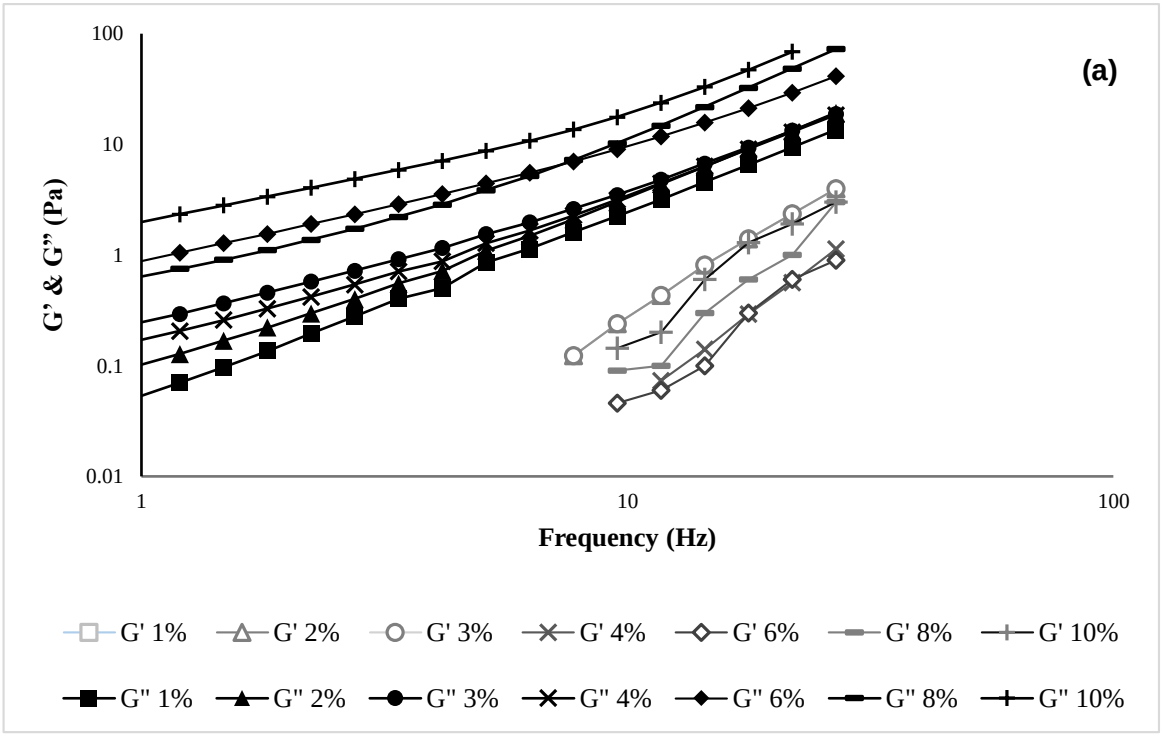


Figure 14. Effect of (a) CPG and (b) PPG concentration on the apparent viscosity of 15% soybean oil-in-water emulsions made with various concentration of puka gum ((□ = 1% PG, ▲ = 2% PG, o = 3% PG, ✖ = 4% PG, ◇ = 6% PG, ▬ = 8% PG, ✚ = 10% PG ; light grey to dark black colour = 1 to 10% PG).



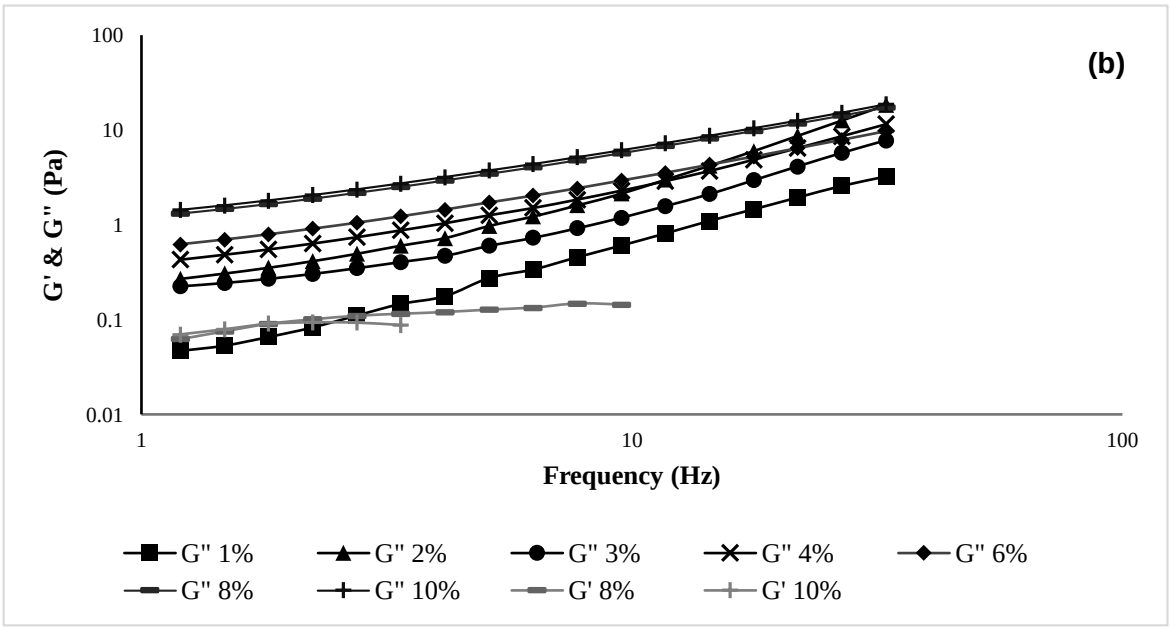


Figure 15. Effect of (a) CPG and (b) PPG concentration on the loss modulus (*G″*) and the storage modulus (*G′*) of 15% soybean oil-in-water emulsions; *G′*= grey colour and *G″*= black colour.

As opposed to the shear thinning behaviour observed for only PG solutions, especially above 10 s^{-1} (Figure 7), the emulsions prepared with CPG and PPG showed more of a Newtonian behaviour at low PG concentrations (≤4% w/w), with the shear-thinning becoming slightly more pronounced above 100 s^{-1} and especially at the highest gum content of 10% w/w. Generally, a stable fluid

emulsion performs as a Newtonian fluid but if flocculation occurs, the emulsion may exhibit a shear-thinning behaviour. This behaviour is also likely to happen because of the rheological properties provided by the non-adsorbed gum at a range of gum concentrations.

Even though the degree of droplet flocculation in an emulsion (by bridging or depletion mechanisms) contributes to an increase in viscosity and to a shear-thinning behaviour (McClements, 2016), a major contribution in the present case, is the systematic increase of various components with increasing gum powder; components such as non-adsorbed PG, protein and fibres, are possibly responsible for the increase in viscosity, controlling also the shear thinning behaviour (normally associated to droplet dissociation, the stretching of polymers and alignment of particles with the flow), which in this case starts to be a bit more obvious at higher PG concentrations (>4% w/w). Pseudoplastic behaviour observed with increasing gum concentration in solutions and in emulsion systems has been shown for other gums such as basil seed gum (Hosseini-Parvar *et al.*, 2010).

Moreover, the lack of a strong interaction or connectivity between droplets and/or non-adsorbed material in the continuous phase is demonstrated by the lack of elasticity developed in the emulsions. As shown in Figure 15, at all PG concentrations, the loss modulus (G'') was higher than the storage modulus (G')—the latter one, not being even detected at <8% w/w PPG—indicating that the liquid-like behaviour dominated the rheology of the PG-stabilised emulsions observed at all frequencies. In general, there was an increase in the magnitude of both G' and G'' of CPG and PPG emulsions with gum content and frequency, indicating the dependency towards frequency, typical of more liquid-like systems. As stated by Rao (2010), when $G''>G'$, the energy used to deform the material is dissipated viscously; thus, the material behaves like a liquid. This has also been shown for GA-soy protein emulsions at high concentrations which behave as viscoelastic liquids ($G'' > G'$) (Salazar-Montoya, Jiménez-Avalos, & Ramos-Ramírez, 2012).

4.2.6 Emulsion Stability

Figure 16 presents the phase separation of emulsions containing <4% w/w of either CPG or PPG after 1-month storage at 20 ˚C, indicated by the creaming layer. The microstructure of the emulsions at all gum concentrations is shown in Figure 17 and Figure 18 with the corresponding particle size distribution for both CPG and PPG-stabilised emulsions. An improved physical stability was exhibited in emulsions with more than 4% w/w PG concentration, which correlates well with the reduction in droplet size observed, as big oil droplets (>10 μm) were confirmed under light microscopy at <4% w/w gum concentration. As discussed above, bridging and coalescence are likely to occur below the saturation point (Figure 17 and Figure 18 at 2% and 3% PG) and stability (or full coverage at the interface) is likely to happen at >4% PG. The quality of the images obtained at high

gum content, especially when using the crude gum (Figure 17 and Figure 18, 6-10% CPG), decreased significantly, probably due to the increasing non-adsorbed material in the continuous phase which interfered with the contrast in the images. Overall, the results confirm that the adsorption of this gum-containing protein provides long term stability above a critical concentration. Polysaccharides provide strong steric stabilization at the interface and therefore protection against coalescence, flocculation and subsequent creaming (Dickinson & Stainsby, 1988; Garti, 1999; Khouryieh, Puli, Williams, & Aramouni, 2015). In our case, also the electrostatic stabilization seems strong as demonstrated above. Therefore, the long-term stability of the PG emulsions is likely to occur via a combination of factors, such as (i) the steric and electrostatic stabilization provided by enough PG adsorbed at the interface and by (ii) the increase in viscosity of the continuous phase with increasing non-adsorbed polysaccharide surrounding the droplets which can confer enough restriction in droplets movement (Acedo-Carrillo, 2006; Dickinson, 1998; Valdez *et al.*, 2006).

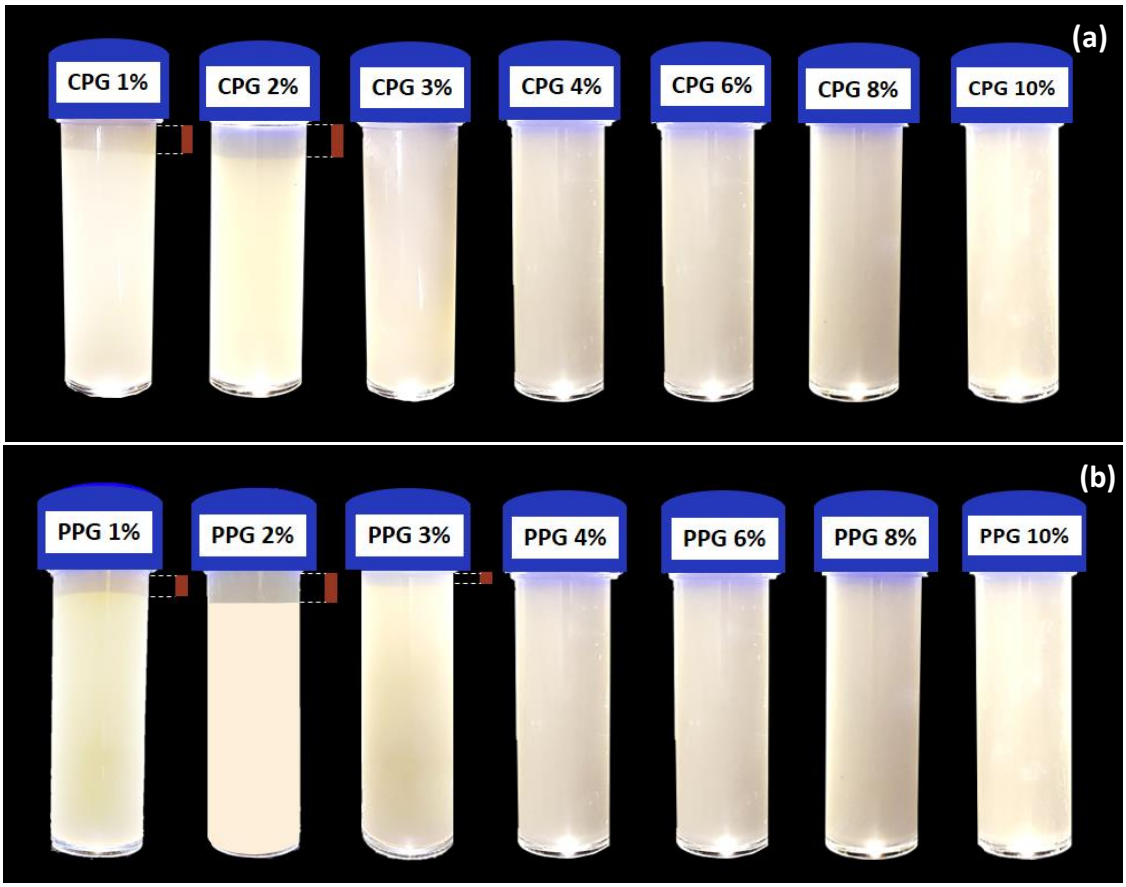


Figure 16. Phase separation of (a) CPG and (b) PPG-stabilised 15% soybean oil-in-water emulsions made with various concentration of puka gum during one-month storage at 20˚C.

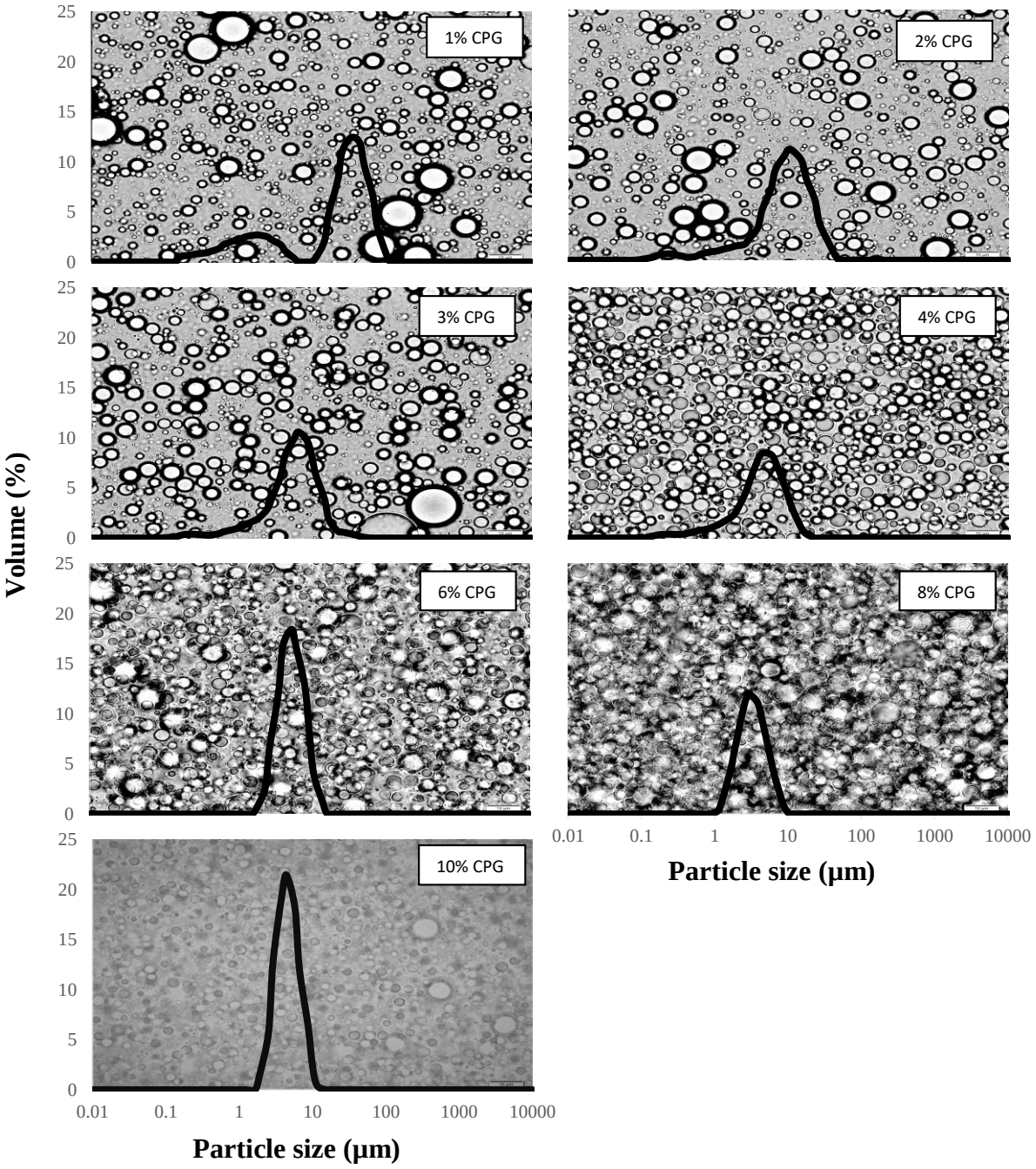


Figure 17. Effect of CPG concentration on the emulsion microstructure of 15% soy oil-in-water emulsions made with various concentration of puka gum; magnification is 100x, scale bar = 10 µm.

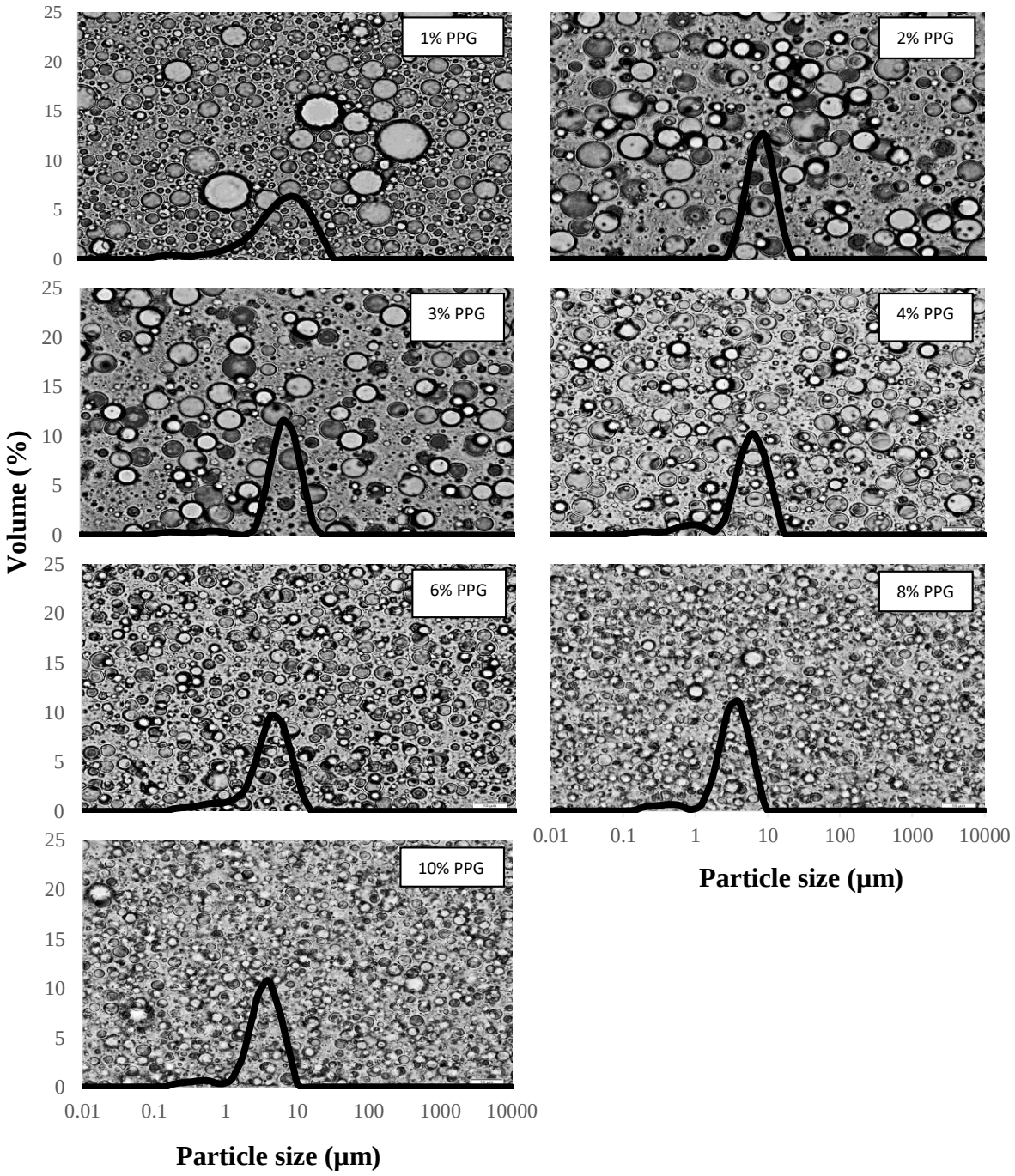


Figure 18. Effect of PPG concentration on the emulsion microstructure of 15% soybean oil-in-water emulsions made with various concentration of puka gum; magnification is 100x, scale bar = 10 µm.

CHAPTER 5 EFFECT OF pH ON THE EMULSIFYING PROPERTIES OF PUKA GUM

5.1 Introduction

This chapter reports the effect of one of the environmental variables — pH, on the emulsifying properties of puka gum (PG). 4% w/w PG-stabilised 15% w/w soybean O/W emulsions were prepared with the methods described in Section 3.4. A set of different pH's, including the native pH of the emulsions (pH 2, pH 3.5, pH 5 [native], pH 6.5 and pH 8.5) were used in the emulsion preparation. The pH adjustment was conducted before the emulsification process of the PG emulsions and followed the method by Hosseini-Parvar *et al*. (2016). The properties of the emulsions were immediately determined by measuring the mean particle size (d_{32}), particle size distribution, the droplet charges (zeta potential) and microstructural characteristics using light microscopy. Rheological properties, including apparent viscosity and viscoelasticity, of freshly prepared emulsions were also determined. Meanwhile, the stability of emulsions was frequently monitored over a period of time by measuring the d_{32} and observing visual phase separation, under storage at 20 ºC for one month. The addition of 2% v/v surfactants, such as sodium dodecyl sulfate (SDS), was added before particle size measurements to distinguish between flocculation or coalescence to conclude the correct cause of particle size growth of the emulsions during storage.

Some studies regarding the effect of various pH conditions on the emulsifying properties of polysaccharides have been previously reported, such as basil seed gum (Hosseini-Parvar, Osano, & Matia-Merino, 2016), gum arabic (Nakauma *et al*., 2008; Qian, Decker, Xiao, & McClements, 2010; Wu, Eskin, Cui, & Pokharel, 2015), citrus pectin (Wu *et al.*, 2015), soybean soluble polysaccharide (Nakauma *et al*., 2008), sugar beet pectin (Nakauma *et al.,* 2008), persian gum (Golkar, Taghavi, & Aghili Dehnavi, 2018) and yellow mustard mucilage (Wu *et al*., 2015). The aim of this study was to investigate the emulsifying properties of PG at various pH conditions, since this has not been done systematically in the literature.

