Emulsifying Properties of a Novel Polysaccharide Extracted from the Seeds Of Basil (Ocimum basilicum L.)

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Abstract

The present study investigated the emulsifying properties of a novel polysaccharide extracted from the seeds of Basil (*Ocimum bacilicum*, L.). Emulsifying properties of basil seed gum (BSG) were evaluated in terms of emulsion droplet size distribution ($d_{32}$ and $d_{43}$), rheological properties (apparent viscosity and viscoelasticity), droplet charge (zeta potential), visual phase separation (at 20°C for 1 month period), and adsorption properties (surface/interfacial tensions). Soya oil-in-water emulsions (30% wt/wt) were formulated and stabilised by BSG containing <1.2% (wt/wt) protein and a major glucomannan fraction. Different BSG concentrations (0.1-1% wt/wt) were tested, as well as the effect of pH (1-12), salt (5-70 mM NaCl), heating (80°C, 30 mins) and purification (removal of proteins from gum) on 0.3% (wt/wt) BSG-stabilised oil-in-water emulsions.

Emulsions with monomodal droplet distributions and with oil droplet size below 1.0 µm ($d_{32}$) were formed with as little as 0.3% (wt/wt) BSG. The emulsifying properties of BSG were sensitive to changes in pH and salt. Generally, small emulsion droplets were formed at pH above 6.0 and low ionic strength. However, larger droplets were formed and zeta potential values decreased at low pH and high ionic strength. Microstructures confirmed the occurrence of coalescence over time. BSG appeared to exhibit strong hydrophobic character as fluorescing dye (usually for proteins) was detected at the interface, as well as polysaccharide inclusions were trapped within coalescing droplets after homogenisation, suggesting its strong adsorption. Heating and purification reduced the emulsifying properties of BSG. Nevertheless, the emulsions remained stable against phase separation.

The rheological properties of BSG emulsions appeared to be dependent on gum concentration and purification, but independent on pH, salt, and heating, which suggests the resistance of BSG to processing conditions, and thereby it could provide strong emulsion stability. Surface/interfacial tension measurements confirmed the adsorption of BSG at the oil-water interfaces. All gum preparations (crude, purified and protein-free) exhibited an ability to lower the tensions at the interface. However, purification of the gum reduced its adsorption activity, indicating that (i) protein plays an important role in gum adsorption, but it is not an absolute driving force for adsorption and (ii) gum itself becomes altered by the purification process.

BSG (0.17 % wt/wt carbohydrate purity) demonstrated excellent emulsifying and stabilising properties when compared to some other polysaccharides. Protein-free BSG produced larger droplets than crude BSG, but still it produced stable emulsions, comparable to other gums, such as sugar beet pectin. This suggests that the emulsifying and stabilising mechanism of the gum is not only ascribed to the surface-active protein moiety, but could also be attributed to the hydrophobic character of the polysaccharide itself. Overall, BSG is a promising gum, which can be considered as a novel hydrocolloid emulsifier.
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Chapter 1

General Introduction

Plant polysaccharides, also known as hydrocolloids or gums play a significant role in food processing, in addition to improving the texture and sensory quality of food products. Non-starch polysaccharides including galactomannans (e.g. guar, fenugreek, and locust bean) and glucomannans (e.g. konjac) are one of the important categories of plant-based polysaccharides (Cui, Ikeda, & Eskin, 2007). They possess different chemical and molecular properties, which make them useful in various food applications. Some of their important functional properties in food include thickening, gelling, binding and emulsifying. In addition, these plant-originated polysaccharides are important sources of dietary fibre (Cui, et al., 2007; Srivastava & Kapoor, 2005), although it can be observed that their rheological properties sometimes make it difficult to fortify at high levels due to complexities in processing. These versatile properties have fuelled the interest of the food industry to continue exploring some novel plant polysaccharides with functional properties, which can be used as potential food ingredients.