5.2 Results

Altering the pH of PG emulsions could noticeably shift the particles size distribution profiles towards the smaller particle size area, as shown in Figure 19. Results also indicated that the average droplet sizes (d_{32}) of PG emulsions were reduced considerably at the range of the observed pH's. PG emulsions at native pH (~5) and pH 6.5 had similar d_{32} (1.91 and 2.23 µm, respectively). Meanwhile,

a smaller particle size (1.78 µm) of the PG emulsion was obtained at acidic conditions (pH 3.5). However, the greatest reduction of d_{32} compared to native pH (1.91 µm) occurred at the acidic conditions (pH 2 = 0.48 µm) followed by emulsions at more alkaline conditions (pH 8.5 = 1.40 µm).

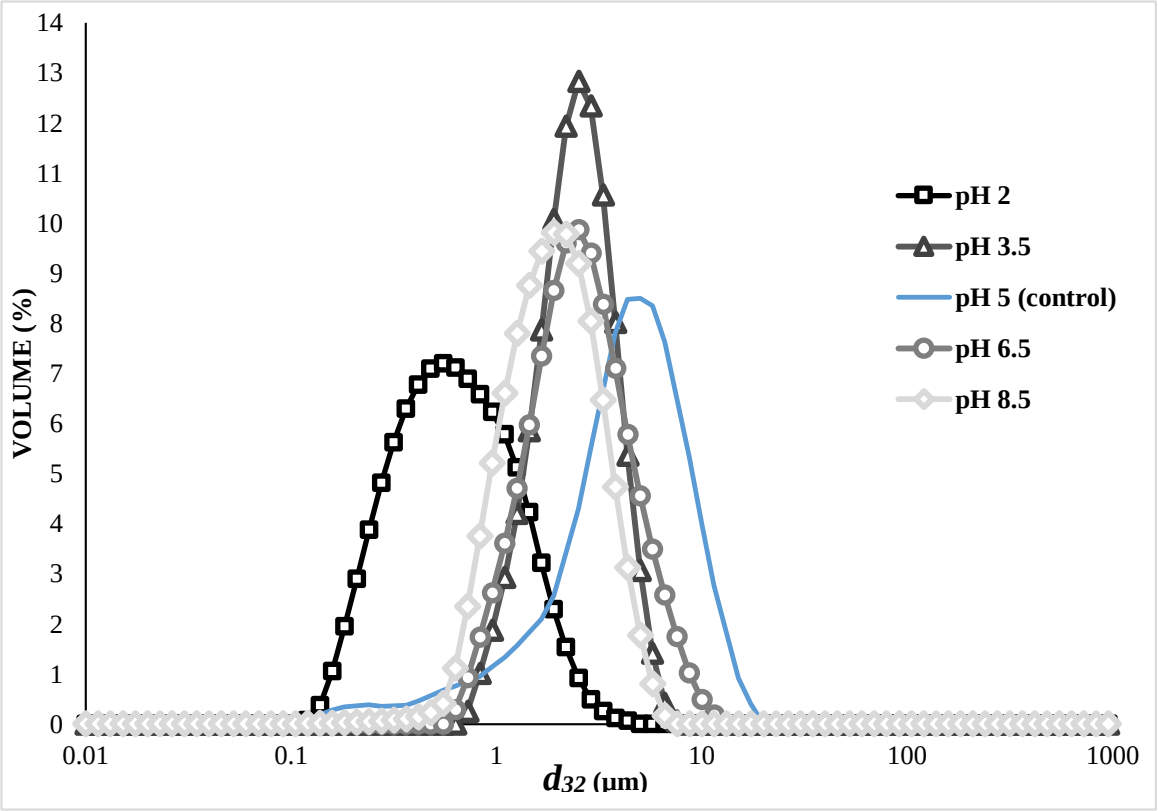


Figure 19. Effect of pH on the particle size distribution of 4% w/w PG-stabilised 15% w/w soybean oil-in-water emulsions at 20°C.

The zeta potential of PG emulsions, as shown in Figure 20, appeared to be negative regardless of the pH environment. Generally, the zeta potential at higher pH's than 3.5, was much less pH-dependent. The highest value of *ca.* -35.25 mV was obtained with the emulsion at native pH (~5), following by *ca.*-29.3 and -29.04 mV at higher pH (pH 6.5 and pH 8.5, respectively). However, there was no significant difference (p>0.05) between pH 6.5 and 8.5 due to the similarity of the zeta potential values of PG emulsions at those conditions. Less negatively charged emulsions were found below the native pH, with values significantly decreased (p<0.05) from -35.35 mV (pH 5) to -23.99 mV at pH 3.5. At pH 2, where a high excess of H^+ ions occurs, there was a higher reduction to *ca.* -4.54 mV. It is likely that the p*K*a of the gum, where the charges on PG are almost negligible is around pH 2 or below.

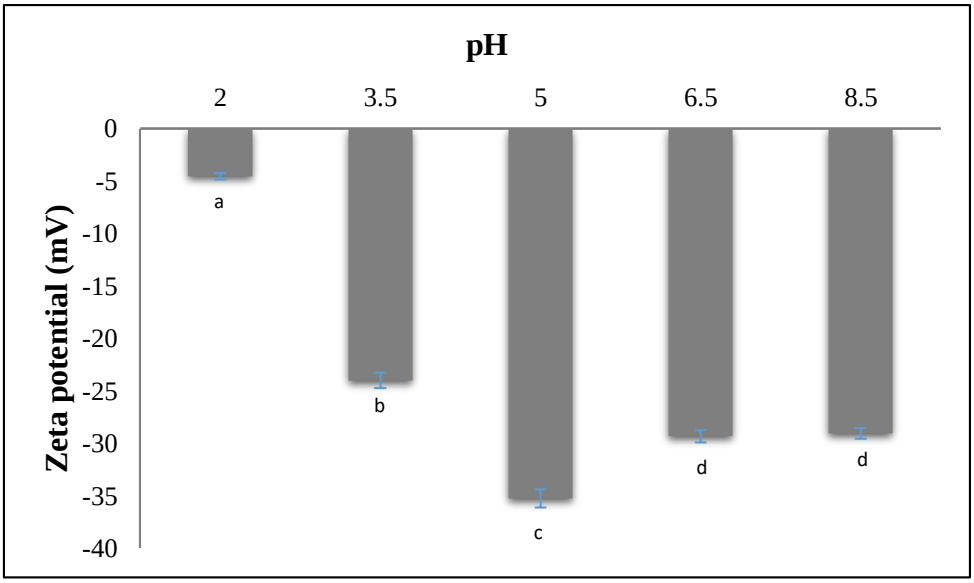


Figure 20. Effect of pH on the zeta potential of 4% w/w PG-stabilised 15% w/w soybean oil-in-water emulsions at 20°C. The graph represents the means ± SEMs. Tukey HSD test was used for statistical comparison between before and after heating treatment at the same puka gum concentration, where significantly different values (P-values < 0.05) are denoted by a different letter.

4% w/w PG-stabilised emulsions exhibited shear-thinning flow behaviour at pH's above 3.5 whereas the flow was very much Newtonian at acidic pH's (< 3.5) as shown in Figure 21a. From a minimum viscosity detected at pH 2 (~6.2 mPa.s), there was a gradual increase in viscosity to a maximum occurring at pH 6.5 (~145 mPas) at 1-10 s^{-1} shear rate. At very alkaline conditions (pH 8.5) the apparent viscosity obtained (~38 mPas) was just below the viscosity at pH 5 (~62 mPas). The effect of pH on the viscoelasticity of PG emulsions is shown in Figure 21b. The storage modulus (G'), which was not even detected at 1 Hz, was consistently lower than the loss modulus (G''). This indicated that the liquid-like behaviour dominated the rheology of PG-stabilised emulsions at all studied pHs. The trends shown for the loss modulus mimicked the viscosity behaviour obtaining the highest G'' values at pH 6.5 and minimum values at pH 2.0. This viscoelastic modulus was highly dependent on the frequency with values converging at frequencies above 30 Hz at all pHs studied.

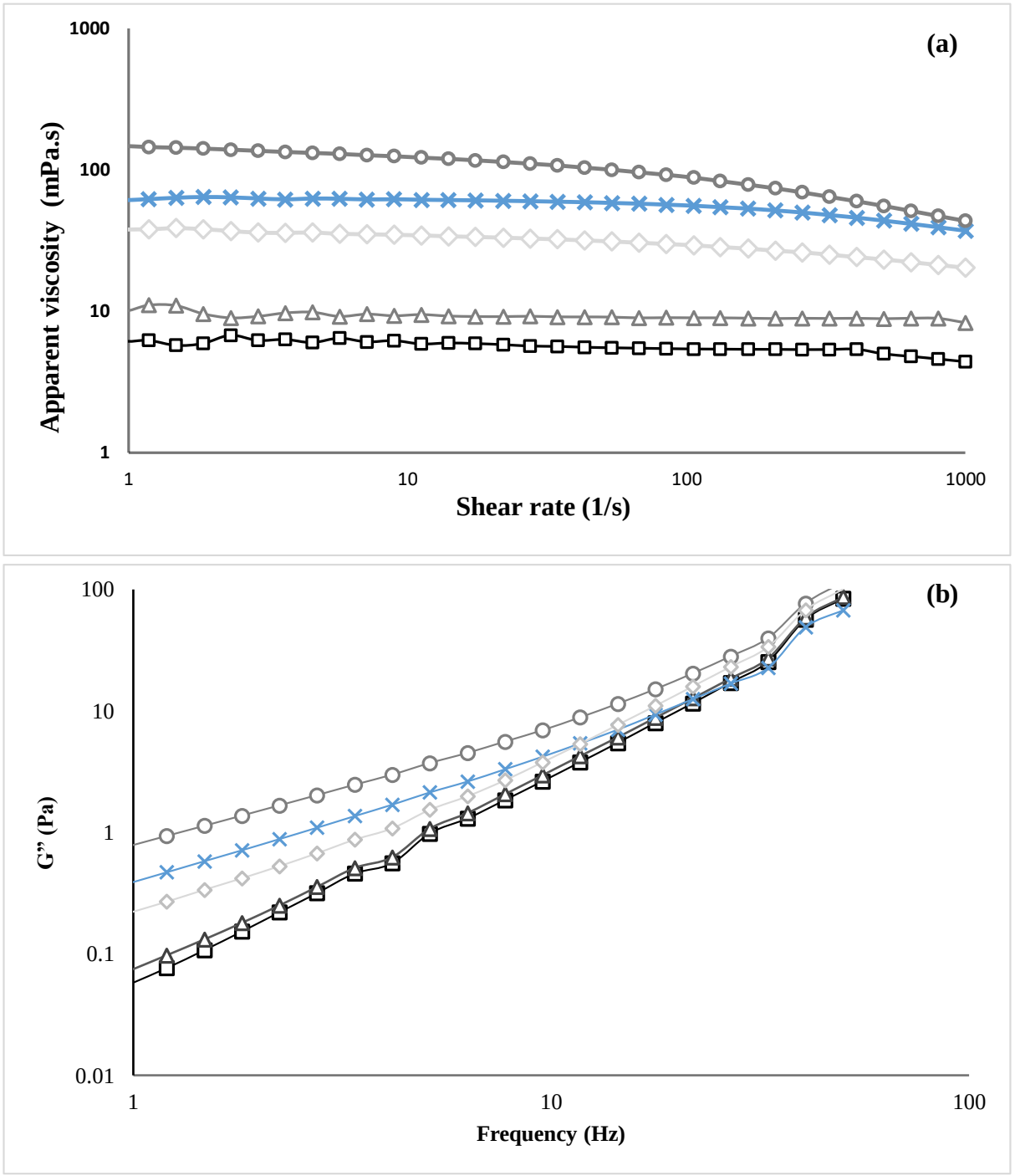


Figure 21. Effect of pH on: (a) the apparent viscosity and (b) loss modulus (*G"*) of 4% w/w PG-stabilised 15% w/w soybean oil-in-water emulsions (= pH 2 ; = pH 3.5 ; = pH 5 (native pH) ; = pH 6.5 ; = pH 8.5).

The microstructure of the 4% w/w PG emulsions at all pH's is shown in Figure 22 with the corresponding particle size distribution. The modification of pH on the 4% w/w PG-stabilised emulsions resulted in a reduction in droplet size and a shift of particle size distribution to the smaller size range compared to the control emulsion at native pH (~5). Some degree of droplet flocculation

or aggregation was detected around native pH (3.5 >pH≤ 6.5) as confirmed by light microscopy. Even though at native pH the emulsions had bigger droplet sizes, than at the other pH conditions, the droplets were still below 20 µm, as shown in Figure 22. Therefore, the microstructure of the PG emulsions was observed to be strongly pH dependant; when the pH was outside the native pH range (either at very acidic conditions such as pH 2 or very alkaline, such as at pH 8.5) the conditions were more favourable to obtain smaller droplet sizes. The addition of SDS showed this flocculation detected strongly at pH 5.0 was mainly a bridging flocculation phenomenon.

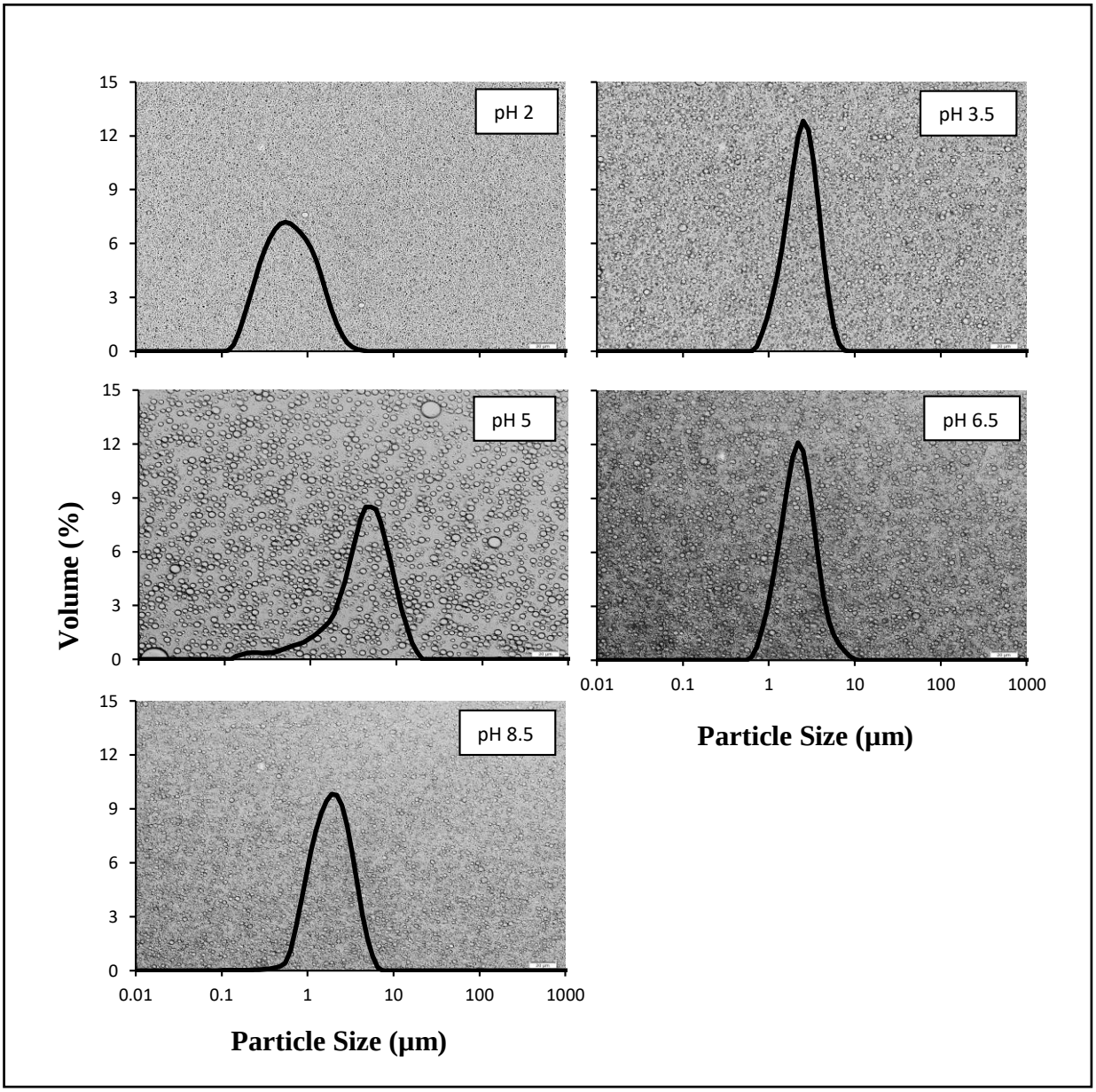


Figure 22. Effect of pH on the emulsion microstructure of 15% soy oil-in-water emulsions made with 4% w/w puka gum; magnification is 40x, scale bar = 20 µm.

The stability of 4% (w/w) PG emulsions during storage, as a result of the effect of pH, was evaluated in terms of the droplet size growth (d_{32}) and visual phase separation. Generally, the average droplet size of emulsions increased slightly towards bigger sizes (2 – 2.5 µm) during 7-day storage, except for emulsions at extreme pH conditions (pH 2 and 8.5), which remained constant throughout the period observed (Figure 23). After 7 days of storage, the droplet size was noticeably larger in emulsions at around native pH (5 – 6.5) from *ca.* 1.90 and 2.24 µm for the freshly prepared emulsion to *ca.* 2.36 and 2.53 µm after one-week storage, respectively. In order to investigate the cause of the increase in droplet size, 2% v/v sodium dodecyl sulfate (SDS) was added to the stored emulsions at pH 6.5 just before measurements. Figure 24 shows the presence of an excess amount of 2% (v/v) SDS in the stored emulsion at pH 6.5 changed the particle size distribution to the smaller size range thus smaller droplet sizes were obtained. This means that bridging flocculation is more likely to be the cause of growth in droplet size as small molecule surfactants, in this case, SDS, when added to the emulsion, displace the adsorbed proteins and polysaccharides, then eventually alter the particle size of the stored emulsions to the former sizes when the fresh emulsion was prepared (day 0). Meanwhile, big droplet sizes associated with coalescence would occur when no changes in the average droplet size with SDS addition, were observed. Visual phase separation of all the PG-stabilised emulsions at the pH range studied here, indicated by a creaming layer, was not been detected during 30 days of storage at 20°C, as shown in Figure 25.

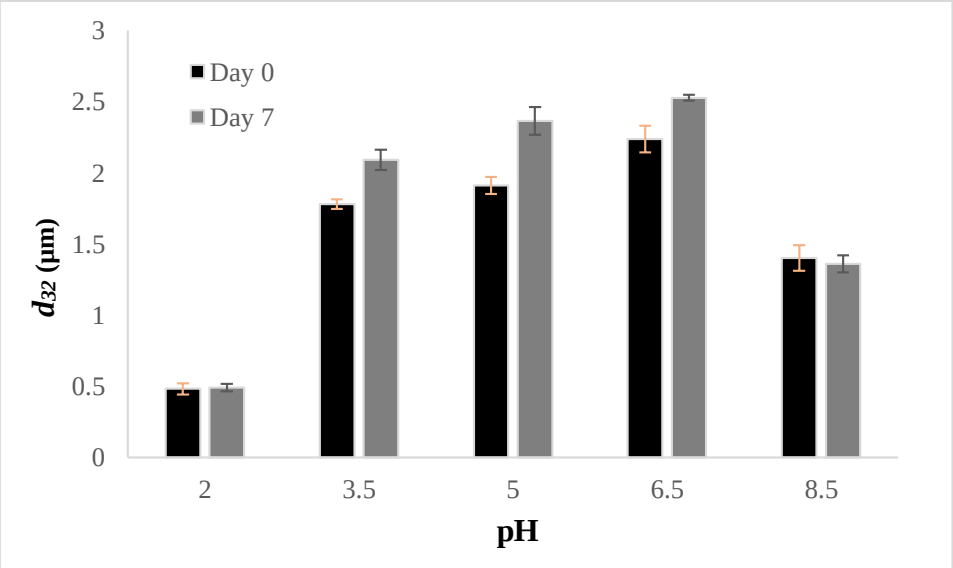


Figure 23. Effect of various pH on the average particle size (d_{32}) of 4% w/w PG-stabilised 15% w/w soybean oil-in-water emulsions at 20°C during 7-day storage. BH = before heating; AH=after heating.

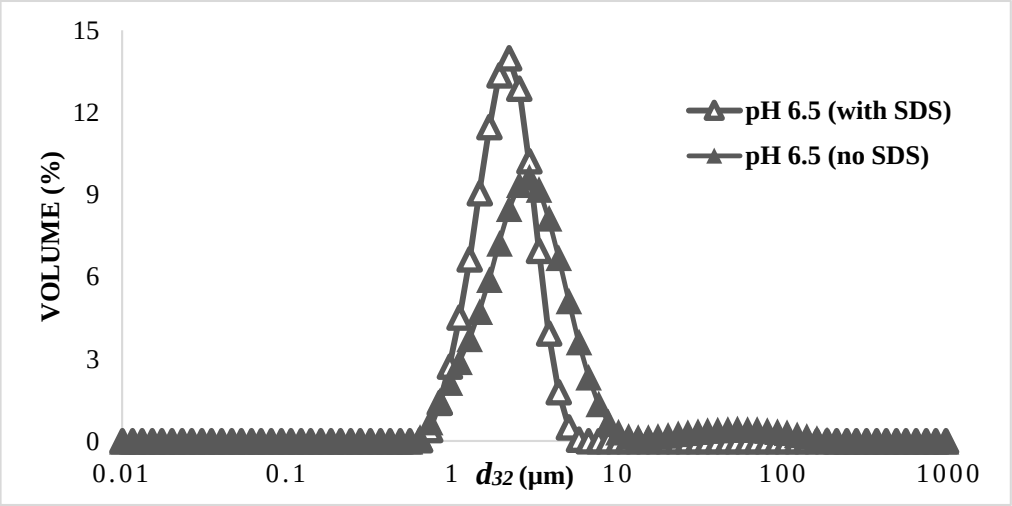


Figure 24. Effect of the addition of 2% v/v SDS on the average particle size (d_{32}) of 4% w/w PG-stabilised 15% (w/w) soybean oil-in-water emulsions at pH 6.5 at one-month.

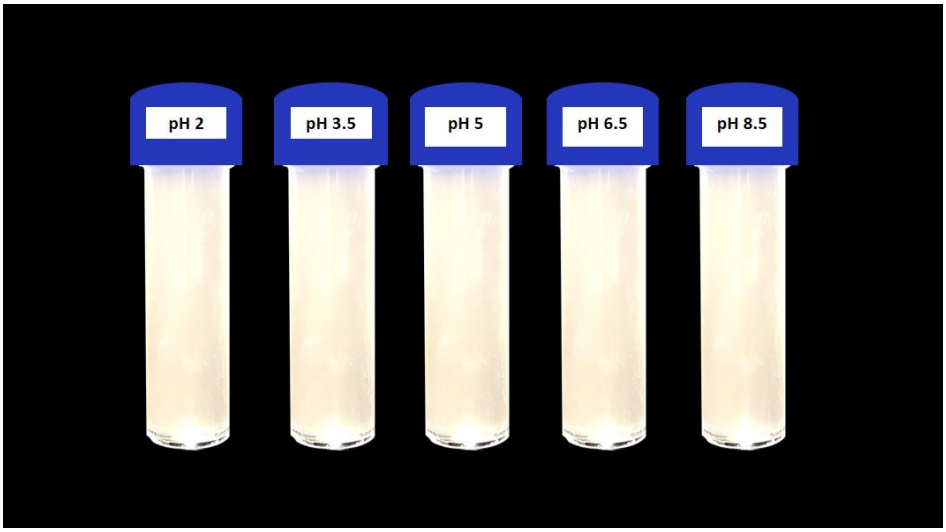


Figure 25. Phase separation profile of 4% w/w PG-stabilised 15% w/w soybean oil-in-water emulsions treated with heating at various pH conditions during one-month storage.

5.3 Discussion

The emulsifying properties of PG, based on the average particle size and particle size distributions obtained, were sensitive to pH changes. This sensitivity meant that the pH changes, from native pH (~5) to lower or higher pH conditions, generally altered the particle size of the emulsions towards smaller sizes at pHs outside this pH 5 range. It is an interesting fact that the particle size (d_{32}) of emulsions stabilized by PG was rapidly decreased when the pH was dropped (<pH 3.5) even though the zeta potential values also decreased dramatically close to zero (especially at pH 2). These results

are not in agreement with other gums like those studied by Hosseini-Parvar *et al*. (2016), where the authors stated that altering the pH of basil seed gum affected the ability of the functional components to adsorb at the interface which resulted in the presence of initially larger droplet sizes at acidic pH's. It seems in the case of PG, acidification improved the emulsification ability of the gum with the associated protein. In the literature, other gums with improved emulsifying capacity at low pH are *Ulva fasciata* polysaccharide, gum arabic and soybean soluble polysaccharide, (Nakauma *et al*., 2008; Niu *et al*., 2016; Shao, Zhu, & Jin, 2017) and this effect has been attributed to no significant changes in its particle size distribution, easily adsorption on to the oil droplet interface and enhanced emulsifying stability, respectively, at low pH.

The huge decrease in zeta potential values, from pH 5 to pH 2 in the current PG, is more likely to be caused by an overall emulsion droplet charge reduction as the pH gets close to the p*Ka* of the gum as stated by Wee, Sims, Goh, and Matia-Merino (2019). However, the less negative value of zeta potential at pH 2 (-4.5 mV) did not necessarily decrease the stability of the PG emulsions. The most likely reason is due to the lowest mean particle sizes obtained at this acidic pH, as mentioned above, which would slow down any creaming purely based on size (droplets in the submicron region). Additionally, it also likely that the stability of PG O/W emulsions towards aggregation is mainly based not on electrostatic but on steric stabilization, making the system more insensitive to pH changes. It is curious the fact that the emulsion at pH 5 had the greatest zeta potential values (-35.35 mV) however, it appeared the most aggregated emulsion. This could indicate that the gum and protein contained in this PG crude powder get adsorbed at the interface providing charges, but some bridging between droplets may have resulted in flocculation. The acidification of the gum seems to improve this, probably due to some disassociation of gum or/and protein that allows better and more uniform adsorption of species occurring at acidic pH. Certainly, the polysaccharide was probably in a more aggregated state before emulsification thus bigger particle sizes were initially formed at these pH's ($3.5 < pH \leq 6.5$).

The addition of SDS to the emulsions, confirmed the presence of bridging flocculation in the emulsions formed, since this breaking agent could disaggregate partially the droplets. Depletion flocculation was also likely to contribute to the emulsion flocculation given the presence of non-adsorbed polysaccharides in the system at these concentrations. These non-adsorbed polysaccharides surround the droplets and favour an attractive depletion force between droplets as a result of an osmotic driving force in the system (McClements, 2016). More work is underway to bring more insight into the molecular changes of the gum occurring under acidification. Regardless of the aggregation state of the emulsions, we can still state that above pH 3.5, the high negative charge of

PG –stabilised emulsions around *ca.* -30 – (-35) mV would correspond to good electrostatic stability. Wee *et al*. (2014) reported that as puka-gum is an arabinogalactan-protein, at the native pH (~5), the carboxylate groups of the proteins and the gum i.e. $–COO^-$ would be in deprotonated form, contributing to electrostatic repulsion.

On the other, under alkaline conditions (pH 8.5), the d_{32} of emulsion stabilised by 4% w/w PG decreased to below 1.5 µm being the droplets highly negatively charged (±30 mV) indicating again an electrostatically stable emulsion. This reduction in the droplet size at alkaline pH's may also be associated with the addition of NaOH on the oil phase itself, aiding the emulsification process (Bansal, Chan, McCallough, & Shah, 1978; Marinova *et al.*, 1996). The smaller droplet sizes of the emulsions at very acidic pH and alkaline conditions (d_{32} = 0.48 µm at pH 2 and 1.4 µm at pH 8.5, respectively) are also likely to have an impact on their rheological properties as confirmed in the apparent viscosity of PG-stabilised emulsions. Lower viscosity at shear rate $1s^{-1}$ was detected in emulsions at pH 2 and pH 8.5 (6.2 mPa.s and 38.1 mPa.s, respectively) as compared to the emulsion with the biggest d_{32} (145.2 mPas at pH 6.5). The high emulsion viscosity at pH 6.5 could be related to a greater amount of non-adsorbed gum which could contribute to a greater viscosity of the continuous phase and therefore to the overall emulsion viscosity. The bigger droplet size seems to indicate that the emulsification is less efficient and therefore the balance of adsorbed versus non-adsorbed gum could be altered, even though surface load measurements were not carried out in this study. Nevertheless, the higher viscosities of the emulsions at pH 5 and above, are consistent with the gum being in a more aggregated state. High viscosities may also lead to more inefficient homogenization as this would hinder the formation of smaller droplets, however overall, emulsion stability against creaming is promoted by the high viscosity of an emulsion (Huang, Kakuda, & Cui, 2001; Li *et al*., 2018; McClements, 2016).

Furthermore, all emulsions at pH ≥5.0 exhibited shear-thinning behaviour, and the apparent viscosity of all PG-stabilised emulsions was dependant on pH changes at all shear rates. The highest emulsion viscosities as described at around native pH (~5 to 6.5), are likely to also be associated with the aggregation of these bigger droplets; it is known that aggregated emulsions will exhibit greater viscosities than unflocculated ones of similar composition. Meanwhile, the acidic emulsions having the smallest $d_{32,}$ and showing no signs of aggregation exhibited a Newtonian behaviour and the lowest viscosities at all shear rates. The highest viscosity at pH 7 was also found by Junqueira *et al*. (2019), in emulsions stabilised by mucilage obtained from *Pereskia aculeata* Miller. The authors attributed this to a disordered spatial structure of emulsion droplets which may lead to a high degree of droplet

aggregation thus resulting in bigger droplets and greater viscosity (Junqueira *et al*., 2019; Owens, Griffin, Khouryieh, & Williams, 2018).

The viscoelasticity of the emulsions was also dependant on pH and frequency. The loss modulus (G'') of the emulsions was significantly higher than the storage modulus (G')—the latter one, not being even detected at the whole range of pH—indicating the lack of elasticity formed in the emulsions due to the lack of strong connectivity or interaction between droplets and/or non-adsorbed material in the continuous phase. Overall, the rheological properties of the emulsions are more likely to be dominated by the presence of PG in the continuous phase as the viscosity and viscoelasticity of PG solutions were similar to those of the emulsions (data not shown).

In terms of emulsion stability, the mean particle size (d_{32}) of PG emulsions increased during storage over a period of time. A rapid increase was showed in emulsions at native pH (~5) and a slight increase at 2 < pH < 8.5. A mixture of bridging and depletion flocculation is likely to be the cause of the growth in the droplet sizes over time (as proven by the addition of SDS in emulsions at pH 6.5). Microstructural images with the corresponding particle size distribution further indicated that flocculation of droplets occurs around native pH (~5 to 6.5). However, no changes in d_{32} at very acidic pH (pH 2) and alkaline conditions were shown, indicating that emulsions remained stable upon storage, in terms of droplet size. Similar to this, Nakauma *et al*. (2008) stated that the emulsion stability of soybean soluble polysaccharide and gum arabic emulsions did not decrease by decreasing the pH even though its zeta potential values decreased.

Furthermore, no visual phase separation, indicated by no creaming layer appearing, was observed during 30-day of storage at all studied pHs. Although flocculation was present in emulsions around native pH, the emulsions still remained stable over time. This is likely to be due to the higher viscosity of the emulsion provided by PG, which keeps the flocs suspended resulting in no creaming happening. Therefore, PG-containing protein moiety seems to provide long term stability at all studied pHs by viscosity effects from the non-adsorbed gum as well as protection against aggregation by steric hindrance and electrostatic repulsion at certain pHs where no bridging happened.

CHAPTER 6 EFFECT OF HEAT ON THE STABILITY OF PUKA GUM STABILISED EMULSIONS

6.1 Introduction

This chapter details the effect of heat treatment on the properties of emulsions stabilized by Puka Gum (PG). A fixed concentration of 4% w/w PG was used to stabilise 15% w/w soybean O/W emulsions prepared following the methods described in Section 3.4. Fresh emulsions at acidic pH, native pH, around neutral pH, and at alkaline conditions (pH 2, pH 5, pH 6.5 and pH 8.5, respectively), were subjected to thermal processing (80 ºC for 30 min). The mean particle size (d_{32}), particle size distribution, the droplet charges (zeta potential), rheological properties and microstructural properties under light microscopy were measured within 24 h after the preparation of the emulsions. The growth in mean droplet size (d_{32}) and visual phase separation were monitored during storage at 20 ºC for one month.

Some studies have been done in terms of testing the thermostability of O/W emulsions stabilized by polysaccharide at certain pHs such as basil seed gum (Hosseini-Parvar *et al*., 2016), gum Arabic (Chanamai & McClements, 2002; Golkar *et al*., 2018; Wu, Eskin, Cui, & Pokharel, 2015), Persian gum (Golkar *et al*., 2018), gum karaya (Shekarforoush *et al*., 2016), yellow mustard mucilage (Wu *et al*., 2015), citrus pectin (Wu *et al*., 2015), soybean soluble polysaccharide (Nakamura, Maeda, & Corredig, 2007) and *Retama reatam* seed galactomannan (Chouaibi *et al*., 2019). However, there is nothing in the literature regarding the thermostability of puka gum emulsions.

The aim of this study was to evaluate how resistant towards heat treatment, the emulsions made using PG at various pH's were. Understanding this is critical since future formulations based on PG-stabilised emulsions, will be processed via pasteurization or UHT treatments so a first study looking at the variation of the properties will provide valuable information.

6.2 Results

The particle size distribution of 4% w/w PG emulsions, affected by heat treatment is shown in Figure 26. With respect to pH conditions, PG emulsions subjected to heating had a slightly larger particle size than the unheated ones. At around native conditions (pH 5 and 6.5), the mean particle size (d_{43}) of PG emulsions increased from 3.78 to 4.29 µm and from 2.27 to 3.73 µm, respectively. Meanwhile, emulsions at alkaline conditions had a larger particle size (2.14 µm) compared to unheated emulsions

(1.81 µm). Only at acidic conditions (pH 2), PG-stabilised emulsions were not susceptible to heating as manifested by the similar curve of particle size distribution and considerably unchanged mean particle size (0.8 µm with or without heat). Regardless of the small changes in terms of mean sizes for all pH's apart from pH 2.0, visually, the size distribution that looked more shifted towards the slightly bigger sizes was the one corresponding to pH 6.5.

Changes in droplet size after heating are often a sign of droplet aggregation, which can lead to coalescence. When subjecting the emulsions to 80 °C for 30 min in our case, the size changes seemed minimum compared to other gums such as basil seed gum where droplet size changed to sizes above 130 µm from 20 µm at pH 2.0 (Osano, 2010). The aggregation of the non-adsorbed PG could also contribute to the detection of slightly bigger sizes if these aggregates scatter the light during the measurements using the Mastersizer.

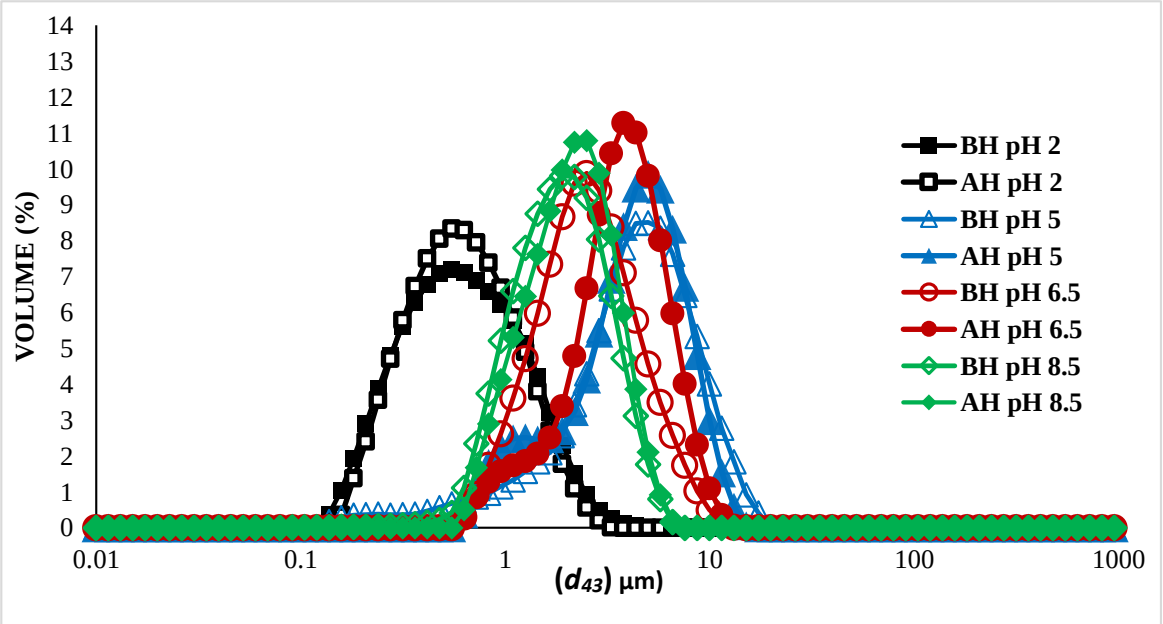


Figure 26. (a) Effect of heat treatment (80°C, 30 min) on the particle size distribution of 4% w/w PG-stabilised 15% w/w soybean oil-in-water emulsions.

In terms of the effect of heat treatment on the droplet charges, generally, the zeta potential values of the heated emulsions only significantly changed ($p < 0.05$) at pH 5.0 and 6.5, as shown in Figure 27. Whereas at pH 5 the zeta decreased, at pH 6.5 increased after heating, reflecting some changes at the interface at this pH range, which coincides with the pH range where some initial flocculation was

detected before any heating. Overall, the zeta potential was heat-independent at the extreme pH's very acidic or very alkaline with statistically insignificant changes.

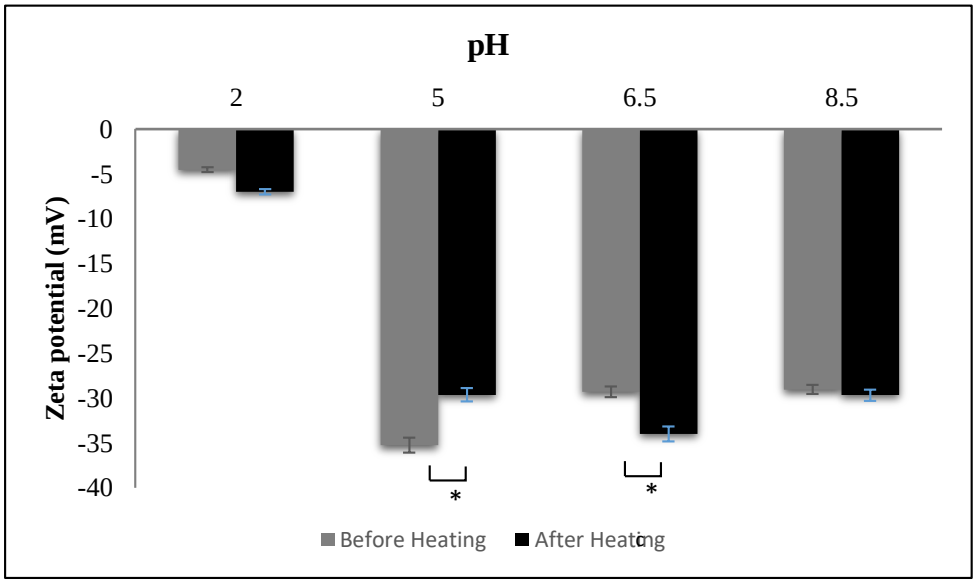


Figure 27. Effect of heat on the zeta potential of 4% w/w PG-stabilised 15% w/w soybean oil-in-water emulsions at 20°C. The graph represents the means ± SEMs. Tukey HSD test was used for statistical comparison between "before" and "after heating treatment" at the same puka gum concentration, where significantly different (P-values < 0.05) are denoted by an asterisk (*).

The apparent viscosity of PG-stabilised emulsions, as affected by heating, is shown in Figure 28. Higher viscosity was observed for all heated emulsions regardless of the pH, with the smallest changes in apparent viscosity after heating being detected in the control emulsions (native pH ~5). As an example, at 1 s^{-1}, the viscosity of emulsions at pH 2 and 6.5 before heating were 6.2 and 145.2 mPa.s, respectively, and the values increased to 17.4 and 237 mPa.s with the heat treatment. As the shear rate increased, shear-thinning behaviour was shown in all emulsions, especially for those at more alkaline conditions (> pH 5). In terms of the viscoelasticity of PG stabilised emulsions, as observed in Figure 29, an increase mainly in the loss modulus (G'') occurred for all the emulsions after heating with the storage modulus (G') being measurable at pH 6.5, with negligible values for the other pH's. Clearly the loss modulus (G'') dominated the viscoelastic behaviour for all the emulsions before and after heating. This modulus was highly frequency-dependent and the values tended to converge at high frequencies (Figure 29).

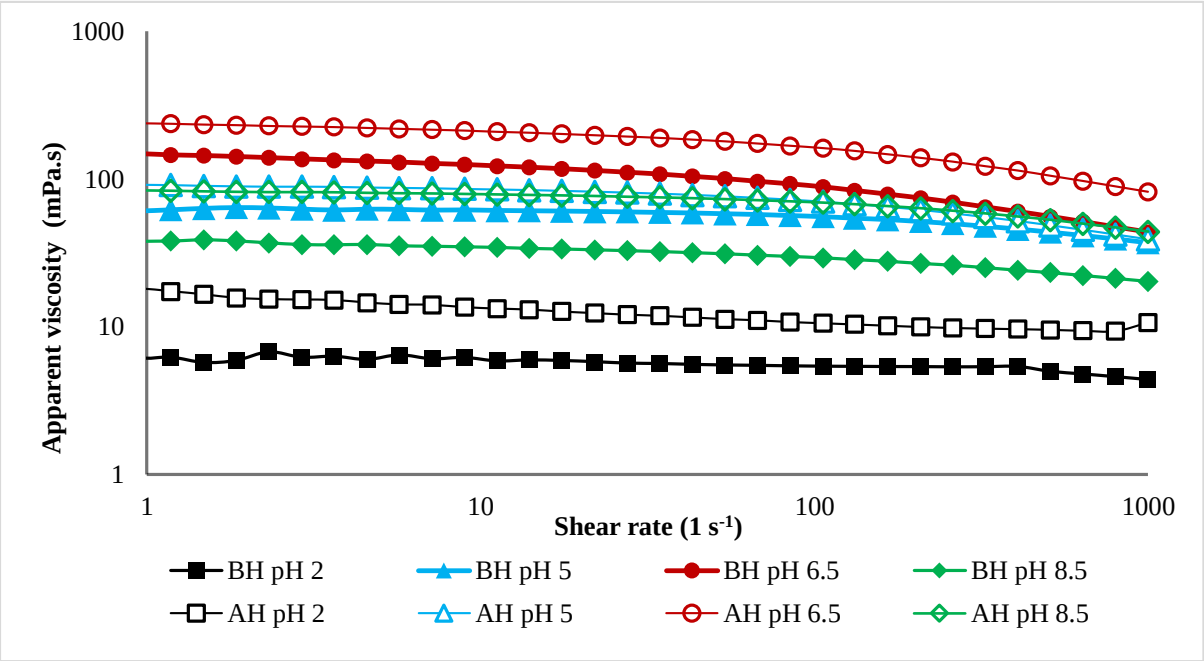


Figure 28. Effect of heating treatment on the apparent viscosity of 4% (w/w) PG-stabilised 15% soybean oil-in-water emulsions; BH = before heating and AH = after heating.

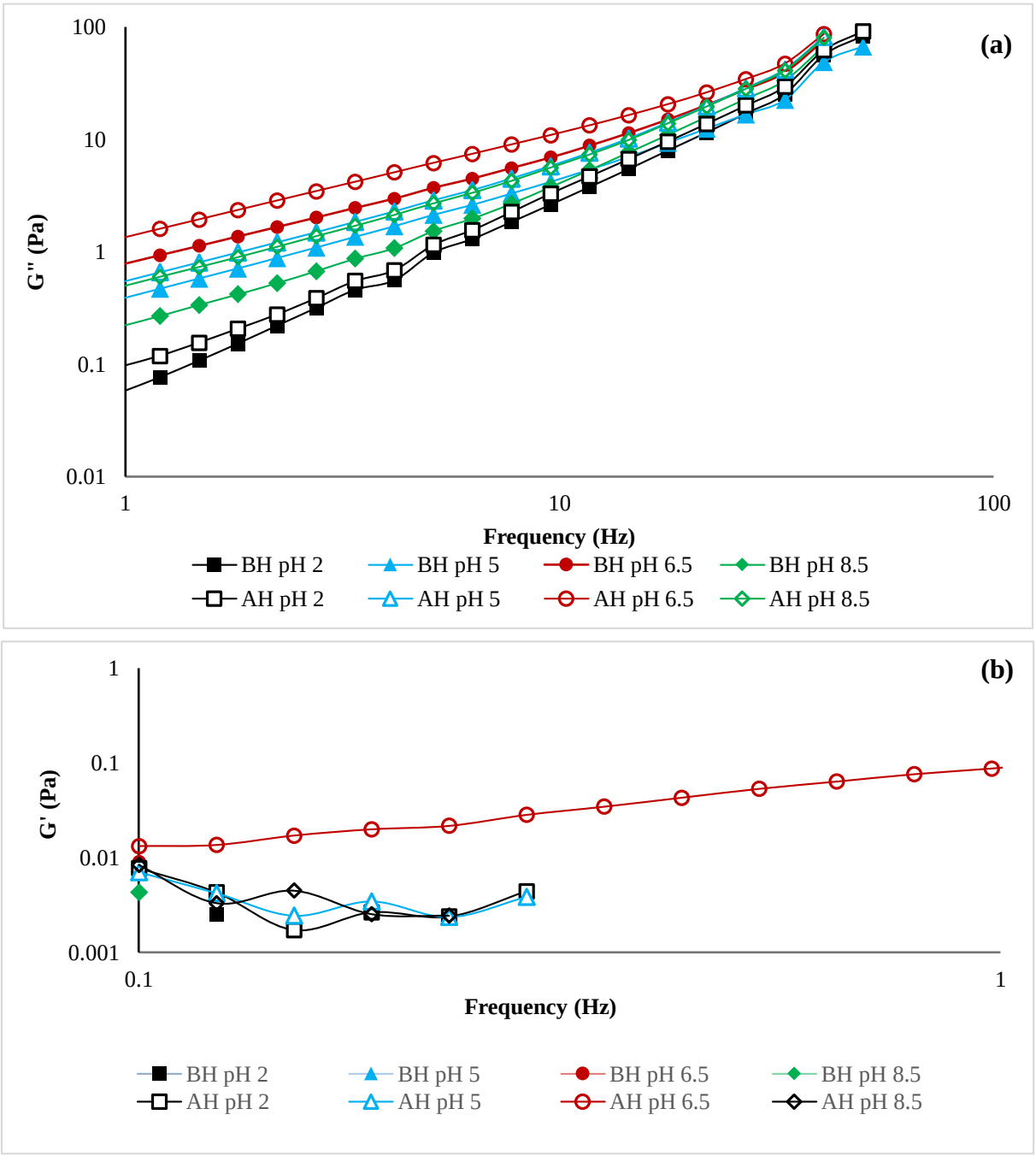


Figure 29. Effect of heating treatment on: (a) the loss modulus (*G''*) and (b) the storage modulus (*G''*) of 4% (w/w) PG-stabilised 15% soybean oil-in-water emulsions; BH = before heating and AH = after heating.