A wide range of food products exist as food emulsions, which can either be oil-in-water (O/W) or water-in-oil (W/O) systems. The formation of a food emulsion, with desirable quality attributes, is dependent on the type and quality of the raw ingredients (e.g. water, oil, emulsifiers, and stabilisers), in addition to the processing conditions (e.g. mixing, homogenisation, and heating) (McClements, 2004). Generally, emulsions are thermodynamically unstable systems due to the unfavourable interaction between the oil and water phases. Hence, they always tend to breakdown over a period of time. An emulsion is said to be kinetically stable, but only if there are no noticeable changes in the droplet size over a period of time. The long-term stability of an emulsion can, therefore, be achieved by using a surface-active material which adsorbs at the oil-water interfaces or by increasing the viscosity of the aqueous phase (Dickinson & Stainsby, 1988). An emulsifier is a surface-active substance which adsorbs to the interface and thereby, it provides stability against droplet flocculation, via strong repulsive interactions (electrostatic and steric) between the emulsion droplets (Dickinson, 1992, 2003; McClements, 2004).
Normally, polysaccharides are used as texture modifiers or stabilisers, in food emulsion systems. However, a number of plant polysaccharides such as gum Arabic and fenugreek gum are also used as food emulsifiers. These polysaccharides have been shown to adsorb at the oil-water interfaces and they are capable of forming stable O/W emulsions (Dickinson, 2003; Garti & Leser, 2001; Huang, Kakuda, & Cui, 2001). Their adsorption properties are believed to be attributed to the hydrophobic groups or proteinaceous moieties associated with them. Hence, surface-active polysaccharides can act both as emulsifiers and stabilisers in many instances. This study is an attempt to explore the functional properties of a new polysaccharide, which has been extracted from the seeds of *Ocimum basilicum* – and which can be potentially used as a novel food ingredient.

Basil (*Ocimum basilicum* L.) is an herbaceous plant popularly grown in India, Iran (Fekri, *et al*., 2008) and in some warm regions of Africa (Simon, *et al*., 1999). Aside from its culinary use, basil seeds have been traditionally used as a natural remedy for the treatment of indigestion, ulcer, diarrhoea, sore throats, and kidney disorders (Simon, *et al*., 1999; Vieira & Simon, 2000). In some regions in Asia, they are incorporated into food products, such as desserts and beverages as a source of dietary fibre (Mathews, Singhal, & Kulkarni, 1993).

The seeds of basil are mainly composed of two major fractions: (i) “glucomannans” (~43 %), and (ii) “(1→4)-linked xylan” (~24.3%) (Anjaneyalu & Channe Gowda, 1979). Recently, Razavi *et al.* (2009) reported the optimum extraction conditions of the polysaccharide from basil seeds in terms of pH, temperature, and water/seed ratio. The extracted basil seed gum (BSG) was found to contain a small fraction of protein (~1.5 %). Moreover, investigation into the rheological properties showed that BSG demonstrated a non-Newtonian pseudoplastic behaviour. It also exhibited a very high zero shear viscosity and a yield stress (Hosseini-Parvar, *et al*., 2009). So far, there has not been any study on the ability of this gum to emulsify an O/W emulsion system. Therefore, the aim of this study was to investigate the emulsifying properties of basil seed gum (BSG) and specifically to:
1. Determine the effect of processing conditions (e.g. gum concentration, pH, ionic strength, and heat) and the effect of purification (removal of protein) on the emulsifying properties of BSG,

2. Determine the adsorption properties (surface/interfacial tensions) of BSG,

3. Compare the emulsifying properties of BSG with other commercial hydrocolloids (e.g. gum Arabic, fenugreek, and sugar beet pectin).
Chapter 2
Review of Literature

2.1 Introduction
The aim of this review is to discuss the formation and stability of emulsions with an emphasis on oil-in-water emulsions stabilised by surface-active biopolymers (e.g. polysaccharides). The mechanisms of emulsion instabilities (and factors affecting their properties and stability) are also highlighted. In addition, naturally-occurring and chemically modified polysaccharides, which exhibit adsorption and emulsifying properties, are discussed. Finally, an investigation into the origin of the surface adsorption and the emulsifying and stabilising capabilities of hydrocolloid emulsifiers are also covered.

2.2 Food emulsions
Emulsions are dispersions of two immiscible liquids, wherein one liquid is uniformly dispersed as small droplets within another. The size of droplets ranges from 0.1µm to a visible size. In general, emulsions are divided into two categories: oil-in-water (O/W) and water-in-oil (W/O) emulsions. O/W emulsions consist of oil droplets dispersed in an aqueous phase, and W/O emulsions are water droplets dispersed in an oil phase. Examples of O/W emulsions include milk, cream, salad dressings, mayonnaise and sauces. Margarine and butter are typical examples of W/O emulsions (McClements, 2005; Morrison & Ross, 2002). The ensuing review is mainly focused on the O/W emulsion systems.