The light microscopy images of the 4% w/w PG emulsions, as depicted in Figure 30, confirmed that heating around native pH resulted in larger droplet sizes. Bimodal particle size distributions were observed in emulsions at pH 5 and pH 6.5, where bigger oil droplets (>20 µm) were detected clearly under light microscopy at pH 6.5, confirming coalescence as a result of heating. Clearly, a certain degree of bridging flocculation and coalescence seems to be markedly seen at pH 5 and pH 6.5 compared to other pH conditions. The microstructure of the heated emulsions at very acidic (pH 2)

and alkaline conditions (pH 8.5) showed smaller oil droplet sizes (<<20 μm), and completely unflocculated emulsions after heating, which correlates well with no changes in monomodal size distributions detected. The fact that the non-heated emulsions were already flocculated at those pH's 5-6.5 is likely to have accelerated the coalescence process given the close proximity of the droplets, as previously reported by Dickinson (1992). An increase in the rate of coalescence is normally influenced by: (i) the 'film thinning′, which causes a close contact between droplets and (ii) the 'film rupture', which mainly is due to a weak interfacial layer leading to coalescence. In our case, it seems that especially at pH 6.5, where clear coalescence is detected, heating accelerates the rearrangements of the adsorbed gum at the interface in the already flocculated emulsions, allowing areas of the oil droplets to be less protected sterically and therefore facilitating the coalescence process.

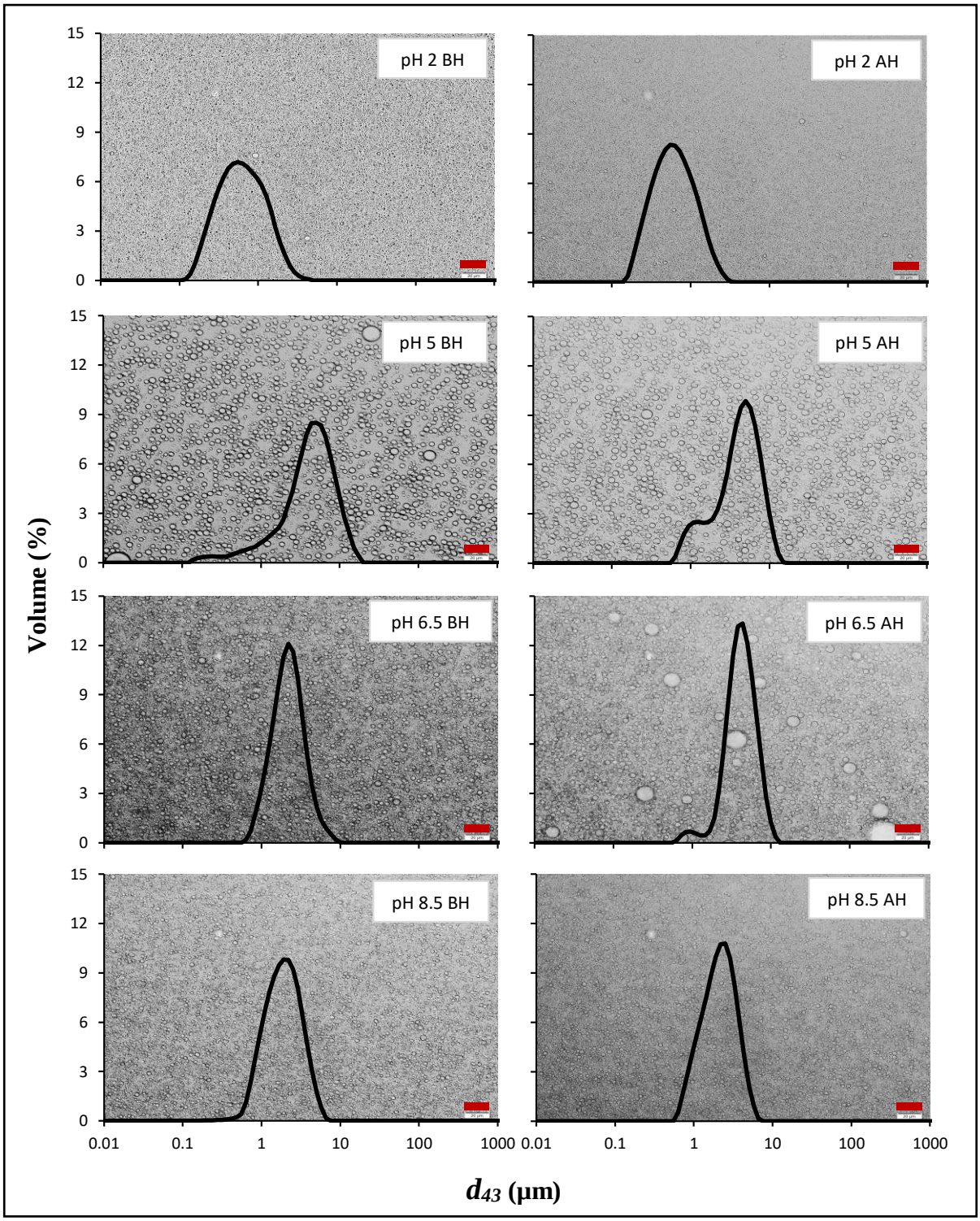


Figure 30. Effect of heat on the emulsion microstructure of 15% soy oil-in-water emulsions made with 4% w/w puka gum; magnification is 40x, scale bar = 20 µm.

In order to get more insights on the thermostability of these PG emulsions (after the heat treatment at 80 °C for 30 min), the mean particle size (d_{32}) and visual phase separation of the emulsions at various pH were investigated during one-month storage, as shown in Figure 31 and Figure 33, respectively.

There were no significant changes in the droplet sizes of heated emulsions over a one-month period, especially on emulsions with high acidity (pH 2) or at alkaline condition (pH 8.5) as compared to the control emulsions. A growth in droplet size was only observed in emulsions at pH 6.5, increasing from *ca.* 2.82 µm at day 1 to *ca.* 3.25 µm at the 4th week of storage. Therefore, only at this (~6.5), the average droplet size was markedly bigger after heating, in agreement with the microstructural changes observed above.

In order to confirm the cause of the increase in droplet size, 2% v/v sodium dodecyl sulfate (SDS) was added to the stored emulsions at pH 5 and 6.5. The addition of 2% v/v SDS to the stored emulsion at pH 6.5, as shown in Figure 32, did not alter significantly the particle size distribution as well as the average droplet size. As explained in Section 5.2, this means that the droplets presumably coalesced over time. In addition, remarkably, no creaming layer, which is associated with emulsion instability, was observed in the heated emulsions across the whole pH range over a month period (Figure 33).

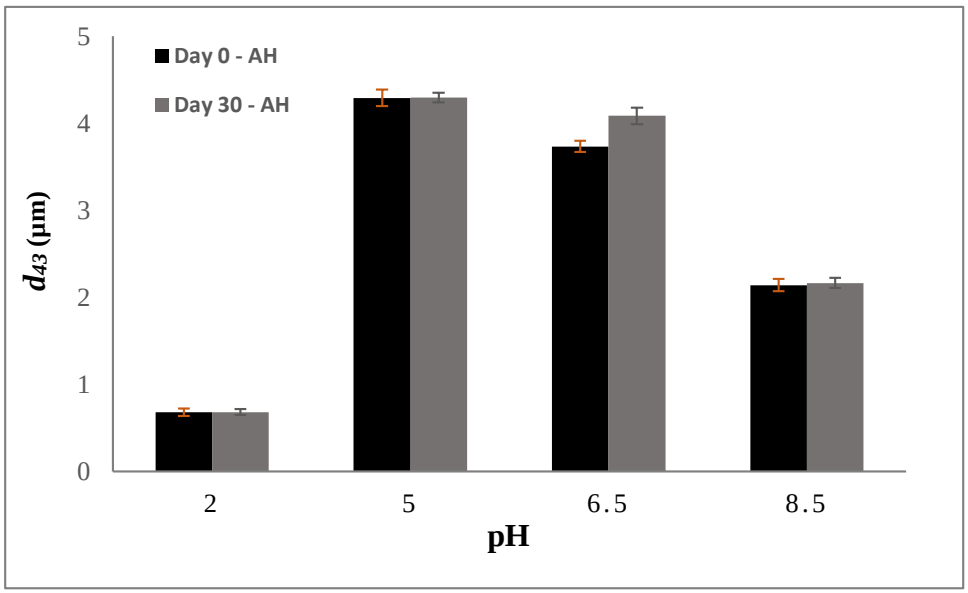


Figure 31. Effect of heating treatment (80°C for 30 min) on the average particle size (d_{43}) of 4% (w/w) PG-stabilised 15% (w/w) soybean oil-in-water emulsions at 20°C during one-month storage. BH = before heating; AH=after heating.

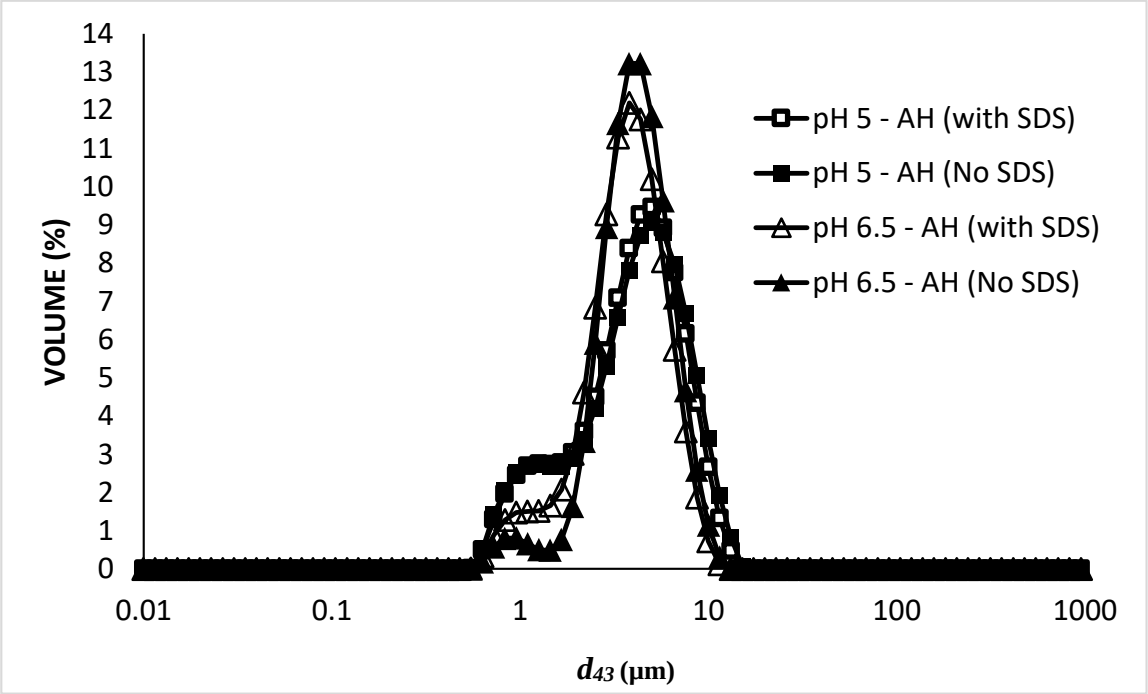


Figure 32. Effect of the addition of 2% v/v SDS on the average particle size (d_{43}) of 4% w/w PG-stabilised 15% w/w soybean oil-in-water emulsions subjected to heating after one-month storage.

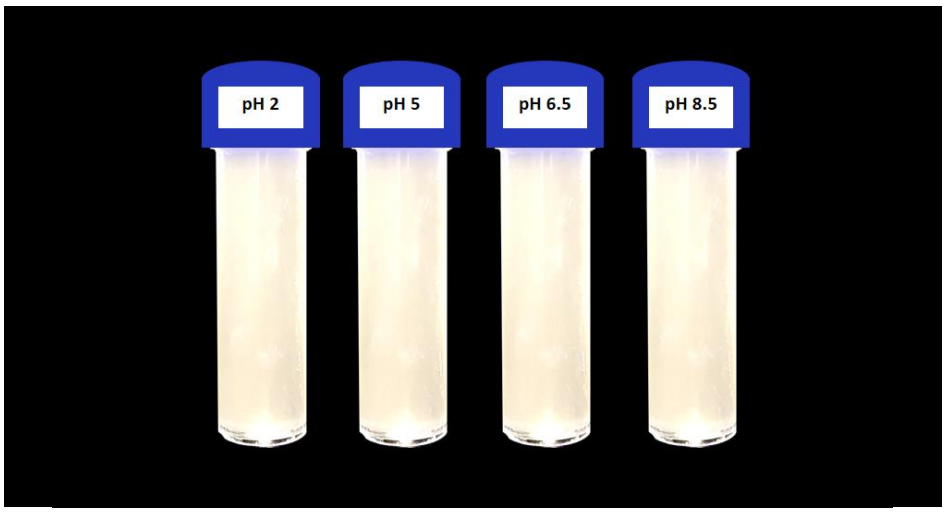


Figure 33. Phase separation profile of 4% w/w PG-stabilised 15% w/w soybean oil-in-water emulsions treated with heating at 80 °C for 30 min during one-month storage at 20 ˚C.

6.3 Discussion

Generally, PG stabilised emulsions at extreme pH's (2 and 8.5) were insensitive to heating (80 ºC for 30 min), in terms of particle size (d_{32}) and particle size distribution. This could be related to the fact that emulsions predominantly stabilised by hydrocolloids do not experience an unfolding of the adsorbed polymer to expose non-polar groups at high temperature, as previously stated by Qian, Decker, Xiao, and McClements (2010). Golkar *et al*. (2018) reported that thermal treatment at 90ºC had no significant effect on the mean particle size of emulsions stabilised by either persian gum or gum arabic. The heat-resistant properties of emulsions stabilised by gums above including PG are likely to be due to the presence of a robust protective film formation around oil droplets. In our case, working with crude PG, the interfacial layer is likely to contain also protein together with the polysaccharide and therefore the protein fraction would be susceptible to heat.

Only heat treatment at 80 ºC for 30 min of emulsions at pH 6.5, caused droplet aggregation leading to coalescence and bimodal size distributions compared to the unheated ones. These results were confirmed by the microstructural analysis of the emulsions where epecially at pH 6.5, larger droplet sizes appeared after heating. McClements (2016) stated that hydrophobic interactions leading to droplet aggregation can be favoured by heating. In the present case, further droplet aggregation and/or coalescence are presumably caused by the presence of hydrophobic interactions through PG. The addition of 2% v/v SDS confirmed that the predominantly origin of large emulsion droplets was coalescence.

Similar results in soybean soluble polysaccharide-stabilised emulsions were reported by Nakamura *et al*. (2007), where larger droplets after heating (90 ºC for 30 min) occurred at pH 7 compared to those under acidic conditions. The heat-induced aggregation is likely to be associated with the hydrophobic fractions (due to the presence of a protein moiety) in the polymer fractions resulting in the alteration of the aggregation state of the polysaccharides. In our case, the fact that the emulsions appeared already in an aggregated state at pH 5.0 and 6.5 before heat treatment, only promoted the coalescence process after heating. The close proximity of the droplets helps the hydrophobic interactions facilitating coalescence. Further investigation of the weaker interfacial layer at this pH is required, especially because the zeta potential values were relatively high, indicating the presence of sufficient adsorbed material.

The viscosity of PG emulsions was slightly affected by the heat treatment. Regardless of the pH, the apparent viscosity of PG emulsions after heating, markedly increased, especially at low shear rates (1-100 s^{-1}). A slightly higher viscosity after heating can have various causes: (i) an increase in droplet

flocculation, where the flocs can dissociate under high shear rate (>100 s^{-1}), especially for the emulsion at native pH and (ii) aggregation of the non-adsorbed gum due to hydrophobic forces becoming stronger with heating. Similar findings by Hosseini-Parvar *et al*. (2016) on heated O/W emulsions stabilised by basil seed gum have been reported. These authors found that the occurrence of droplet aggregation induced by heating could lead to a slight increase in the apparent viscosity of heated basil seed gum-stabilised emulsion at a low shear rate and low pH.

In terms of emulsion stability, heated PG-stabilised emulsions, at extreme pH's (2 and 8.5), did not exhibit an increase in average droplet size (d_{43}) during 30-day of storage. Although there was a slight increase in the mean particle size of emulsion at pH 6.5, phase separation was absent over a month of storage suggesting the heat-resistant nature of PG emulsions. Therefore, no phase separation observed is presumably due to higher apparent viscosity and viscoelasticity of the continuous phase after heating, resulting in controlled droplet movements and eventually to the physical stability of the emulsions.

CHAPTER 7 CONCLUSIONS AND RECOMMENDATIONS

This study aimed to investigate the emulsifying properties of puka gum (PG) by evaluating the effect of a series of concentrations (1-10% w/w) of crude and purified gum and varying the pH of the emulsions (pH 2-8.5). In addition, the stability of the PG-stabilised emulsions towards thermal treatment (80 ºC for 30 min) at certain pH was tested. 15% w/w soybean oil was used for the emulsion preparation. The emulsifying properties were discussed based on measuring the following: particle size (d_{32}), particle size distribution, droplet charge (zeta potential), rheological properties (viscosity and viscoelasticity) and microstructural properties of the emulsions. The emulsion stability of the PG emulsions, was monitored based on: (i) the changes in droplet size (d_{32}) over time—in the presence or not of sodium dodecyl sulphate (SDS)— and (ii) visual phase separation, also evaluated after 30-day of storage at 20 ºC. The effect of pH and heat on the emulsifying properties of PG was tested using a fixed PG concentration (4% w/w) in the final emulsion. In addition, the chemical composition and the rheological properties—affected by shear over time—of puka gum solutions were also analysed. These are the following main findings of the present work:

7.1 Conclusions

- The apparent viscosity of 4% w/w PG solutions was found to be shear-thinning with a Newtonian plateau at low shear rates. The shear-thinning exhibited by PG solutions was not as pronounced as for other gums, and only appeared at high shear rates (>100 s^{-1}).
- The viscosity of PG solutions was also time-dependent during the first 200 min of dispersing the gum at high shear rates (between 100 s^{-1} and 500 s^{-1}) and during the first 400 min at low shear rate (1 s^{-1} and 10 s^{-1}). This is, when constant shear rates were applied over a period of time, the PG solution exhibited shear-thinning behaviour with a tendency to reach almost Newtonian plateau after a given time, indicating the sensitivity of the gum to shear. Further examination showed that after applying a very high shear rate (800 s^{-1} for 300 min) and then resting the sample for 5 hours, the PG exhibited irreversible viscosity loss due to the intense shear, presumably resulting in chain breakage and molecular weight reduction.
- Stable emulsions against flocculation and coalescence and subsequent creaming could be created by PG. A stable emulsion, which is defined by the monomodal particle size distribution of small oil droplets (<2 μm) with little or no phase separation for at least 30 days, can be produced by using a low concentration of PG (4% w/w). PG-stabilized emulsions exhibited shear-thinning behaviour with a Newtonian plateau at low shear, at 4% w/w PG.

The ζ-potential of puka gum emulsions was approximately -35 mV indicating electrostatic stabilization.

- Similar emulsification properties of crude puka gum and purified puka gum indicated that the purification process may not be required, even though the composition varies in terms of total protein, carbohydrate, minerals, glucuronic acid and anhydrous monosaccharides.
- Emulsions stabilised by 4% w/w PG remained stable against phase separation at all studied pH´s. Very small droplet sizes (d_{32} = 0.48 and 1.40 µm) were formed at pH 2 and 8.5, respectively. Acidification is likely to improve the emulsification ability of PG by allowing better and more uniform adsorption of the gum. Very alkaline conditions also favoured the formation of very small sizes. However, emulsions appeared heavily flocculated by bridging around the native pH of the gum between pH 5 and pH 6.5.
- Electrostatic stability indicated by a greater magnitude of zeta potential (-30 – (-35) mV), occurred in emulsions at pH >3.5. The big droplet charge reduction in emulsions under very acidic condition (pH 2) was likely caused by the pH close to the *pKa* of PG. Even with high negative zeta potential values at pH 5 and 6.5 emulsions were aggregated which indicates that bridging of charged material occurs at this pH range.
- Dependency on varying the pH is shown in the apparent viscosity of PG emulsions at all shear rates. The viscoelasticity of the emulsions was also dependant on pH at low frequency (1-10 Hz), then become independent to pH at a higher frequency. The loss modulus (G'') of the emulsions was significantly higher than the storage modulus (G'), indicating liquid-like behaviour dominated the rheology of PG-stabilised emulsions at all pHs.
- A mixture of bridging and depletion flocculation only appears in emulsions around native pH, as confirmed by the microstructure images of emulsion at pH 6.5 and changes in droplet size in stored emulsion after adding SDS. However, a stable emulsion over time, in terms of no visual phase separation, could be created by PG at all studied pHs. This concludes that PG emulsions are resistant to creaming for a wide range of pH and the long-term stability provided by PG is mainly due to the viscosity effects from the non-adsorbed gum.
- The physical stability of PG-stabilised emulsions was generally not affected by heating. Only a slight increase in particle size was detected after heating at all pH´s. It was at pH 6.5, where the greatest increase in the particle size distribution and d_{43} were detected. Coalescence was the origin of the larger emulsion droplets after heating at around native pH, indicated by the bimodal size distribution and bigger droplet sizes appearing in the microstructure images. Flocculation occurred at around native pH in unheated emulsions and this presumably can cause an increase in the rate of coalescence in heated emulsions at around this pH. Moreover,

at pH 6.5, the aggregation driven by the protein fraction could be maximum, leading to a greater degree of coalescence.

- The apparent viscosity of the heated PG emulsions slightly increased, which could have various causes: (i) an increase in droplet flocculation, where the flocs can dissociate under high shear rate (>100 s^{-1}), especially for the emulsion at native pH and (ii) aggregation of the non-adsorbed gum due to hydrophobic forces becoming stronger with heating. In addition, no phase separation was observed in all heated emulsions during 30-day of storage, which is likely due to the higher apparent viscosity and viscoelasticity of the continuous phase after heating, which can control the droplet movements and eventually the physical stability of emulsions.
- Overall, puka gum— as a novel surface-active hydrocolloid—represents a promising natural emulsifier for emulsion-based food and beverage products, which indicates that PG confers emulsion stability under extreme environmental conditions.

7.2 Recommendations

- Further work is recommended to be done on the characterisation of the irreversible loss of viscosity that occurs in PG solutions as studies regarding this rheological property, especially in polysaccharides, are very limited. The molecular characterisation of this gum as it changes with shear is key to understand the best conditions to process this ingredient in the food industry.
- Other aspects of the emulsification properties of this gum, such as the adsorption mechanism in O/W emulsions could be studied in detail, generating more knowledge regarding this novel plant-based polysaccharide.
- Furthermore, future experimental work to compare PG with other commercial emulsifiers is needed. As a first step, the incorporation of PG into various food systems involving emulsification processes, such as in ice cream, yoghurt, salad dressings and soft drinks could be of interest.

References

Abbasi, S. (2017). Challenges towards characterization and applications of a novel hydrocolloid: Persian gum. *Current Opinion in Colloid & Interface Science, 28*, 37-45. doi:https://doi.org/10.1016/j.cocis.2017.03.001

Abbasi, S., & Mohammadi, S. (2013). Stabilization of milk–orange juice mixture using Persian gum: Efficiency and mechanism. *Food Bioscience, 2*, 53-60. doi:https://doi.org/10.1016/j.fbio.2013.04.002

Abbasi, S., & Rahimi, S. (2015). Persian gum. In *Encyclopedia of Biomedical Polymers and Polymeric Biomaterials, 11 Volume Set* (pp. 5919-5928): CRC Press.

Abdolmaleki, K., Mohammadifar, M. A., Sheikhi, Z., Matinfar, G., & Nayebzadeh, K. (2019). The Role of Oil Phase in the Stability and Physicochemical Properties of Oil-in-Water Emulsions in the Presence of Gum Tragacanth. *Journal of the American Oil Chemists' Society, 96*(7), 795-803. doi:10.1002/aocs.12248

Akhtar, M., Dickinson, E., Mazoyer, J., & Langendorff, V. (2002). Emulsion stabilizing properties of depolymerized pectin. *Food Hydrocolloids, 16*(3), 249-256. doi:https://doi.org/10.1016/S0268-005X(01)00095-9

Alba, K., & Kontogiorgos, V. (2017). Pectin at the oil-water interface: Relationship of molecular composition and structure to functionality. *Food Hydrocolloids, 68*, 211-218. doi:10.1016/j.foodhyd.2016.07.026

Alpizar-Reyes, E., Carrillo-Navas, H., Gallardo-Rivera, R., Varela-Guerrero, V., Alvarez-Ramirez, J., & Pérez-Alonso, C. (2017). Functional properties and physicochemical characteristics of tamarind (Tamarindus indica L.) seed mucilage powder as a novel hydrocolloid. *Journal of Food Engineering, 209*, 68-75. doi:https://doi.org/10.1016/j.jfoodeng.2017.04.021

Anjaneyalu, Y. V., & Gowda, D. C. (1979). Structural studies of an acidic polysaccharide from Ocimum basilicum seeds. *Carbohydrate Research, 75*, 251-256. doi:https://doi.org/10.1016/S0008-6215(00)84644-3

Atgié, M., Masbernat, O., & Roger, K. (2018). Emulsions stabilized by gum arabic: Composition and packing within interfacial films. *Langmuir, 35*(4), 962-972

BahramParvar, M., Razavi, S. M. A., & Mazaheri Tehrani, M. (2012). Optimising the ice cream formulation using basil seed gum (Ocimum basilicum L.) as a novel stabiliser to deliver

improved processing quality. *International Journal of Food Science & Technology, 47*(12), 2655-2661. doi:10.1111/j.1365-2621.2012.03148.x

Bai, L., Huan, S., Li, Z., & McClements, D. J. (2017). Comparison of emulsifying properties of food-grade polysaccharides in oil-in-water emulsions: Gum arabic, beet pectin, and corn fiber gum. *Food Hydrocolloids, 66*, 144-153. doi:10.1016/j.foodhyd.2016.12.019

Bai, L., Liu, F., Xu, X., Huan, S., Gu, J., & McClements, D. J. (2017). Impact of polysaccharide molecular characteristics on viscosity enhancement and depletion flocculation. *Journal of Food Engineering, 207*, 35-45. doi:https://doi.org/10.1016/j.jfoodeng.2017.03.021

Bansal, V. K., Chan, K. S., McCallough, R., & Shah, D. O. (1978). The Effect Of Caustic Concentration On Interfacial Charge, Interfacial Tension And Droplet Size: A Simple Test For Optimum Caustic Concentration For Crude Oils. *Journal of Canadian Petroleum Technology, 17*(01). doi:10.2118/78-01-05

Barak, S., & Mudgil, D. (2014). Locust bean gum: Processing, properties and food applications—A review. *International Journal of Biological Macromolecules, 66*, 74-80. doi:https://doi.org/10.1016/j.ijbiomac.2014.02.017

Behbahani, M. S., & Abbasi, S. (2017). Stabilization of flixweed seeds (Descurainia sophia L.) drink: Persian refreshing drink. *Food Bioscience, 18*, 22-27. doi:https://doi.org/10.1016/j.fbio.2017.03.001

Breckenridge J., Holthaus D. (2019). (TIC Gums, White Marsh, MD, USA). Personal Communication.

Brummer, Y., Cui, W., & Wang, Q. (2003). Extraction, purification and physicochemical characterization of fenugreek gum. *Food Hydrocolloids, 17*(3), 229-236. doi:https://doi.org/10.1016/S0268-005X(02)00054-1

Bueche, F. (1960). Mechanical degradation of high polymers. *Journal of Applied Polymer Science, 4*(10), 101-106

Carnachan, S. M., Bell, T. J., Hinkley, S. F. R., & Sims, I. M. (2019). Polysaccharides from New Zealand Native Plants: A Review of Their Structure, Properties, and Potential Applications. *Plants, 8*(6), 163. Retrieved from https://www.mdpi.com/2223-7747/8/6/163

Castel, V., Rubiolo, A. C., & Carrara, C. R. (2017). Droplet size distribution, rheological behavior and stability of corn oil emulsions stabilized by a novel hydrocolloid (Brea gum) compared

with gum arabic. *Food Hydrocolloids, 63*, 170-177. doi:https://doi.org/10.1016/j.foodhyd.2016.08.039

Castel, V., Rubiolo, A. C., & Carrara, C. R. (2018). Improvement of emulsifying properties of Brea gum by controlled thermal treatment. *Food Hydrocolloids, 85*, 93-101. doi:https://doi.org/10.1016/j.foodhyd.2018.07.010

Chanamai, R., & McClements, D. J. (2002). Comparison of Gum Arabic, Modified Starch, and Whey Protein Isolate as Emulsifiers: Influence of pH, $CaCl_2$ and Temperature. *Journal of food science, 67*(1), 120-125. doi:10.1111/j.1365-2621.2002.tb11370.x

Chang, Y., & McClements, D. J. (2015). Interfacial deposition of an anionic polysaccharide (fucoidan) on protein-coated lipid droplets: Impact on the stability of fish oil-in-water emulsions. *Food Hydrocolloids, 51*, 252-260. doi:https://doi.org/10.1016/j.foodhyd.2015.05.014

Charoen, R., Jangchud, A., Jangchud, K., Harnsilawat, T., Naivikul, O., & McClements, D. J. (2011). Influence of biopolymer emulsifier type on formation and stability of rice bran oil-in-water emulsions: whey protein, gum arabic, and modified starch. *Journal of Food Science*, *76*(1), E165-E172

Chen, H. M., Fu, X., & Luo, Z. G. (2016). Effect of molecular structure on emulsifying properties of sugar beet pulp pectin. *Food Hydrocolloids, 54*, 99-106. doi:10.1016/j.foodhyd.2015.09.021

Chen, H.-H., Xu, S.-Y., & Wang, Z. (2007). Interaction between flaxseed gum and meat protein. *Journal of Food Engineering, 80*(4), 1051-1059. doi:https://doi.org/10.1016/j.jfoodeng.2006.08.017

Chen, J., Li, J., Sun, A.-d., Zhang, B.-l., Qin, S.-g., & Zhang, Y.-q. (2014). Supercritical CO_2 extraction and pre-column derivatization of polysaccharides from Artemisia sphaerocephala Krasch. seeds via gas chromatography. *Industrial Crops and Products, 60*, 138-143. doi:https://doi.org/10.1016/j.indcrop.2014.06.013

Chivero, P., Gohtani, S., Yoshii, H., & Nakamura, A. (2016). Assessment of soy soluble polysaccharide, gum arabic and OSA-Starch as emulsifiers for mayonnaise-like emulsions. *LWT - Food Science and Technology, 69*, 59-66. doi:10.1016/j.lwt.2015.12.064

Chouaibi, M., Rezig, L., Lakoud, A., Boussaid, A., Hassouna, M., Ferrari, G., & Hamdi, S. (2019). Exploring potential new galactomannan source of Retama reatam seeds for food, cosmetic and pharmaceuticals: characterization and physical, emulsifying and antidiabetic properties.

International Journal of Biological Macromolecules, 124, 1167-1176. doi:10.1016/j.ijbiomac.2018.12.007

Chung, C., Degner, B., & McClements, D. J. (2013). Designing reduced-fat food emulsions: Locust bean gum–fat droplet interactions. *Food Hydrocolloids, 32*(2), 263-270. doi:https://doi.org/10.1016/j.foodhyd.2013.01.008

Cortés-Camargo, S., Gallardo-Rivera, R., Barragán-Huerta, B. E., Dublán-García, O., Román-Guerrero, A., & Pérez-Alonso, C. (2018). Exploring the Potential of Mesquite Gum–Nopal Mucilage Mixtures: Physicochemical and Functional Properties. *Journal of Food Science, 83*(1), 113-121. doi:10.1111/1750-3841.13937

Crispín-Isidro, G., Hernández-Rodríguez, L., Ramírez-Santiago, C., Sandoval-Castilla, O., Lobato-Calleros, C., & Vernon-Carter, E. J. (2019). Influence of purification on physicochemical and emulsifying properties of tamarind (Tamarindus indica L.) seed gum. *Food Hydrocolloids, 93*, 402-412. doi:https://doi.org/10.1016/j.foodhyd.2019.02.046

Crispín-Isidro, G., Hernández-Rodríguez, L., Ramírez-Santiago, C., Sandoval-Castilla, O., Lobato-Calleros, C., & Vernon-Carter, E. J. (2019). Influence of purification on physicochemical and emulsifying properties of tamarind (Tamarindus indica L.) seed gum. *Food Hydrocolloids, 93*, 402-412. doi:https://doi.org/10.1016/j.foodhyd.2019.02.046

Csáki, K. F. (2011). Synthetic surfactant food additives can cause intestinal barrier dysfunction. *Medical Hypotheses, 76*(5), 676-681. doi:https://doi.org/10.1016/j.mehy.2011.01.030

Cui, S. W., & Chang, Y. H. (2014). Emulsifying and structural properties of pectin enzymatically extracted from pumpkin. *LWT - Food Science and Technology, 58*(2), 396-403. doi:https://doi.org/10.1016/j.lwt.2014.04.012

de Barros Fernandes, R. V., Borges, S. V., Silva, E. K., da Silva, Y. F., de Souza, H. J. B., do Carmo, E. L., . . . Botrel, D. A. (2016). Study of ultrasound-assisted emulsions on microencapsulation of ginger essential oil by spray drying. *Industrial Crops and Products, 94*, 413-423.

Dickinson, E. (1992). An introduction to food colloids. *Oxford University Press*.

Dickinson, E. (2009). Hydrocolloids as emulsifiers and emulsion stabilizers. *Food Hydrocolloids, 23*(6), 1473-1482. doi:https://doi.org/10.1016/j.foodhyd.2008.08.005

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids, 17*(1), 25-39. doi:10.1016/S0268-005X(01)00120-5

Dickinson, E. (2018). Hydrocolloids acting as emulsifying agents – How do they do it?. *Food Hydrocolloids, 78*, 2-14. doi:10.1016/j.foodhyd.2017.01.025

Drusch, S. (2007). Sugar beet pectin: A novel emulsifying wall component for microencapsulation of lipophilic food ingredients by spray-drying. *Food Hydrocolloids, 21*(7), 1223-1228. doi:https://doi.org/10.1016/j.foodhyd.2006.08.007

Endreß, H. U., & Christensen, S. H. (2009). 12 - Pectins. In G. O. Phillips & P. A. Williams (Eds.), *Handbook of Hydrocolloids (Second Edition)* (pp. 274-297): Woodhead Publishing.

Esfahani, R., Jafari, S. M., Jafarpour, A., & Dehnad, D. (2019). Loading of fish oil into nanocarriers prepared through gelatin-gum Arabic complexation. *Food Hydrocolloids, 90*, 291-298.

Esmaeelian, M. (2016). Formulation and fuzzy modelling of emulsion stability of Neroli essential oil, gum Arabic and maltodextrin. *Bioscience biotechnology research communications, 9*(2), 189-194.

Fadavi, G., Mohammadifar, M. A., Zargarran, A., Mortazavian, A. M., & Komeili, R. (2014). Composition and physicochemical properties of zedo gum exudates from Amygdalus scoparia. *Carbohydrate Polymers, 101*, 1074-1080. doi:https://doi.org/10.1016/j.carbpol.2013.09.095

Farzi, M., Emam-Djomeh, Z., & Mohammadifar, M. A. (2013). A comparative study on the emulsifying properties of various species of gum tragacanth. *International Journal of Biological Macromolecules, 57*, 76-82. doi:https://doi.org/10.1016/j.ijbiomac.2013.03.008

Foster, T. (2008). Plant heritage New Zealand: te whakapapa o ngā rakau. *Raupo*

Freitas, R. A., Martin, S., Santos, G. L., Valenga, F., Buckeridge, M. S., Reicher, F., & Sierakowski, M. R. (2005). Physico-chemical properties of seed xyloglucans from different sources. *Carbohydrate Polymers, 60*(4), 507-514. doi:https://doi.org/10.1016/j.carbpol.2005.03.003

Funami, T., Nakauma, M., Ishihara, S., Tanaka, R., Inoue, T., & Phillips, G. O. (2011). Structural modifications of sugar beet pectin and the relationship of structure to functionality. *Food Hydrocolloids, 25*(2), 221-229. doi:https://doi.org/10.1016/j.foodhyd.2009.11.017

Funami, T., Nakauma, M., Katayama, T., Sakata, M., Ogasawara, T., Nishino, M., & Sasaki, Y. (2018). Outputs through the collaborative works with Prof. G. O. Phillips on hydrocolloid emulsifiers. *Food Hydrocolloids, 78*, 47-54. doi:https://doi.org/10.1016/j.foodhyd.2017.07.039

Funami, T., Zhang, G., Hiroe, M., Noda, S., Nakauma, M., Asai, I., . . . Phillips, G. O. (2007). Effects of the proteinaceous moiety on the emulsifying properties of sugar beet pectin. *Food Hydrocolloids, 21*(8), 1319-1329. doi:https://doi.org/10.1016/j.foodhyd.2006.10.009

Gahruie, H. H., Eskandari, M. H., Khalesi, M., Van der Meeren, P., & Hosseini, S. M. H. (2020). Rheological and interfacial properties of basil seed gum modified with octenyl succinic anhydride. *Food Hydrocolloids, 101*, 105489

García-Márquez, E., Román-Guerrero, A., Cruz-Sosa, F., Lobato-Calleros, C., Álvarez-Ramírez, J., Vernon-Carter, E. J., & Espinosa-Andrews, H. (2015). Effect of layer (calcium phosphate–chitosan)-by-layer (mesquite gum) matrix on carotenoids-in-water-emulsion properties. *Food Hydrocolloids, 43*, 451-458. doi:https://doi.org/10.1016/j.foodhyd.2014.07.005

Garti, N. (1999). Hydrocolloids as emulsifying agents for oil-in-water emulsions. *Journal of Dispersion Science and Technology***,** 20, 327-355

Garti, N., & Leser, M. E. (2001). Emulsification properties of hydrocolloids. *Polymers for Advanced Technologies*, 12, 123-135

Garti, N., Madar, Z., Aserin, A., & Sternheim, B. (1997). Fenugreek Galactomannans as Food Emulsifiers. *LWT - Food Science and Technology, 30* (3), 305-311. doi:https://doi.org/10.1006/fstl.1996.0179

Gemede, H. F., Haki, G. D., Beyene, F., Rakshit, S. K., & Woldegiorgis, A. Z. (2018). Indigenous Ethiopian okra (Abelmoschus esculentus) mucilage: A novel ingredient with functional and antioxidant properties. *Food Science & Nutrition, 6* (3), 563-571. doi:10.1002/fsn3.596

Gidley, M. J., Lillford, P. J., Rowlands, D. W., Lang, P., Dentini, M., Crescenzi, V., . . . Grant Reid, J. S. (1991). Structure and solution properties of tamarind-seed polysaccharide. *Carbohydrate Research, 214*(2), 299-314. doi:https://doi.org/10.1016/0008-6215(91)80037-N

Golkar, A., Nasirpour, A., Keramat, J., & Desobry, S. (2015). Emulsifying Properties of Angum Gum (*Amygdalus scoparia Spach*) Conjugated to β-Lactoglobulin through Maillard-Type Reaction. *International Journal of Food Properties, 18*(9), 2042-2055. doi:10.1080/10942912.2014.962040

Golkar, A., Taghavi, S. M., & Aghili Dehnavi, F. (2018). The emulsifying properties of persian gum (*Amygdalus scoparia Spach*) as compared with gum Arabic. *International Journal of Food Properties, 21*(1), 416-436. doi:10.1080/10942912.2018.1454464

Guzey, D., & McClements, D. J. (2006). Formation, stability and properties of multilayer emulsions for application in the food industry. *Advances in Colloid and Interface Science, 128-130*, 227-248. doi:https://doi.org/10.1016/j.cis.2006.11.021

Han, Y., Cheng, Z., Zhang, Y., Zhang, N., Zhu, X., Chen, X., . . . Huang, Y. (2020). Effect of metal ions and pH on the emulsifying properties of polysaccharide conjugates prepared from low-grade green tea. *Food Hydrocolloids, 102*, 105624. doi:https://doi.org/10.1016/j.foodhyd.2019.105624

Hashtjin, A. M., & Abbasi, S. (2015). Nano-emulsification of orange peel essential oil using sonication and native gums. *Food Hydrocolloids, 44*, 40-48. doi:https://doi.org/10.1016/j.foodhyd.2014.08.017

Hernández-Morales, M. d. l. Á., Maldonado-Astudillo, Y. I., Jiménez-Hernández, J., Salazar, R., Ramírez-Sucre, M. O., Ibarz, A., . . . Ortuño-Pineda, C. (2018). Physicochemical and rheological properties of gum seed and pulp from Hymenaea courbaril L. *CyTA - Journal of Food, 16*(1), 986-994. doi:10.1080/19476337.2018.1513955

Hifney, A. F., Fawzy, M. A., Abdel-Gawad, K. M., & Gomaa, M. (2016). Industrial optimization of fucoidan extraction from Sargassum sp. and its potential antioxidant and emulsifying activities. *Food Hydrocolloids, 54*, 77-88. doi:https://doi.org/10.1016/j.foodhyd.2015.09.022

Holtzinger, J., Green, J., Lamb, G., Atkinson, D., & Spikes, H. (2012). New Method of Measuring Permanent Viscosity Loss of Polymer-Containing Lubricants. *Tribology Transactions, 55*(5), 631-639. doi:10.1080/10402004.2012.688931

Hosseini-Parvar, Matia-Merino, & Golding. (2015). Effect of basil seed gum (BSG) on textural, rheological and microstructural properties of model processed cheese. *Food Hydrocolloids, 43*, 557-567. doi:https://doi.org/10.1016/j.foodhyd.2014.07.015

Hosseini-Parvar, Matia-Merino, Goh, Razavi, & Mortazavi. (2010). Steady shear flow behavior of gum extracted from Ocimum basilicum L. seed: Effect of concentration and temperature. *Journal of Food Engineering, 101*(3), 236-243. doi:https://doi.org/10.1016/j.jfoodeng.2010.06.025

Hosseini-Parvar, Osano, & Matia-Merino. (2016). Emulsifying properties of basil seed gum: Effect of pH and ionic strength. *Food Hydrocolloids, 52*, 838-847. doi:10.1016/j.foodhyd.2015.09.002

Hou, Z., Gao, Y., Yuan, F., Liu, Y., Li, C., & Xu, D. (2010). Investigation into the Physicochemical Stability and Rheological Properties of β-Carotene Emulsion Stabilized by Soybean Soluble Polysaccharides and Chitosan. *Journal of Agricultural and Food Chemistry, 58*(15), 8604-8611. doi:10.1021/jf1015686

Huang, X., Kakuda, Y., & Cui, W. (2001). Hydrocolloids in emulsions: particle size distribution and interfacial activity. *Food Hydrocolloids, 15*(4-6), 533-542

Jafari, S. M., Beheshti, P., & Assadpour, E. (2013). Emulsification properties of a novel hydrocolloid (Angum gum) for d-limonene droplets compared with Arabic gum. *International Journal of Biological Macromolecules, 61*, 182-188. doi:https://doi.org/10.1016/j.ijbiomac.2013.06.028

Jarzębski, M., Smułek, W., Kościński, M., Białopiotrowicz, T., & Kaczorek, E. (2018). Verbascum nigrum L. (mullein) extract as a natural emulsifier. *Food Hydrocolloids, 81*, 341-350. doi:https://doi.org/10.1016/j.foodhyd.2018.02.050

Jin, Q., Li, X., Cai, Z., Zhang, F., Yadav, M. P., & Zhang, H. (2017). A comparison of corn fiber gum, hydrophobically modified starch, gum arabic and soybean soluble polysaccharide: Interfacial dynamics, viscoelastic response at oil/water interfaces and emulsion stabilization mechanisms. *Food Hydrocolloids, 70*, 329-344. doi:10.1016/j.foodhyd.2017.03.005

Junqueira, L. A., Amaral, T. N., Félix, P. C., Botrel, D. A., Prado, M. E. T., & de Resende, J. V. (2019). Effects of Change in PH and Addition of Sucrose and NaCl on the Emulsifying Properties of Mucilage Obtained from Pereskia aculeata Miller. *Food and Bioprocess Technology, 12*(3), 486-498. doi:10.1007/s11947-018-2223-1

Kang, J., Cui, S. W., Phillips, G. O., Chen, J., Guo, Q., & Wang, Q. (2011). New studies on gum ghatti (Anogeissus latifolia) part II. Structure characterization of an arabinogalactan from the gum by 1D, 2D NMR spectroscopy and methylation analysis. *Food Hydrocolloids, 25*(8), 1991-1998. doi:https://doi.org/10.1016/j.foodhyd.2010.11.021

Kang, J., Guo, Q., Phillips, G. O., & Cui, S. W. (2014). Understanding the structure–emulsification relationship of gum ghatti – A review of recent advances. *Food Hydrocolloids, 42*, 187-195. doi:10.1016/j.foodhyd.2014.04.010