Emulsion formation involves the homogenisation of two immiscible liquids in the presence of a surface active material (McClements, 2005). There are two stages of homogenisation: primary homogenisation and secondary homogenisation. In a primary homogenisation, the separate oil and water phases are combined to form a coarse emulsion: whereas, in a secondary homogenisation, the size of droplets is reduced in an existing emulsion. Homogenisation requires energy: the majority of which gets dissipated in the form of heat. In secondary homogenisation, the energy is needed to deform and break up the droplets - a process which can be achieved by intense agitation (Walstra & Van Vliet, 2008). A high pressure homogeniser is
commonly used to produce emulsions with fine droplet sizes. However, a high speed mixer is used to produce a coarse emulsion prior to processing in a high pressure homogeniser. The coarse emulsion is passed into the homogeniser, thus forcing it through a narrow valve, where a combination of disruptive forces is applied, which results in the disruption of large droplets into smaller ones (McClements, 2005).

2.2.1 Emulsion instability
Thermodynamically, emulsions are unstable systems whose stability depends on their ability to withstand the changes affecting their properties over time. This is the kinetic stability, which involves the maintenance of one single phase, with no changes in droplet size, over a period of time. Emulsions are subject to several types of physical mechanisms, which can affect their long-term stability, i.e. their droplet size and distribution (McClements, 2005; Norde, 2003). Physical instabilities can include creaming, coalescence, partial coalescence, flocculation, Ostwald ripening, and phase inversion (Rousseau, 2002) as illustrated below in Figure 1.

![Types of instabilities in O/W emulsions](image)

**Figure 1:** Types of instabilities in O/W emulsions (McClements, 2005)

2.2.1.1 Creaming
The movement of dispersed droplets to the top of an O/W emulsion is due to a difference in density between the dispersed phase (above certain size) and the aqueous phase. This movement and subsequent aggregation of droplets is termed ‘creaming’
Creaming of droplets leads to the formation of a concentrated emulsion layer on top of the original emulsion (Friberg, 1992). This process is only reversible if the cream layer is resistant to coalescence. Creaming has an undesirable effect on the overall quality of an emulsion. The rate of creaming can be controlled by: (i) minimising the density difference between the droplets and the aqueous phase; (ii) reducing the droplet size; (iii) modifying the rheology of the continuous phase; and (iv) increasing the droplet concentration. Stokes’ Law explains the rate of creaming by the mathematical equation (Dickinson, 1992) described as follows:

\[
\nu = \frac{2gr^2(\rho_2 - \rho_1)}{9\eta}
\]

where, \(\nu\) = creaming velocity, \(g\) = local acceleration of free fall (=9.8ms\(^2\)), \(r\) = droplet radius, \(\eta\) = shear viscosity, \(\rho\) = density and the subscripts 1 and 2 refer to the aqueous and dispersed phases, respectively.

Based on Stokes’ equation, emulsions with smaller droplets are more resistant to creaming than emulsions containing larger droplets. Normally, polysaccharides are added into emulsions, in order to prevent creaming by increasing the viscosity of the continuous phase. Hence, polysaccharides are known as emulsion stabilisers. Also, increasing the dispersed phase volume fraction will minimise the movement of droplets (Ford, et al., 2004; McClements, 2005).

### 2.2.1.2 Flocculation

Flocculation is a type of droplet aggregation found in emulsion systems. This is a phenomenon in which two or more droplets come together to form an aggregate, but where droplets retain their identity (McClements, 2005). In terms of structure, flocs can be categorised as (i) open packing and (ii) close packing (see Figure 2). The open packing structure is formed, when the droplets attached firmly to each other after collision and they are unable to undergo any structural rearrangement. This type of floc traps large amounts of continuous phase within it. On the other hand, the droplets in a close packing structure do not always stick together after collision and they may undergo structural rearrangements and hence, the droplets can pack more closely together. This type of floc has a more compact structure and it traps a lesser amount of the continuous phase.
Flocculation can either be a reversible (weak flocculation) or an irreversible (strong flocculation) process. Usually, emulsions which contain weakly flocculated aggregates, exhibit shear-thinning flow behaviour. The flocculated droplets are deformed and disrupted with increasing shear stress, which results in a decrease in the apparent viscosity of the emulsion (see Figure 3). Strongly flocculated emulsions on the other hand, have elastic properties below the yield stress. A network of aggregated droplets can be stable against creaming, but it may be unstable to syneresis, or the release of the serum phase (Dickinson, 1992; McClements, 2005).