Katayama, T., Ido, T., Sasaki, Y., Ogasawara, T., Al-assaf, S., & Phillips, G. (2008). Characteristics of the Adsorbed Component of Gum Ghatti Responsible for Its Oil-Water Interface Advantages. Foods and Food Ingredients Journal of Japan

Khalloufi, S., Alexander, M., Douglas Goff, H., & Corredig, M. (2008). Physicochemical properties of whey protein isolate stabilized oil-in-water emulsions when mixed with flaxseed gum at neutral pH. *Food Research International, 41*(10), 964-972. doi:https://doi.org/10.1016/j.foodres.2008.07.019

Kim, Y. D., & Morr, C. V. (1996). Microencapsulation Properties of Gum Arabic and Several Food Proteins: Spray-Dried Orange Oil Emulsion Particles. *Journal of Agricultural and Food Chemistry, 44*(5), 1314-1320. doi:10.1021/jf9503927

Kokubun, S., Yadav, M. P., Moreau, R. A., & Williams, P. A. (2014). Components responsible for the emulsification properties of corn fibre gum. *Food Hydrocolloids, 41*, 164-168. doi:10.1016/j.foodhyd.2014.04.011

Lahaye, M., & Robic, A. (2007). Structure and Functional Properties of Ulvan, a Polysaccharide from Green Seaweeds. *Biomacromolecules, 8*(6), 1765-1774. doi:10.1021/bm061185q

Leroux, J., Langendorff, V., Schick, G., Vaishnav, V., & Mazoyer, J. (2003). Emulsion stabilizing properties of pectin. *Food Hydrocolloids, 17*(4), 455-462. doi:https://doi.org/10.1016/S0268-005X(03)00027-4

Li, J., Hu, X., Li, X., & Ma, Z. (2016). Effects of acetylation on the emulsifying properties of Artemisia sphaerocephala Krasch. polysaccharide. *Carbohydrate Polymers, 144*, 531-540. doi:https://doi.org/10.1016/j.carbpol.2016.02.039

Li, J., Hu, X., Yan, X., Li, X., Ma, Z., & Liu, L. (2018). Effects of hydrolysis by xylanase on the emulsifying properties of Artemisia sphaerocephala Krasch. polysaccharide. *Food Hydrocolloids, 76*, 158-163. doi:https://doi.org/10.1016/j.foodhyd.2016.12.015

Liu, J., Shim, Y. Y., Poth, A. G., & Reaney, M. J. T. (2016). Conlinin in flaxseed (Linum usitatissimum L.) gum and its contribution to emulsification properties. *Food Hydrocolloids, 52*, 963-971. doi:https://doi.org/10.1016/j.foodhyd.2015.09.001

Liu, J., Shim, Y. Y., Tse, T. J., Wang, Y., & Reaney, M. J. T. (2018). Flaxseed gum a versatile natural hydrocolloid for food and non-food applications. *Trends in Food Science & Technology, 75*, 146-157. doi:https://doi.org/10.1016/j.tifs.2018.01.011

Liu, Z., Guo, X., & Meng, H. (2020). Added ferulic acid enhances the emulsifying properties of pectins from different sources. *Food Hydrocolloids, 100*, 105439. doi:https://doi.org/10.1016/j.foodhyd.2019.105439

López-Franco, Y. L., de la Barca, A. M. C., Valdez, M. A., Peter, M. G., Rinaudo, M., Chambat, G., & Goycoolea, F. M. (2008). Structural Characterization of Mesquite (Prosopis velutina) Gum and its Fractions. *Macromolecular Bioscience, 8*(8), 749-757. doi:10.1002/mabi.200700285

López-Franco, Y., Higuera-Ciapara, I., Goycoolea, F. M., & Wang, W. (2009). 18 - Other exudates: tragancanth, karaya, mesquite gum and larchwood arabinogalactan. In G. O. Phillips & P. A. Williams (Eds.), *Handbook of Hydrocolloids (Second Edition)* (pp. 495-534): Woodhead Publishing.

Ma, F., Bell, A. E., & Davis, F. J. (2015). Effects of high-hydrostatic pressure and pH treatments on the emulsification properties of gum arabic. *Food Chemistry, 184*, 114-121. doi:https://doi.org/10.1016/j.foodchem.2015.03.075

Ma, F., Wang, R., Li, X., Kang, W., Bell, A. E., Zhao, D., . . . Chen, W. (2020). Physical properties of mucilage polysaccharides from Dioscorea opposita Thunb. *Food Chemistry, 311*, 126039. doi:https://doi.org/10.1016/j.foodchem.2019.126039

Ma, F., Zhang, Y., Wen, Y., Yao, Y., Zhu, J., Liu, X., . . . Tikkanen-Kaukanen, C. (2017). Emulsification properties of polysaccharides from Dioscorea opposita Thunb. *Food Chemistry, 221*, 919-925. doi:https://doi.org/10.1016/j.foodchem.2016.11.073

Mahfoudhi, N., Chouaibi, M., Donsì, F., Ferrari, G., & Hamdi, S. (2012). Chemical composition and functional properties of gum exudates from the trunk of the almond tree (Prunus dulcis). *Food Science and Technology International, 18*(3), 241-250. doi:10.1177/1082013211415173

Mahfoudhi, N., Sessa, M., Chouaibi, M., Ferrari, G., Donsì, F., & Hamdi, S. (2014). Assessment of emulsifying ability of almond gum in comparison with gum arabic using response surface methodology. *Food Hydrocolloids, 37*, 49-59. doi:https://doi.org/10.1016/j.foodhyd.2013.10.009

Marinova, K. G., Alargova, R. G., Denkov, N. D., Velev, O. D., Petsev, D. N., Ivanov, I. B., & Borwankar, R. P. (1996). Charging of Oil−Water Interfaces Due to Spontaneous Adsorption of Hydroxyl Ions. *Langmuir, 12*(8), 2045-2051. doi:10.1021/la950928i

Mathews, S., Singhal, R. S., & Kulkarni, P. R. (1993). Ocimum basilicum: A new non-conventional source of fibre. *Food Chemistry, 47*(4), 399-401. doi:https://doi.org/10.1016/0308-8146(93)90185-I

Mathur, N. K. (2012). Industrial galactomannan polysaccharides. Boca Raton, Fla.: CRC Press

McClements, D. J. (2004). *Food emulsions: Principles, practices, and techniques: Second edition*: CRC Press.

McClements, D. J. (2012). Advances in fabrication of emulsions with enhanced functionality using structural design principles. *Current Opinion in Colloid & Interface Science, 17*(5), 235-245. doi:https://doi.org/10.1016/j.cocis.2012.06.002

McClements, D. J. (2016). *Food emulsions : principles, practices, and techniques* (Third edition ed.): CRC Press, Taylor & Francis Group.

McClements, D. J., & Gumus, C. E. (2016). Natural emulsifiers — Biosurfactants, phospholipids, biopolymers, and colloidal particles: Molecular and physicochemical basis of functional performance. *Advances in Colloid and Interface Science, 234*, 3-26. doi:10.1016/j.cis.2016.03.002

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

McGarvie, D., & Parolis, H. (1981). Methylation analysis of the mucilage of Opuntia ficus-indica. *Carbohydrate Research, 88*(2), 305-314. doi:https://doi.org/10.1016/S0008-6215(00)85543-3

Michael, P., Cheekolu, M., Panwar, P., Devlin, M., Davidson, R., Johnson, D., & Martini, A. (2018). Temporary and Permanent Viscosity Loss Correlated to Hydraulic System Performance. *Tribology Transactions, 61*(5), 901-910. doi:10.1080/10402004.2018.1439210

Mikkonen, K. S., Xu, C., Berton-Carabin, C., & Schroën, K. (2016). Spruce galactoglucomannans in rapeseed oil-in-water emulsions: Efficient stabilization performance and structural partitioning. *Food Hydrocolloids, 52*, 615-624. doi:https://doi.org/10.1016/j.foodhyd.2015.08.009

Mirhosseini, H., Tan, C. P., Aghlara, A., Hamid, N. S. A., Yusof, S., & Chern, B. H. (2008). Influence of pectin and CMC on physical stability, turbidity loss rate, cloudiness and flavor release of orange beverage emulsion during storage. *Carbohydrate Polymers, 73*(1), 83-91. doi:https://doi.org/10.1016/j.carbpol.2007.11.002

Morelli, A., Massironi, A., Puppi, D., Creti, D., Domingo Martinez, E., Bonistalli, C., . . . Chiellini, F. (2019). Development of ulvan-based emulsions containing flavour and fragrances for food

and cosmetic applications. *Flavour and Fragrance Journal, 34*(6), 411-425. doi:10.1002/ffj.3519

Naji-Tabasi, S., & Razavi, S. M. A. (2016). New studies on basil (Ocimum bacilicum L.) seed gum: Part II—Emulsifying and foaming characterization. *Carbohydrate Polymers, 149*, 140-150. doi:https://doi.org/10.1016/j.carbpol.2016.04.088

Naji-Tabasi, S., & Razavi, S. M. A. (2017). Functional properties and applications of basil seed gum: An overview. *Food Hydrocolloids, 73*, 313-325. doi:https://doi.org/10.1016/j.foodhyd.2017.07.007

Naji-Tabasi, S., Razavi, S. M. A., Mohebbi, M., & Malaekeh-Nikouei, B. (2016). New studies on basil (Ocimum bacilicum L.) seed gum: Part I – Fractionation, physicochemical and surface activity characterization. *Food Hydrocolloids, 52*, 350-358. doi:https://doi.org/10.1016/j.foodhyd.2015.07.011

Nakamura, A., Maeda, H., & Corredig, M. (2007). Influence of Heating on Oil-in-Water Emulsions Prepared with Soybean Soluble Polysaccharide. *Journal of Agricultural and Food Chemistry, 55*(2), 502-509. doi:10.1021/jf062634g

Nakamura, A., Yoshida, R., Maeda, H., Furuta, H., & Corredig, M. (2004). Study of the role of the carbohydrate and protein moieties of soy soluble polysaccharides in their emulsifying properties. *Journal of Agricultural and Food Chemistry, 52*(17), 5506-5512.

Nakauma, M., Funami, T., Noda, S., Ishihara, S., Al-Assaf, S., Nishinari, K., & Phillips, G. O. (2008). Comparison of sugar beet pectin, soybean soluble polysaccharide, and gum arabic as food emulsifiers. 1. Effect of concentration, pH, and salts on the emulsifying properties. *Food Hydrocolloids, 22*(7), 1254-1267. doi:10.1016/j.foodhyd.2007.09.004

Neckebroeck, B., Verkempinck, S. H. E., Vaes, G., Wouters, K., Magnée, J., Hendrickx, M. E., & Van Loey, A. M. (2020). Advanced insight into the emulsifying and emulsion stabilizing capacity of carrot pectin subdomains. *Food Hydrocolloids, 102*, 105594. doi:https://doi.org/10.1016/j.foodhyd.2019.105594

Ngouémazong, E. D., Christiaens, S., Shpigelman, A., Van Loey, A., & Hendrickx, M. (2015). The Emulsifying and Emulsion-Stabilizing Properties of Pectin: A Review. *Comprehensive Reviews in Food Science and Food Safety, 14*(6), 705-718. doi:10.1111/1541-4337.12160

Nilsson, L. and B. Bergenståhl (2007). Adsorption of hydrophobically modified anionic starch at oppositely charged oil/water interfaces. *Journal of colloid and interface science,* 308(2): 508-513

Niu, F., Niu, D., Zhang, H., Chang, C., Gu, L., Su, Y., & Yang, Y. (2016). Ovalbumin/gum arabic-stabilized emulsion: Rheology, emulsion characteristics, and Raman spectroscopic study. *Food Hydrocolloids, 52,* 607-614. doi:https://doi.org/10.1016/j.foodhyd.2015.08.010

Nussinovitch, A., & Hirashima, M. (2014). *Cooking innovations : using hydrocolloids for thickening, gelling, and emulsification*. Boca Raton, [Florida]: Taylor & Francis/CRC Press.

Osano. (2010). *Emulsifying properties of a novel polysaccharide extracted from the seeds of basil (Ocimum basilicum L.) : a thesis presented in partial fulfilment of the requirements for the degree of Master of Technology in Food Technology at Massey University, Palmerston North, New Zealand*: Massey University.

Osano, J., Hosseini-Parvar, S., Matia-Merino, L., & Golding, M. (2014). Emulsifying properties of a novel polysaccharide extracted from basil seed (Ocimum bacilicum L.): Effect of polysaccharide and protein content. *Food Hydrocolloids, 37,* 40-48. doi:10.1016/j.foodhyd.2013.09.008

Osano, J., Matia-Merino, L., Hosseini-Parvar, S., Golding, M., & Goh, K. (2010). Adsorption properties of basil (Ocimum basilicum L.) seed gum. *USM R & D, 18*(113), e117.

Owens, C., Griffin, K., Khouryieh, H., & Williams, K. (2018). Creaming and oxidative stability of fish oil-in-water emulsions stabilized by whey protein-xanthan-locust bean complexes: Impact of pH. *Food Chemistry, 239,* 314-322. doi:https://doi.org/10.1016/j.foodchem.2017.06.096

Ozturk, B., & McClements, D. J. (2016). Progress in natural emulsifiers for utilization in food emulsions. *Current Opinion in Food Science, 7,* 1-6. doi:10.1016/j.cofs.2015.07.008

Pereira, G. A., Silva, E. K., Peixoto Araujo, N. M., Arruda, H. S., Meireles, M. A. A., & Pastore, G. M. (2019). Mutamba seed mucilage as a novel emulsifier: Stabilization mechanisms, kinetic stability and volatile compounds retention. *Food Hydrocolloids, 97,* 105190. doi:https://doi.org/10.1016/j.foodhyd.2019.105190

Prajapati, V. D., Jani, G. K., Moradiya, N. G., & Randeria, N. P. (2013). Pharmaceutical applications of various natural gums, mucilages and their modified forms. *Carbohydrate Polymers, 92*(2), 1685-1699. doi:https://doi.org/10.1016/j.carbpol.2012.11.021

Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2010). Comparison of Biopolymer Emulsifier Performance in Formation and Stabilization of Orange Oil-in-Water Emulsions. *Journal of the American Oil Chemists' Society, 88*(1), 47-55. doi:https://doi-org.ezproxy.massey.ac.nz/10.1007/s11746-010-1658-y

Qian, K. Y., Cui, S. W., Wu, Y., & Goff, H. D. (2012). Flaxseed gum from flaxseed hulls: Extraction, fractionation, and characterization. *Food Hydrocolloids, 28*(2), 275-283. doi:https://doi.org/10.1016/j.foodhyd.2011.12.019

Qin, L., Xu, S.-y., & Zhang, W.-b. (2005). Effect of enzymatic hydrolysis on the yield of cloudy carrot juice and the effects of hydrocolloids on color and cloud stability during ambient storage. *Journal of the Science of Food and Agriculture, 85*(3), 505-512. doi:10.1002/jsfa.1882

Rahimi, S. (2012). Determination of some chemical, physical, physicochemical, structural, and rheological properties of Persian gum.

Raikos, V., Duthie, G., & Ranawana, V. (2017). Comparing the efficiency of different food-grade emulsifiers to form and stabilise orange oil-in-water beverage emulsions: influence of emulsifier concentration and storage time. *International Journal of Food Science & Technology, 52*(2), 348-358.

Rao, M. A. (2010). *Rheology of fluid and semisolid foods: principles and applications*: Springer Science & Business Media.

Rashid, F., Hussain, S., & Ahmed, Z. (2018). Extraction purification and characterization of galactomannan from fenugreek for industrial utilization. *Carbohydrate Polymers, 180*, 88-95. doi:https://doi.org/10.1016/j.carbpol.2017.10.025

Razavi, S. M. A., & Naji-Tabasi, S. (2017). Chapter 16 - Rheology and Texture of Basil Seed Gum: A New Hydrocolloid Source. In J. Ahmed, P. Ptaszek, & S. Basu (Eds.), *Advances in Food Rheology and Its Applications* (pp. 405-435): Woodhead Publishing.

Ren, W., Zhao, S., Lian, Y., Yang, Y., Tian, G., Zhao, C., . . . Zheng, J. (2020). Effects of hydrosoluble calcium ions and organic acids on citrus oil emulsions stabilized with citrus pectin. *Food Hydrocolloids, 100*, 105413. doi:https://doi.org/10.1016/j.foodhyd.2019.105413

Rincón, F., de Pinto, G. L., & Beltrón, O. (2006). Note. Behaviour of a Mixture of Acacia glomerosa, Enterolobium cyclocarpum and Hymenaea courbaryl Gums in Ice Cream Preparation. *Food Science and Technology International, 12*(1), 13-17. doi:10.1177/1082013206060445

Rodrigues, D. C., Cunha, A. P., Silva, L. M. A., Rodrigues, T. H. S., Gallão, M. I., & Azeredo, H. M. C. (2018). Emulsion films from tamarind kernel xyloglucan and sesame seed oil by different emulsification techniques. *Food Hydrocolloids, 77*, 270-276. doi:https://doi.org/10.1016/j.foodhyd.2017.10.003

Rubio-Senent, F., Rodríguez-Gutiérrez, G., Lama-Muñoz, A., García, A., & Fernández-Bolaños, J. (2015). Novel pectin present in new olive mill wastewater with similar emulsifying and better biological properties than citrus pectin. *Food Hydrocolloids, 50*, 237.

Sáenz, C., Sepúlveda, E., & Matsuhiro, B. (2004). Opuntia spp mucilage's: a functional component with industrial perspectives. *Journal of Arid Environments, 57*(3), 275-290. doi:https://doi.org/10.1016/S0140-1963(03)00106-X

Salazar-Montoya, J. A., Jiménez-Avalos, H. A., & Ramos-Ramírez, E. G. (2012). Effects of Gum Arabic Concentration and Soy Proteins on the Flow and Viscoelasticity of their Dispersion. *International Journal of Food Properties, 15*(4), 891-902. doi:10.1080/10942912.2010.506099

Sav, A. R., et al. (2013). Investigational Studies on Highly Purified Fenugreek Gum as Emulsifying Agent. *Journal of Dispersion Science and Technology***,** 34(5): 657-662

Schmidt, U. S., Schütz, L., & Schuchmann, H. P. (2017a). Interfacial and emulsifying properties of citrus pectin: interaction of pH, ionic strength and degree of esterification. *Food Hydrocolloids, 62*, 288.

Schmidt, U. S., Schütz, L., & Schuchmann, H. P. (2017b). Interfacial and emulsifying properties of citrus pectin: Interaction of pH, ionic strength and degree of esterification. *Food Hydrocolloids, 62*, 288-298. doi:10.1016/j.foodhyd.2016.08.016

Scoparo, C. T., Souza, L. M., Dartora, N., Sassaki, G. L., Santana-Filho, A. P., Werner, M. F. P., . . . Iacomini, M. (2016). Chemical characterization of heteropolysaccharides from green and black teas (Camellia sinensis) and their anti-ulcer effect. *International Journal of Biological Macromolecules, 86*, 772-781. doi:https://doi.org/10.1016/j.ijbiomac.2016.02.017

Shao, P., Shao, J., Jiang, Y., & Sun, P. (2016). Influences of Ulva fasciata polysaccharide on the rheology and stabilization of cinnamaldehyde emulsions. *Carbohydrate Polymers, 135*, 27-34. doi:10.1016/j.carbpol.2015.08.075

Shao, P., Zhu, Y., & Jin, W. (2017). Physical and chemical stabilities of β-carotene emulsions stabilized by Ulva fasciata polysaccharide. *Food Hydrocolloids, 64*, 28-35. doi:https://doi.org/10.1016/j.foodhyd.2016.10.023

Sharma, G., Sharma, S., Kumar, A., Al-Muhtaseb, A. a. H., Naushad, M., Ghfar, A. A., . . . Stadler, F. J. (2018). Review: Guar gum and its composites as potential materials for diverse applications: A review. *Carbohydrate Polymers, 199*, 534-545. doi:10.1016/j.carbpol.2018.07.053

Sharma, M., Mondal, D., Mukesh, C., & Prasad, K. (2014). Preparation of tamarind gum based soft ion gels having thixotropic properties. *Carbohydrate Polymers, 102*, 467-471. doi:https://doi.org/10.1016/j.carbpol.2013.11.063

Shekarforoush, E., Mirhosseini, H., Amid, B. T., Ghazali, H., Muhammad, K., Sarker, M. Z. I., & Paykary, M. (2016). Rheological Properties and Emulsifying Activity of Gum Karaya (Sterculia Urens) in Aqueous System and Oil in Water Emulsion: Heat Treatment and Microwave Modification. *International Journal of Food Properties, 19*(3), 662-679. doi:10.1080/10942912.2015.1038836

Shekarforoush, E., Mirhosseini, H., Sarker, M. Z. I., Kostadinović, S., Ghazali, H. M., Muhamad, K., & Samaram, S. (2016). Soy Protein–Gum Karaya Conjugate: Emulsifying Activity and Rheological Behavior in Aqueous System and Oil in Water Emulsion. *Journal of the American Oil Chemists' Society, 93*(1), 1-10.

Siew, C. K., Williams, P. A., Cui, S. W., & Wang, Q. (2008). Characterization of the surface-active components of sugar beet pectin and the hydrodynamic thickness of the adsorbed pectin layer. *J. Agric. Food Chem., 56*, 8111.

Sims, I. M., & Furneaux, R. H. (2003). Structure of the exudate gum from Meryta sinclairii. *Carbohydrate Polymers, 52*(4), 423-431.

Spotti, M. L., Cecchini, J. P., Spotti, M. J., & Carrara, C. R. (2016). Brea Gum (from Cercidium praecox) as a structural support for emulsion-based edible films. *LWT - Food Science and Technology, 68*, 127-134. doi:https://doi.org/10.1016/j.lwt.2015.12.018

Srivastava, M., & Kapoor, V. P. (2005). Seed Galactomannans: An Overview. *Chemistry & Biodiversity, 2*(3), 295-317. doi:10.1002/cbdv.200590013

Stewart, s., & mazza, g. (2000). Effect of flaxseed gum on quality and stability of a model salad dressing1. *Journal of food quality, 23*(4), 373-390. Doi:https://doi.org/10.1111/j.1745-4557.2000.tb00565.x

Teimouri, S., Abbasi, S., & Scanlon, M. G. (2018). Stabilisation mechanism of various inulins and hydrocolloids: Milk–sour cherry juice mixture. *International Journal of Dairy Technology, 71*(1), 208-215. doi:10.1111/1471-0307.12376

Tesch, S., et al. (2002). Stabilization of emulsions by OSA starches. *Journal of Food Engineering*, 54(2): 167-174

Tomoda, M., Shimada, K., Saito, Y., & Sugi, M. (1980). Isolation and Structural Features of a Mucilage," Okra-mucilage F," from the Immature Fruits of Abelmoschus esculentus. *Chemical and Pharmaceutical Bulletin, 28*(10), 2933-2940.

Trubiano, P. C., & Lacourse, N. L. (1988). Emulsion-Stabilizing Starches. In *Flavor Encapsulation* (Vol. 370, pp. 45-54): American Chemical Society.

Trujillo-Ramírez, D., Lobato-Calleros, C., Román-Guerrero, A., Hernández-Rodríguez, L., Alvarez-Ramirez, J., & Vernon-Carter, E. J. (2018). Complexation with whey protein hydrolysate improves cacao pods husk pectin surface active and emulsifying properties. *Reactive and Functional Polymers, 123*, 61-69. doi:https://doi.org/10.1016/j.reactfunctpolym.2017.12.011

Ustyuzhanina, N. E., Bilan, M. I., Dmitrenok, A. S., Shashkov, A. S., Ponce, N. M. A., Stortz, C. A., . . . Usov, A. I. (2020). Fucosylated chondroitin sulfate from the sea cucumber Hemioedema spectabilis: Structure and influence on cell adhesion and tubulogenesis. *Carbohydrate Polymers, 234*, 115895. doi:https://doi.org/10.1016/j.carbpol.2020.115895

Vasile, F. E., Martinez, M. J., Pizones Ruiz-Henestrosa, V. M., Judis, M. A., & Mazzobre, M. F. (2016). Physicochemical, interfacial and emulsifying properties of a non-conventional exudate gum (Prosopis alba) in comparison with gum arabic. *Food Hydrocolloids, 56*, 245-253. doi:https://doi.org/10.1016/j.foodhyd.2015.12.016

Verbeken, D., Dierckx, S., & Dewettinck, K. (2003). Exudate gums: occurrence, production, and applications. *Applied Microbiology and Biotechnology, 63*(1), 10-21. doi:10.1007/s00253-003-1354-z

Verkempinck, S. H. E., Kyomugasho, C., Salvia-Trujillo, L., Denis, S., Bourgeois, M., Van Loey, A. M., . . . Grauwet, T. (2018). Emulsion stabilizing properties of citrus pectin and its interactions

with conventional emulsifiers in oil-in-water emulsions. *Food Hydrocolloids, 85*, 144-157. doi:10.1016/j.foodhyd.2018.07.014

Wang, J., Ye, Z., & Zhu, S. (2007). Topology-engineered hyperbranched high-molecular-weight polyethylenes as lubricant viscosity-index improvers of high shear stability. *Industrial & engineering chemistry research, 46*(4), 1174-1178

Weber, f. E., Taillie, s. A., & Stauffer, k. R. (1974). Functional characteristics of mustard mucilage. *Journal of Food Science, 39*(3), 461-466. doi:10.1111/j.1365-2621.1974.tb02925.x

Wee, M. S. M., Nurhazwani, S., Tan, K. W. J., Goh, K. K. T., Sims, I. M., & Matia-Merino, L. (2014). Complex coacervation of an arabinogalactan-protein extracted from the Meryta sinclarii tree (puka gum) and whey protein isolate. *Food Hydrocolloids, 42*, 130-138. doi:https://doi.org/10.1016/j.foodhyd.2014.03.005

Wee, M. S. M., Sims, I. M., Goh, K. K. T., & Matia-Merino, L. (2019). Molecular, rheological and physicochemical characterisation of puka gum, an arabinogalactan-protein extracted from the Meryta sinclairii tree. *Carbohydrate Polymers, 220*, 247-255. doi:https://doi.org/10.1016/j.carbpol.2019.05.076

Wefers, D., Bindereif, B., Karbstein, H. P., & van der Schaaf, U. S. (2018). Whey protein-pectin conjugates: Linking the improved emulsifying properties to molecular and physico-chemical characteristics. *Food Hydrocolloids, 85*, 257-266. doi:https://doi.org/10.1016/j.foodhyd.2018.06.030

Weiping, W., & Branwell, A. (2000). Tragacanth and Karaya in: GO Philips and PA Williams,(eds). Hand Book of Hydrocolloids. In: Cambridge: Wood Head Publishing Ltd.

Wielinga, W. (2009). Galactomannans. In *Handbook of hydrocolloids* (pp. 228-251): Elsevier.

Williams, P. A., Phillips, G. O., Stephen, A. M., & Churms, S. C. (2006). 13 Gums and Mucilages. *Food polysaccharides and their applications*, 455.

Wu, Y., Cui, W., Eskin, N. A. M., & Goff, H. D. (2009). An investigation of four commercial galactomannans on their emulsion and rheological properties. *Food Research International, 42*(8), 1141-1146. doi:https://doi.org/10.1016/j.foodres.2009.05.015

Wu, Y., Eskin, N. A. M., Cui, W., & Pokharel, B. (2015). Emulsifying properties of water soluble yellow mustard mucilage: A comparative study with gum Arabic and citrus pectin. *Food Hydrocolloids, 47*, 191-196. doi:https://doi.org/10.1016/j.foodhyd.2015.01.020

Xiang, S., Yao, X., Zhang, W., Zhang, K., Fang, Y., Nishinari, K., . . . Jiang, F. (2015). Gum Arabic-stabilized conjugated linoleic acid emulsions: Emulsion properties in relation to interfacial adsorption behaviors. *Food Hydrocolloids, 48*, 110-116. doi:10.1016/j.foodhyd.2015.01.033

Yadav, M. P., Johnston, D. B., & Hicks, K. B. (2009). Corn fiber gum: New structure/function relationships for this potential beverage flavor stabilizer. *Food Hydrocolloids, 23*(6), 1488-1493. doi:10.1016/j.foodhyd.2008.08.012

Yadav, M. P., Johnston, D. B., Hotchkiss, A. T., & Hicks, K. B. (2007). Corn fiber gum: A potential gum arabic replacer for beverage flavor emulsification. *Food Hydrocolloids, 21*(7), 1022-1030. doi:10.1016/j.foodhyd.2006.07.009

Yadav, M. P., Moreau, R. A., Hotchkiss, A. T., & Hicks, K. B. (2012). A new corn fiber gum polysaccharide isolation process that preserves functional components. *Carbohydrate Polymers, 87*(2), 1169-1175. doi:10.1016/j.carbpol.2011.08.092

Yamatoya, K., Tabuchi, A., Suzuki, Y., & Yamada, H. (2020). Chapter 20 - Tamarind seed polysaccharide: Unique profile of properties and applications. In K. Pal, I. Banerjee, P. Sarkar, D. Kim, W.-P. Deng, N. K. Dubey, & K. Majumder (Eds.), *Biopolymer-Based Formulations* (pp. 445-461): Elsevier.

Yang, X., Gong, T., Lu, Y.-h., Li, A., Sun, L., & Guo, Y. (2020). Compatibility of sodium alginate and konjac glucomannan and their applications in fabricating low-fat mayonnaise-like emulsion gels. *Carbohydrate Polymers, 229*, 115468. doi:https://doi.org/10.1016/j.carbpol.2019.115468

Yang, X., Nisar, T., Hou, Y., Gou, X., Sun, L., & Guo, Y. (2018). Pomegranate peel pectin can be used as an effective emulsifier. *Food Hydrocolloids, 85*, 30-38. doi:https://doi.org/10.1016/j.foodhyd.2018.06.042

Yosefi, F., Abbasi, S., & Ezatpanah, H. (2012). Effect of persian gum concentration, oil content, whey protein concentrate, and ph on the stability of emulsions prepared by ultrasonic homogenizer. *Journal of Research and Innovation in Food Science and Technology, 1*(3), 199-218. Retrieved from https://www.sid.ir/en/Journal/ViewPaper.aspx?ID=304630

Youssef, M. K., Wang, Q., Cui, S. W., & Barbut, S. (2009). Purification and partial physicochemical characteristics of protein free fenugreek gums. *Food Hydrocolloids, 23*(8), 2049-2053. doi:https://doi.org/10.1016/j.foodhyd.2009.03.017

Zhang, J., Zhang, Y.-K., Liu, Y., & Wang, J.-H. (2019). Emulsifying Properties of Tremella Fuciformis: A Novel Promising Food Emulsifier. *International journal of food engineering, 15*(3-4).

Zhang, Q., Gao, Y., Zhai, Y. A., Liu, F. Q., & Gao, G. (2008). Synthesis of sesbania gum supported dithiocarbamate chelating resin and studies on its adsorption performance for metal ions. *Carbohydrate Polymers, 73*(2), 359-363. doi:https://doi.org/10.1016/j.carbpol.2007.11.028

Zhao, G., Kan, J., Li, Z., & Chen, Z. (2005). Structural features and immunological activity of a polysaccharide from Dioscorea opposita Thunb roots. *Carbohydrate Polymers, 61*(2), 125-131. doi:https://doi.org/10.1016/j.carbpol.2005.04.020

Zhao, S., Gao, W., Tian, G., Zhao, C., DiMarco-Crook, C., Fan, B., . . . Zheng, J. (2018). Citrus Oil Emulsions Stabilized by Citrus Pectin: The Influence Mechanism of Citrus Variety and Acid Treatment. *Journal of Agricultural and Food Chemistry, 66*(49), 12978-12988. doi:10.1021/acs.jafc.8b04711

Zhou, W. W., Meng, L., Li, X., Ma, L., & Dai, R. (2010). Effect of the interaction between carrageenan, gellan gum and flaxseed Gum on quality attributes of starch-free emulsion-type sausage. *Journal of Muscle Foods, 21*(2), 255-267.

Zhu, W., Lingfeng, Z., Guoban, Z., Gensuo, Z., & Yan, Z. (1996). Investigation of the Thermal Decomposition Kinetics of Modified Carboxyl Hydroxypropyl Sesbania Gum [J]. *Acta Physico-chimica Sinica, 7*.

Zhu, Y., Ren, X., Bao, Y., Li, S., Peng, Z., Zhang, Y., & Zhou, G. (2020). Emulsification of oil-in-water emulsions with eggplant (Solanum melongena L.). *Journal of Colloid and Interface Science, 563*, 17-26. doi:https://doi.org/10.1016/j.jcis.2019.12.055

Appendix:

Plant based-polysaccharides emulsifiers.

No	Name	Source	Chemical Structure	Concentration range tested (%, w/w)	Emulsification	Emulsion stability (ES)	Application in Food Emulsions	References
1	Corn Fibre Gum (CFG)	Low value by product of corn milling	A highly branched polysaccharide, an arabinoxylan (AX) (β-1,4 linked D-xylopyranose (XYP) with α-L arabinofuranosyl (Araf) units linked	0.001 to 15%	d_{43}**:** 5 µm at 5% CFG, a decrease from 5.1 to 4.6 µm **Zeta potential:** -15 at 1% CFG, pH 7, 25˚C, 24 hours (h)	**pH & Ionic strength:** emulsion stability not affected **Creaming Index (CI):** <10% at 0.4% CFG and 90% at 1.2% CFG, 7 days of storage, 25˚C	A potential gum arabic replacer	Bai, Liu, *et al*., 2017; Bai, Huan, Li, & McClements, 2017; Chivero, Gohtani, Yoshii, & Nakamura, 2016; Jin *et al*., 2017; Kokubun, Yadav, Moreau, & Williams, 2014; Yadav, Johnston, & Hicks, 2009; Yadav, Johnston, Hotchkiss, & Hicks, 2007
2	Soybean Soluble Polysaccharide (SSPS)	By product of soy protein isolate production	Negatively charged, highly branched backbone : rhamnogalacturonan (RG) and neutral branched chains with a protein fraction; Molecular weight (Mw) 50 kDa ; 5.6% protein	1 to 15%	d_{32}**:** 0.66 µm at 4% SSPS **Zeta potential:** -45.5 at 4% SSPS	**Heat:** d_{32} rose from 0.66 to 1.08 µm at 60˚C for 3 days **pH:** d_{32} value was independent of pH range 3-6, above pH 6 the diameter increased to 1.25 µm	Acid milk drinks, mayonnaise-like product	Jin *et al*., 2017 ; Nakauma *et al*., 2008
3	Gum Arabic (GA)	Tree gum exudates from *Acacia senegal* or *Acacia seyal*	Branched heteropolyelectrolyte with 3 major components: arabinogalactan (AG), glycoprotein (GP), and arabinogalactan protein complex (AGP); backbone: 1→3-linked-β galactopyranose (Gal) units; sidechains: 1→6-linked Gal units;	0.001 to 30%	d_{43}**:** ~0.5 µm at 5% GA, a decrease from 0.82 to 0.65 µm **Min. mass ratio (oil to emulsifier) to produce small droplets:** 5:5 **zeta potential:** -20 mV at 1% GA, pH 7, 25˚C, 24h	**pH:** at pH 2-8, Z-average ~600-650 nm and zeta potential values -10 to -30 mV **Ionic strength:** stable Z-average (~600-650 nm & <1000 nm) and zeta potential values (-20mV) at addition of NaCl (0-500 mM) & $CaCl_2$ (0-50 mM), respectively. **Heat:** 30 to 90˚C, 20 min increased Z-average from 600 to 622 nm **Creaming Index (CI):** <10% at any GA concentrations (0-2%), 7 days storage, 25˚C	Soft drink-like products, oil flavours	Bai, Liu, *et al*., 2017; Bai, Huan, Li, & McClements, 2017; Dickinson, 2003, 2018; Jin *et al*., 2017; Ma, Bell, & Davis, 2015; Qian, Decker, Xiao, & McClements, 2010; Xiang *et al*., 2015

No	Name	Source	Chemical Structure	Concentration range tested (%, w/w)	Emulsion formation	Emulsion stability (ES)	Application in Food Emulsions	References
4	Galactomannan	Found in the endosperm of *Legume* family	Water-soluble non-ionic polysaccharides; D-mannose (Man) units linked by β (1–4) glycoside & D-Gal units are joined through (1–6) glycoside linkage	0.7%	**Particle size:** ~10 μm	**Heat:** stable at 15-50˚C **Storage:** no phase separation after 4 weeks	Ice cream, soft drinks, juices	Dickinson, 2003; Garti & Leser, 2001; Wielinga, 2009
4.1	Fenugreek gum (FG)	The endosperm of fenugreek (*Trigonellafoenum-graecum* Linn.)	Ratio of mannose to galactose (M/G ratio) 1.2; 2.62% protein	0.1 to 2%	**Emulsion capacity (EC):** 97.36% **Particle size:** ~3 μm	**ES:** 96.36% (GG > FG > TG > LBG) at 0.5% FG; 80ºC 30 min **Storage:** after 2 weeks, ES value was 96% at 0.5% FG	Ice cream, soft drinks, juices	Garti, 1999; Garti, Madar, Aserin, & Sternheim, 1997; Rashid, Hussain, & Ahmed, 2018; Wielinga, 2009; Wu, Cui, Eskin, & Goff, 2009
4.2	Guar gum (GG)	The endosperm of *Cyamopsis tetragonolobus* / *Ceratonia siliqua* / *Cyamopsis psoraloides*	M/G ratio 1.2, 3.46% protein, hydroxyl groups present in the structure, β-D-glucopyranosyl groups polymerised by 1→4 linkages; 33-40% Gal	0.1 to 2%	**EC:** 100% at 0.5% GG	**ES:** 99.36% (0.5% GG) at 80˚C 30 min	Ice cream, soft drinks, juices	
4.3	Tara gum (TG)	From the endosperm of the seeds of *Caesalpinia spinose*	M/G ratio 3.0 ; 0.71% protein; β-D-glucopyranosyl groups polymerised by 1→4 linkages; 25% Gal	0.1 to 2%	**EC:** 60%	**ES:** 45% at 80˚C 30 min	Ice cream, soft drinks, juices	

No	Name	Source	Chemical Structure	Concentration range tested (%, w/w)	Emulsion formation	Emulsion stability (ES)	Application in Food Emulsions	References
4.4	Locust bean gum (LBG)	From the seeds of the carob tree	A linear chain of (1→4)-linked β-D-mannopyranosyl units with (1→6)-linked α-D-galactopyranosyl residues as side chains; M/G ratio 3.7 ; 4.57% protein; 17-26% galactose	0.05 to 2%	d_{43}: 0.24 µm at 0.05-0.15% LBG **EC:** ~40%	**ES:** ~40% at 80˚C 30 min	Reduced-fat food emulsions, model food sauces, ice cream, low-fat yoghurt	Barak & Mudgil, 2014; Chung, Degner, & McClements, 2013; Garti, 1999; Wu *et al*., 2009 Wielinga, 2009
4.5	*Retama reatam* seed galactomannan (RRSG)	Galactomannan extracted from the seed of *Retama reatam*	95.52% total carbohydrate (Man and Gal, with a M/G ratio of 1.85) and 0.71 to 4.57% protein Mw 1.56 × 10^6 g/mol	0.25 to 1.5%	d_{43}: 41nm at 1% RRSG	**Heat:** no significant changes in the microstructure of emulsion after heating at 80 ˚C at 1% RRSG, pH 4.50; an increase in temperature from 20 to 80 ˚C increased the d_{43} by 5 nm (~45 to 50 nm)	No studies	Chouaibi *et al*., 2019
4.6	Spruce galactoglucomannan (GGM)	Galactoglucomannan extracted from *Picea abies*	Backbones of β-(1→4)-D-mannopyranosyl and β-(1→4)-D-glucopyranosyl units that carry single α-D-galactopyranosyl residues that are (1→6)-linked to Man units and O-acetyl substituents	1%	d_{32}: 393 nm at 1% GGM	**Storage:** showed creaming after 1 day of storage	No studies	Mikkonen, Xu, Berton-Carabin, & Schroën, 2016 ; Mikkonen, 2009; Willfor, 2008
4.7	Sesbania gum (SG)	The endosperm of Sesbania seeds	Contain mostly galactomannan and a small amount of protein (2.75%) and fat; β(1→4) glycosidic bonds linked Man and α(1→6) glycosidic bonds linked Gal; Mw: ~3x10^5 Da; M/G ratio 2:1	0.1 to 0.5%	**EC:** 94% at 0.1% SG	**ES:** 92% at 0.1% gum at 80 °C for 30 min	A substitute for GG	Jia *et al*, 2019; Zhang, Gao, Zhai, Liu, & Gao, 2008; Zhu, Lingfeng, Guoban, Gensuo, & Yan, 1996

No	Name	Source	Chemical Structure	Concentration range tested (%, w/w)	Emulsion formation	Emulsion stability (ES)	Application in Food Emulsions	References
5	Citrus pectin (CP)	Extracted from citrus fruit peels	α-1→4 linked galacturonic acid (GalA) chains with 1→2 linked l-rhamnose (Rha), GalA 75.1%, medium methoxylated pectins (MMP): 50); methylated RG-I fractions; 2.6 to 6.4% protein; Degree of acetylation (DAc): 10%	1%	**Particle size:** 6.66 um; exhibited a bimodal size profile **Zeta potential:** 0 to -20 mV at pH 2 and -30 to -80 mV at pH 7 (1% CP solution)	**Ionic strength:** an addition of $CaCl_2$ (0-200 mM) increased d_{32} from 1.47 to 3.5 µm **Heat:** gradually de-stabilized under thermal treatment (at 60 °C for 7 days) **Zeta potential:** -10 to -30 mV at pH 3 and -40 to -60 mV at pH 7 during 4 day of storage, 4 ˚C **Addition of citric acid (0-3%):** d_{32}=1.5 to 0.8 µm **Visual separation:** showed cream layer after day 1 and no dramatically increase droplet size during 2 weeks of storage	Acid dairy drinks	Endreß & Christensen, 2009; Funami *et al*., 2007; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Liu, Guo, & Meng, 2020; Ren *et al*., 2020; Verkempinck *et al*., 2018
6	Apple Pectin (AP)	Extracted from apple peels	α-1→4 linked GalA chains with 1→2 linked l-Rha, GalA 75.1%, MMP (50); methylated RG-I fractions, 2.6 to 6.4% protein; DAc: 10%	1%	d_{43}**:** 6.98 um	**Heat:** d_{43} of emulsions increased from 6µm to 10µm at 60 °C after 7 day of storage	Acid dairy drinks	Endreß & Christensen, 2009; Funami *et al*., 2007; Leroux *et al*., 2003; Liu *et al*., 2020
7	Carrot Pectin (CRP)	Extracted from carrot pulp		1%	d_{43}**:** 1 to 2.5 µm **Zeta potential:** -40 mV at pH 8 (1% CRP)	**pH: at pH 2.5 to 6,** d_{43} was 2.5 µm after 14 days of storage, wide particle size distribution (PSD) with 45% volume >1µm at pH 6 **Instability index**: 0.45% at pH 6 and 0.5% at pH 2.5 after 14 days of storage	No studies	Neckebroeck *et al*., 2020

No	Name	Source	Chemical Structure	Concentration range tested (%, w/w)	Emulsion formation	Emulsion stability (ES)	Application in Food Emulsions	References
8	Sugar beet Pectin (SBP)	Extracted from sugar beet pulp- a byproduct of sugar extraction	A heteropolysaccharide with a main chain of 1→4-linked α-D- GalA units interrupted by 1→2-linked L-rhamnopyranosyl (Rhap) residues substituted with neutral sugars (D-galactose, L-arabinose, D-xylose), has higher feruloyl esters; higher DAc: 25%	Gum to oil ratio (1:5) 0.001 to 5%	d_{43}**:** 0.1 - 1 µm at 2% SBP, a decrease from 0.25 to 0.18 µm over the range of homogenization pressure ($6.2x10^7$-$1.3x10^8$ Pa) **zeta potential:** -32.5 at 1% SBP, pH 7, 25˚C, 24h	**pH:** d_{32} almost independent of pH <6 (~1-2 µm), zeta potential was -20 to -63.1 mV at pH 2 to 6 (1.5% SBP) **Ionic strength:** the addition of 500 mM NaCl at 1.5% SBP increased the d_{32} values from 0.55 to 1.55 and less negative zeta potential from -60.2 to -26.7; the addition of 500 mM $CaCl_2$ increased the d_{32} values from 0.55 to 1.99 µm and less negative zeta potential from -60.2 to -22.8 mV **Heat:** gradually de-stabilized at 60 °C for 7 d from 0.622 to 2.71 µm **Creaming Index (CI):** <10% at <0.5% SBP and ~85% at 1.2% SBP, 7 days storage, 25 ˚C	Acid dairy drinks	Alba & Kontogiorgos, 2017; Bai, Liu, *et al*., 2017; Bai, Huan, Li, & McClements, 2017; Chen, Fu, & Luo, 2016; Dickinson, 2003, 2018; Endreß & Christensen, 2009; Garti & Leser, 2001; Karnik & Wicker, 2018; Liu *et al*., 2020; Nakauma *et al*., 2008; Schmidt, Schütz, & Schuchmann, 2017a; Siew, Williams, Cui, & Wang, 2008

No	Name	Source	Chemical Structure	Concentration range tested (%, w/w)	Emulsion formation	Emulsion stability (ES)	Application in Food Emulsions	References
9	Pomegranate peel pectin	Extracted from pomegranate fruit (*Punica granatum L.*) peels	Mainly composed of 4.5% Rha, 13.4% xylose, mannose 1.8%, 60.8% glucose, and 19.5% galactose	0.5 to 2%	**EC:** 98% at 2% pectin	**ES:** 97% at 2% pectin **pH:** stable EC and ES values at pH 2-6 and decreased significantly until pH 10. The highest EC and ES values were 75% (pH 6) and 85% (pH 4), respectively **Ionic strength:** the highest ES values was 65% at addition of Na^{+}(60 mg/g pectin); addition of Ca^{2+} (100 mg/g pectin) decreased ES from 66 to 56%	No studies	Yang *et al*., 2018
10	Pumpkin pectin	Extracted from pumpkin (*Cucurbita mixta*) by enzyme treatment	Degree of esterification 47.30% ; Degree of acetylation 6.87%; GalA 75.02% ; Protein 4.58%; The backbone of homogalacturonan (1 → 4)-linked α-d-GalpA, and rhamnogalacturonan regions.	0.1%	**EC:** 63.7%	**ES:** 58.3%	No studies	Cui & Chang, 2014
11	Alperujo pectin	Semi solid by product of the olive oil industry	The total sugar content (uronic acid and neutral sugar) was 27.7%, mainly composed of GalA (32 and 48%) and Ara (24 and 17%), with minor proportions of 15% Glc, 10% Gal, 10% Rha, 7% Man	0.1%	**EA:** 47.9%	**Heat:** ES value was 67% at 80 °C for 30 min	No studies	Rubio-Senent, Rodríguez-Gutiérrez, Lama-Muñoz, García, & Fernández-Bolaños, 2015