The two important driving forces for droplet flocculation are Brownian motion (perikinetic flocculation) and applied shear forces (orthokinetic flocculation). In
quiescent systems, the aggregations of droplets are mainly due to gravity (large droplets) and Brownian motion (small droplets). The shear-induced flocculation takes place, when the emulsion system is subjected to mechanical agitation. Hence, the rate at which flocculation occurs, depends on the (i) collision frequency and (ii) attractive forces between emulsion droplets, which depend on the interfacial layers (Dickinson, 1992; McClements, 2005).

In this review, only the mechanisms of flocculation which are prevailing in emulsions stabilised by biopolymers are discussed. Three types of droplet-droplet interactions are considered. These include: (i) bridging flocculation, (ii) depletion flocculation and (iii) electrostatic flocculation.

2.2.1.2.1 Bridging flocculation
Bridging flocculation occurs when an emulsion system is stabilised by a biopolymer molecule, at an insufficient amount, which partially covers the surface of the emulsion droplets (Dickinson, 1992; McClements, 2005). Usually, macromolecular biopolymers, such as polysaccharides and proteins, provide better stability against flocculation, by forming a thick protective layer around the emulsion droplet. However, when a biopolymer chain, which contains more than one possible point of attachment to the droplet surface, is added at very low concentrations, it is likely that the polymer molecule on one droplet becomes shared by another, through partial adsorption. As a consequence, the attachment of one polymer chain, to more than one droplet, leads to bridging flocculation (see Figure 4).

Figure 4: Bridging flocculation in an O/W emulsion. A biopolymer chain is shared by more than one droplet leading to bridging between droplets
Additionally, bridging flocculation may occur at high homogenisation pressure when the surface area of the emulsion droplets increases relative to the amount of biopolymer available to stabilise them. It can also happen either during (or after) the homogenisation process, when a biopolymer is poorly associated to a droplet and then some of its segments may desorb and become firmly attached to a neighbouring droplet. Hence, this type of bridging flocculation can be prevented by ensuring that there is a sufficient amount of biopolymer to provide complete coverage of the droplet surface (Friberg, 1992; McClements, 2005).

Bridging flocculation may also take place when polymer molecules having opposite electrical charge, to that of the already stabilised emulsion droplets are added to the emulsion in small quantities. An example of this type of bridging flocculation is described when pectin is added to milk protein-stabilised emulsions at a certain pH. At a pH below the isoelectric point of milk proteins, the emulsion droplets are positively charged and the negatively charged pectin molecules act as a bridge, which connects two or more droplets together into flocs. This bridging flocculation can be controlled by: (i) increasing the amount of pectin so all droplets are fully covered by the casein-pectin layer and (ii) ensuring that the biopolymer stabilising the droplets and the biopolymers at the aqueous phase are of similar charges so they do not interact (i.e. pH > pI of the protein).

Furthermore, electrostatic bridging of emulsion droplets may occur when the electrically charged droplets are cross-linked by molecules of the opposite charge. For instance, droplets stabilised by a negatively charged biopolymers can be bridged by multivalent cations (e.g. Ca$^{2+}$ and Fe$^{3+}$). In this case, flocculation can be avoided by using neutral or non-charged biopolymer emulsifiers that provide stability mainly, via a steric mechanism (McClements, 2005). In summary, bridging flocculation is not only associated with the insufficient concentration of an initial amount of biopolymer molecules for emulsification, but it may also be attributed to the interactions between adsorbed polymers at the interface and the small quantities of biopolymers added in the aqueous phase when attractive forces between both biopolymers exist. Additionally, the presence of counter-ions may cause bridging between charged droplets.
2.2.1.2.2 Depletion flocculation

The depletion flocculation mechanism, in an O/W emulsion system, is associated with the osmotic pressure gradient caused by the non-adsorbing biopolymer, from between approaching droplets (see Figure 5). Generally, droplet flocculation arises when the emulsion droplets come close together and unadsorbed biopolymers are excluded (depleted) from the narrow region of the continuous phase between the droplets. As a consequence, there is an osmotic pressure gradient which forces the solvent to move out from between the droplets into the bulk continuous phase. The resulting effect is that droplets are attracted to each other, thus causing flocculation and subsequent loss of emulsion stability (Dickinson, 1992; McClements, 2005; Robins, Watson, & Wilde, 2002).