No	Name	Source	Chemical Structure	Concentration range tested (%, w/w)	Emulsion formation	Emulsion stability (ES)	Application in Food Emulsions	References
12	Basil Seed Gum (BSG)	Gum extracted from *Ocimum basilicum* L. seeds	Two major fractions: glucomannans (~43%) and (1→4)-linked xylan (~24.3%)	0.3 to 1%	d_{32} < 1.0 µm at 0.3% BSG	**pH:** uniform PSD at pH 7; highly negatively charged at alkaline conditions with zeta potential –81.2 mV (pH 12) compared to -2.5 mV (pH 2); d_{43} increased during 1-month storage, 20˚C, at all pH with biggest changes at lower pH (1-2) from 20 to 30 µm, remained stable at pH 7 **Ionic strength:** the addition of NaCl increased d_{32} from 0.99 to 16.1 µm but no more changes at 0.24 M; decrease zeta potential from -57.8 to -14.7 mV (0-0.24 M), the biggest changes of d_{43} during 1-month storage was with 0.24 M NaCl (from <5 to ~12.5 µm) **Heat:** form larger droplets at 80 ˚C for 30 min with d_{43} from ~60 to 140 µm at pH 1 (before and after heating respectively) **1-month storage at 20˚C:** d_{43} values were <5 µm for 0.09 to 1% BSG	Model processed cheese, ice cream	BahramParvar, Razavi, & Mazaheri Tehrani, 2012; Hosseini-Parvar, Matia-Merino, & Golding, 2015; Hosseini-Parvar, Osano, & Matia-Merino, 2016; Naji-Tabasi & Razavi, 2016, 2017; Naji-Tabasi, Razavi, Mohebbi, & Malaekeh-Nikouei, 2016; Osano, Hosseini-Parvar, Matia-Merino, & Golding, 2014; J. Osano, Matia-Merino, Hosseini-Parvar, Golding, & Goh, 2010

No	Name	Source	Chemical Structure	Concentration range tested (%, w/w)	Emulsion formation	Emulsion stability (ES)	Application in Food Emulsions	References
13	Flaxseed Gum (FXG)	Gum derived from flaxseed (*Linum usitatissimum L.*) - a bio product of the flax oil industry	An anionic hetero-polysaccharide :75% arabinoxylans with β-D-(1, 4)-xylan backbone arabynoxylans and RG-I backbone (diglycosyl) linked [1→2)-α-l-Rhap-(1 → 4)-α-d-GalpA	0.4 to 0.8%	d_{43}**:** 2.4 µm at 1% FXG **EAI:** 98.7% at 0.8% FXG	**pH:** stable at pH >2.8, at neutral pH exhibited visual phase separation after 4-day of storage, exhibited >60% serum at 0.35% FXG after day 20 at neutral pH	Milk-based drinks, model salad dressing, meat protein, porcine myofibrillar protein, cloudy carrot juice, dairy desserts	Chen, Xu, & Wang, 2007; Qin, Xu, & Zhang, 2005; Khalloufi, Alexander, Douglas Goff, & Corredig, 2008; Liu, Shim, Poth, & Reaney, 2016; Liu, Shim, Tse, Wang, & Reaney, 2018; Qian, Cui, Wu, & Goff, 2012; Stewart & Mazza, 2000; Zhou, Meng, Li, Ma, & Dai, 2010
14	Brea Gum (BG)	An exudate from *Cercidium praecox* tree	Consist mainly of acid polysaccharides (l-arabinose, d-xylose, glucuronic and 4-O-methyl-D-glucuronic acids); 7.52% protein; Mw 2.79 × 10^3 kDa	5 to 20%	Monomodal PSD **Z-average:** 529.6 nm at 20% BG	Z-average slightly increased from ~400 to ~430 nm after 7-day of storage at room temperature, no phase separation during >10 month of storage using thermal treated BG (24 h, 110˚C) **CI:** 0.77% at 20% BG	Emulsion composite films	Castel, Rubiolo, & Carrara, 2017, 2018; Spotti, Cecchini, Spotti, & Carrara, 2016
15	Polysaccharides from *Dioscorea opposita Thunb* /Chinnese Yam (DOMP)	Extracted polysaccharides from *Dioscorea opposita Thunb* plant using alcohol precipitation method	Highly branched neutral polysaccharide-protein complex; a main chain of (1→3)-α-glucopyranose and β-galactopyranose-[(1→2)-α-mannopyranose]$_3$-(1→2)-α-mannopyranose-(1-6) as a side chain; Mw 143 kDa	0.2 to 1%	**Z-average:** 1.77 – 2.55 µm with different varieties of 0.2% DOMP	**zeta potential:** -27.5 to -64.4 mV at different varieties of 0.2% DOMP **pH:** the smallest oil droplets at any DOMP concentrations (0.2 – 1%) were formed at pH 9 (390 – 540 nm, respectively)	No studies	Ma *et al*., 2020; Ma *et al*., 2017; Zhao, Kan, Li, & Chen, 2005

No	Name	Source	Chemical Structure	Concentration range tested (%, w/w)	Emulsion formation	Emulsion stability (ES)	Application in Food Emulsions	References
16	Gum Tragacanth	Exuded from different species of *Astragalus*: *Astragalus* ***gossypinus***, *A. compactus* and *A. rahensis*	Heterogeneous branched anionic biopolymer, Mw: 8.4×10^5 kDa; two major components: tragacanthic acid (neutral AG: Gal, Ara, Fuc, Man) and bassorin fraction (pectic component: chain of 1→4-linked α-D-galacturonic acid substituted with β-D-xylopyranosyl)	0.2 to 1.3%	d_{43}: 33.08, 9.76 and 22.45 μm at 0.3% *A. gossypinus*, 0.5% *A. compactus* and 0.4% *A. rahensis respectively*	**Storage:** no phase separation (ES=90%) after 90 days storage at room temperature (0.5% of *A. gossypinus*) **CI:** 55-85% after 45-day of storage 50 ˚C	Frozen desserts	Abdolmaleki, Mohammadifar, Sheikhi, Matinfar, & Nayebzadeh, 2019; Farzi, Emam-Djomeh, & Mohammadifar, 2013; López-Franco, Higuera-Ciapara, Goycoolea, & Wang, 2009; Weiping & Branwell, 2000
17	Mesquite Gum (MG)	An exudate from *Prosopis spp*. trees (mesquite trees)	A highly branched complex heteropolyelectrolyte; a backbone of β-D-Gal residues linked by 1→3 and/or 1→6 linkages with β-D-configuration and residues of glucuronic acid and 4-O-methyl-D-glucoronic acid bound to D-Gal by α-D-(1→4) and β-D-(1→6) linkages ; 2-4.8% protein; Mw 2.12×10^6	2 to 5%	**EC:** 98.40% **Oil holding capacity:** 1.04 g oil/g gum d_{32}**:** 7 μm at 2.5% MG d_{43}**:** 12 μm at 5% MG **zeta potential:** -43.50 mV at 2% MG-stabilised emulsions	**pH:** emulsification index (E_{24}) (40%) remained stable after 24 h at pH 5-9 **Heat:** ES=67.12% after heating 85 ˚C, 30 min **Storage:** Emulsion yield after 24h was 40% **CI:** 6% at 5% MG after 6 hours of storage	Emulsify orange peel oil in an oil-gum solids ratio of 0.25	Alftren, 2012; beristain, 2002; Cortés-Camargo *et al*., 2018; García-Márquez *et al*., 2015; López-Franco *et al*., 2008; Vasile, Martinez, Pizones Ruiz-Henestrosa, Judis, & Mazzobre, 2016

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18	Persian gum/ Angun Gum /gum Zed, Zedo, Angun, and gharacia (PSG)	Gum exudate from *Amygdalus scoparia* or *Prunus scoparia* tree	An AG, a heteropolysaccharide (acidic and anionic); (1 → 3)-α-glucopyranose and -β-galactopyranose-[(1 → 2)-α-mannopyranose]3-(1 → 2)-α-mannopyranose-(1–6)- as a side chain; Mw: 16-42 kDa; 0.69 - 1.02% protein	0.5 to 2%	The optimum EC at SFPG: oil ratio of 2:1; 0.75 and 1% PSG was able to stabilise 5-20% O/W emulsions d_{32}**:** 9.89 µm at 1.5% PSG **zeta potential:** more negative with an increase in PSG concentration ~-30 mV at 0.5-1% PSG	**pH:** the lowest d_{32} (13 µm) at pH 4, zeta potential (-30 mV) for all pH 2-7 **Ionic strength:** no significant effect on d_{32} (14-15 µm) when 0-200 mM NaCl was added; *D32* decreased from 13.11 to 5.70 µm as the $CaCl_2$ concentration increased (0-300 mM) **Heat:** heating 25–90°C, the d_{32} changed from 11.04 to14.54 µm ; at 20 to 80 °C for 30 min, respectively; average droplet size was 0.80 µm	Encapsulant in spray drying encapsulation; orange peel essential oil nano emulsions; milk–sour cherry juice mixtures	Abbasi, 2017; Abbasi & Mohammadi, 2013; Golkar, Taghavi, & Dehnavi, 2018; Hashtjin & Abbasi, 2015; Jafari, Beheshti, & Assadpour, 2013 Yosefi, Abbasi, & Ezatpanah, 2012
19	Fucoidan	Polysaccharides extracted from brown seaweed groups, such as *Sargassum spp*. and sea cucumber	A group of fucose containing sulfated polysaccharides; An anionic polysaccharide trisaccharide→3)-β-D-GalNAc-(1→4)-β-D-GlcA(3-O-α-L-Fuc)-(1→	0.06 to 0.15%	E_{24}**:** 50 - 78.13%	**pH:** exhibited more negative zeta potential (-30 to -40 mV) from pH 2 to 6; CI was 85% at pH 2 but smaller CI (<10%) at pH 3-9; no significant change of d_{32} (~0.5 µm) over pH range	Stabilising protein-coated O/W emulsions, fish oil encapsulation, for multilayer emulsion system	Hifney, Fawzy, Abdel-Gawad, & Gomaa, 2016; Chang & McClements, 2015; Tako *et al.*, 2000 ; Kim *et al.*, 2015; Ustyuzhanina *et al.*, 2020
20	Gum Ghatti (GG)	An exuded gum from the *Anogeissus latifolia* tree	A backbone of 1→6 linked Gal*p* (hairy region), Ara*f*-1→4-Glc*p*-1→6-Gal*p* as smooth region. Side chains are terminated by Ara*f*, Rha*p*, Ara*p*, Gal*p* and Glc*p*A residues; Mw: ~1.0×10^6 g/; 4% protein	25%	High oil-load system up to 50% without the aid of weighting agent on medium chain triglycerides emulsions; d_{43}**:** 0.96 µm at 3% gum GG d_{32}**:** 0.85 µm at 3% gum GG	**Storage:** d_{32} remained constant (0.55-0.88µm) at all GG concentrations (2.5-20%) during 2-month storage at 25˚C	Beverage emulsions, mayonnaise type dressing, butter cream	Funami *et al.*, 2018; Kang, Guo, Phillips, & Cui, 2014; Kang *et al.*, 2011; Katayama, Ido, Sasaki, Ogasawara, Al-assaf, & Phillips, 2008

No	Name	Source	Chemical Structure	Concentration range tested (%, w/w)	Emulsion formation	Emulsion stability (ES)	Application in Food Emulsions	References
21	Gum Karaya (GK)	A gum from *Sterculia urens* tree	α-D-galacturonic acid and α-L-rhamnose residues as a backbone and 1→2 linked β-D-galactose or 1→3 linked β-D-glucoronic acid; contains 8% acetyl groups	1%	d_{32}**:** 0.2 μm d_{43}**:** 0.7 μm in emulsion containing protein–gum conjugate (1:1)	**High ES (**E_{24}:69%) on heated microwave solution GK at 1000 W for 12 mins after 30 days storage	No studies	Shekarforoush, Mirhosseini, Amid, *et al*., 2016; Shekarforoush, Mirhosseini, Sarker, *et al*., 2016; Verbeken, Dierckx, & Dewettinck, 2003
22	Artemisia sphaerocephala Krasch. polysaccharide (ASKP)	Extracted from the outer layer of the seed of Artemisia	Consist of two fractions: 60P (high Mw: 551 kDa) arabino-glucuronoxylan and 60S (low Mw: 39 kDa) galactoglucomannan	0.4 to 1.6%	**Z-average:** 1.7 μm at 0.8% ASKP **zeta potential:** -30 mV at 1.2 -1.6% ASKP	After storage at 60˚C for 3 days, d_{32} changed (2.08-3.50 μm)	No studies	Chen *et al*., 2014; Guo *et al*, 2011a ; Guo *et al*, 2011b ; Guo *et al*, 2012 ; Li, Hu, Li,& Ma, 2016 ; Li *et al*., 2018
23	Eggplant flesh pulp (EFP)	Pulp from peeled purple eggplant (*Solanum melongena* L.) flesh	Compose of galacturonic acid, galactose, glucose, xylose, rhamnose and mannose; 11.65% soluble protein	0.5 to 1.5%	Showed trimodal distributions at all concentrations	**CI:** >25% at concentration <1.25%, no changes on CI at 1.5% EFP during 7 days of storage; no significant changes of d_{32} (55 and 50 μm) at 1.25 and 1.5% EFP, respectively during 7-day of storage	Meatballs	Zhu *et al*., 2020
24	Tea Polysaccharide conjugates (TPC-W)	Water extraction from low-grade tea leaves and bud of the plant *Camellia sinensis*	Covalently bind Polysaccharides to a polypeptide backbone to form conjugates; main chain of (1→3)- β-Galp substituted at O-6 by (1→6)-linked β-Galp with side chains of α-Araf and terminal unit of α-Rha*p*, α-Fuc*p* and α-Ara*f*	0.5 to 3%	d_{32}**:** 6 and <1 μm at 0.5 and 3% TPC-W, respectively **zeta potential:** -25 mV at 3% TPC-W	**pH:** higher pH exhibited small droplet size, the smallest d_{32} was ~3μm (2% TPC-W) at pH 8; zeta potential (~22.5 mV) remained stable over the pH range **Ionic strength:** Addition of $CaCl_2$ (500 mM) and NaCl (50 mM) increased d_{32} up to 5μm and less negative zeta potential (~18 mV)	No studies	Han *et al*., 2020; Scoparo *et al*., 2016

No	Name	Source	Chemical Structure	Concentration range tested (%, w/w)	Emulsion formation	Emulsion stability (ES)	Application in Food Emulsions	References
25	Gum seed and pulp from Coapinole or Jatoba fruit (*Hymenaea courbaril* L)	Isolate of pulp and seed hydrocolloid from mature Jatoba fruit	A xyloglucan polysaccharide, 5.94% protein; Mw: 220 - 500 kDa; β-(1→4)-glucose linkages with substitutions in the carbons (1→6)-α-D-xylopyranose and β-D-galactopyranosyl-(1→2)-α-D-xylopyranose	0.5 to 2%	**EC:** ~70% at 2% hydrocolloid **Particle size:** 5-10 μm	**CI:** 43% and 50% at 1% hydrocolloid after 3-day of storage at 35˚C and 40 ˚C, respectively	Ice cream	Freitas *et al*., 2005; Hernández-Morales *et al*., 2018 Rincón, de Pinto, & Beltrón, 2006
26	Tamarind seed gum (TSG)	Extracted gum from tamarind (*Tamarindus indica* L.) kernel seed, a by-product of tamarind pulp industry	A galactoxyloglucan and neutral polysaccharide; β-(1→4)-D-glucan backbone with side chains of α-(1→4)-D-xylopyranose and (1→6) linked [β-D-galactopyranosyl-(1→2)-α-D-xylopyranosyl]; Mw: 720-880 kDa; 12-22% protein	0.2 to 2%	d_{32}**:** 1.35 μm at 2% low purified TSG (LP-TSG) **zeta potential:** -35.1 mV for 0.05% Free soluble protein-high purified TSG solution **EA: 90% at** 1% TSG	**Creaming velocity:** 0.06% at 2% LP-TSG **zeta potential:** -11.07 mV at 2% LP-TSG stabilised emulsions **ES:** no significant changes of d_{32} value (<2.5 μm) at any concentrations of LP-TSG emulsions over 21 days of storage at 4˚C **ES:** 91% at 0.2% TSG	Mayonnaise, salad dressing and emulsion films	Alpizar-Reyes *et al*., 2017; Crispín-Isidro *et al*., 2019; Freitas *et al*., 2005; Gidley *et al*., 1991; Rodrigues *et al*., 2018; Sharma, Mondal, Mukesh, & Prasad, 2014; Yamatoya, Tabuchi, Suzuki, & Yamada, 2020
27	Nopal mucilage / cactus pear plant	A mucilage extracted from *Opuntia ficus indica* planr (a member of cactaceae family)	A mixture of acidic and neutral polysaccharides, (1→4) linked α-D-galacturonic acid and (1→2) linked β-L-rhamnose with chains of (1→6)-β-D-galactose Mw 2.3×10^4 to 4.3×10^6 Da; 6.34% protein	1%	**EC:** 51.94% **Oil holding capacity:** 1.75 g oil/g gum **zeta potential:** -10mV at pH 7	**pH:** the highest E_{24} was >25% at pH 5, higher pH decreased E_{24} **Heat:** ES after heating treatment 51.96%	No studies	Cortes-camargo, 2017; McGarvie & Parolis, 1981; Sáenz, Sepúlveda, & Matsuhiro, 2004

No	Name	Source	Chemical Structure	Concentration range tested (%, w/w)	Emulsion formation	Emulsion stability (ES)	Application in Food	References
28	Mutamba seed mucilage (MSM)	A mucilage extracted from mutamba (*Guazuma ulmifolia Lam*.) seeds	1.28% protein; consists of 50% neutral monosaccharide (galactose and rhamnos) and 37% uronic acid (galacturonic and glucuronic acids)	1%	d_{32}**:** 4.67 µm ; bimodal distribution	**ES:** 20% during 24 hours of storage **Destabilisation index:** <2 compared to GA (>6) at 1% gum, 6 h storage, RT	Model orange peel O/W emulsions, encapsulation of volatile compounds	Pereira *et al*., 2019
29	Okra mucilage (OM)	A mucilage extracted from Okra fruits (*Abelmoschus esculentus*)	Acidic polysaccharides; α-(1→2)-rhamnose and α-(1→4)-galacturonic acid residues	0.25 to 2%	**EC:** 42.22 to 74.45%; the highest EC was achieved at 1% OM	**ES:** 42.22 to 74.45%; the highest ES was achieved at 1% OM	Coconut milk emulsion	Gemede, Haki, Beyene, Rakshit, & Woldegiorgis, 2018; Noorlaila, 2015; Tomoda, Shimada, Saito, & Sugi, 1980
30	*Tremella fuciformis* polysaccharide (TFP)	The polysaccharides are obtained *from Tremella fuciformis* fruit body (an edible and medicinal fungus in China)	Contains 0.72% uronic acid, 0.60% protein and 92.17% total sugar (rhamnose, xylose, mannose and glucose in the molar ratio of 1.13: 1: 4.70: 0.81)	0.5 to 4 %	**EA:** 100% Mean droplet size: 10 µm at 0.5% TFP **zeta potential:** 33.05mV at 0.5% TFP	**pH:** had smallest droplet size (14 µm) at pH 10 **ES:** 100% after 21 days at 21 ˚C **Freeze-thaw stability:** 100% at 4% TFP	No studies	Zhang, Zhang, Liu, & Wang, 2019 Zhang, 2017

No	Name	Source	Chemical Structure	Concentration range tested (%, w/w)	Emulsion formation	Emulsion stability (ES)	Application in Food	References
31	Puka Gum (PG)	An exudate from the wounded trunk of Puka tree (*Meryta sinclairii*)	Comprises of ~2% protein and ~95% carbohydrates (mostly arabinose and galactose); a highly branched backbone of β-1→3-linked galactopyranosyl residues linked with Araf oligosaccharides terminated variously by Rhap, Arap, Galp, and GlcpA residues; Mw ~4.5 × 106 g/mol (7x Mw of GA)	1 to 10%	~0.8 μm was obtained using 10% puka gum and 15% weighted orange oil d_{32}**:** 2 μm at 10% PG-stabilised emulsions	**ES:** all the beverage emulsions showed ringing during 5 days of storage, but a high level of turbidity was enhanced	Orange oil flavour emulsion concentrates	Breckenridge, 2019; Carnachan, Bell, Hinkley, & Sims, 2019; Sims & Furneaux, 2003; Wee, Sims, Goh, & Matia-Merino, 2019
32	*Ulva fascinata* polysaccharide (UFP)	Polysaccharide extracted from edible green algae (genus Ulva)	A sulphated and Anionic heteropolysaccharide, mainly aldobiouronic acid (6.5-19% uronic acid), β-D-glucuronic acid (1→ 4)-α-L-rhamnose-3-sulphate, α-L-iduronic acid (1→ 4)-α-L-rhamnose-3-sulphate	0.1 to 4%	d_{32}**:** < 1.0 μm at 3% UFP, 22um at 1% UFP (soft drinks) **zeta potential:** −26.30 to −38.37 mV at 0.1-4% UFP)	**pH:** zeta potential soft drinks -54 mV at pH 6.5 **No separation** after 30 days at >1% UFP	An emulsifying and clouding agent for orange terpene-based soft drinks	Lahaye & Robic, 2007; Morelli *et al*., 2019; Shao, Shao, Jiang, & Sun, 2016

No	Name	Source	Chemical Structure	Concentration range tested (%, w/w)	Emulsion formation	Emulsion stability (ES)	Application in Food	References
33	Yellow mustard mucilage (YM)	Mucilage extracted from the seed coat (dried layer on) of yellow mustard seeds (*Sinapis alba* L.)	β-1→4 linked glucosyl backbone, 61.1% of glucose and other sugars (galactose, rhamnose, mannose and xylose), 6.9% uronic acid	0.1 to 2%	d_{32}**:** ~1700 nm at 2% YM **zeta potential:** more negative with higher YM concentration. -33 mV at 0.5% YM	**ES:** 100% at 1% YM after 21 day of storage RT **pH:** Exhibited significantly smaller d_{32} (1000 nm) and less negative zeta potential ~33 mV at pH 4 **Freeze-thaw stability:** had higher value (98%) at 0.5% WSM compared to 2.0% GA and 1.0% pectin emulsions **No phase separation** (1-2% mucilage) after 21 days at 25 ˚C	Model salad dressing	Weber, taillie, & stauffer, 1974; Wu, Cui, Eskin, & Goff, 2009; Wu, Eskin, Cui, & Pokharel, 2015