The magnitude of the effect of the depletion flocculation is dependent on the non-adsorbed biopolymer concentration. In a dilute solution, below the critical biopolymer concentration, the osmotic gradient is too weak to induce flocculation. However, as the concentration is increased, the osmotic force becomes strong enough to cause flocculation. The critical concentration value reduces with increasing droplet concentration/size and with the increasing molecular weight or size of the non-adsorbing biopolymer. Theoretically, the depletion potential ($\Delta G_D$) can be expressed as follows (Dickinson, 1992):

**Equation 2**

$$\Delta G_D = \frac{2\pi a \delta^2 (\mu_s - \mu_s^\circ) [1 + (2\delta/3a)]}{V_S}$$

where, $\delta$ = the depletion thickness layer, which is approximately equal to the radius of gyration of the unadsorbed biopolymer molecule in dilute solution, $V_S$ = the molar volume of the solvent, $\mu_s - \mu_s^\circ$ = the difference in solvent chemical potential between the biopolymer solution and pure solvent, and $a$ = the radius of the particle.
The influence of depletion flocculation, on the creaming stability of protein-stabilised O/W emulsions containing different concentrations of non-adsorbing biopolymer (protein), has been reported by Dickinson et al. (1997). A concentrated (35 or 45 vol %) O/W emulsion was stabilised, by using varying amounts of protein (1-6 wt % sodium caseinate) as the sole emulsifying agent (see Figure 6). At protein concentrations of 2 (wt/wt) %, emulsion droplets were stable (sufficient amount of protein to avoid bridging) and they were protected against flocculation and coalescence, due to the thick protein interfacial layer providing electrostatic and steric stability. However, with a further increase of protein content (>3wt %), the creaming stability was reduced, as indicated by the increase in the rate of phase separation. This instability is attributed to depletion flocculation caused by the unadsorbed caseinate in the aqueous phase.
Moreover, polysaccharides, such as xanthan gum and guar gum, which are widely used as food stabilisers, have been found to induce flocculation of the emulsion droplets, by a depletion mechanism. The presence of these polysaccharides at very low gum concentrations (<0.075%), increased the rate of creaming. However, at higher gum concentrations (>0.1%), the creaming rate was significantly reduced, due to the increased viscosity of the continuous phase (Velez, Fernandez, & Munoz, 2003).

2.2.1.2.3 Electrostatic flocculation

Electrostatic flocculation may occur, when the charge of the electrically stabilised emulsion droplets are neutralised and the electrostatic repulsion between the droplets is no longer strong enough to prevent flocculation. Electrostatic repulsion originates
from the interaction between the emulsion droplets of like charges, which prevents them from coming together to aggregate. Hence, electrically charged emulsifiers, such as proteins and polysaccharides, can stabilise emulsion droplets against flocculation, via electrostatic repulsion, in addition to the steric stability provided by thick viscoelastic layers of polymers. The stability of these emulsions largely depends on the pH and ionic strength changes in the system.

Electrostatic flocculation, in emulsions stabilised by biopolymers, can be influenced by pH conditions. At pH values, where biopolymers have no net charge, the electrostatic repulsion between emulsion droplets is too weak to prevent droplets from aggregating. On the other hand, at pH values, where biopolymers have a high net charge, the electrostatic repulsion between droplets is strong, which prevents them from flocculation (Dickinson, 1992; McClements, 2005). Figure 7 shows the effect of pH on the flocculation stability of protein-stabilised O/W emulsions. Extensive flocculation (growth in droplet size) occurs at pH values, near to the isoelectric point of the whey proteins (pH≈5), due to the screening of electrical charges, which leads to droplet flocculation.

![Figure 7: Changes in the droplet size of O/W emulsions stabilised by whey protein isolate, at different pH conditions (McClements, 2005)](image)

Moreover, electrostatic flocculation, can happen when the ionic strength of the aqueous phase is increased. The addition of salt tends to decrease the electrical double layer, by screening the electrostatic repulsion between droplets, until it is no longer sufficiently strong enough to prevent flocculation. Electrostatic flocculation can,
therefore, be avoided by: adjusting the environmental conditions of pH and ionic strength in order to maximise the electrical charge on the adsorbed biopolymer and (ii) using biopolymers, which may provide stability mainly through a non-electrostatic mechanism (e.g. steric repulsion). Comparing the stability of biopolymers (proteins and polysaccharides) against electrostatic flocculation, polysaccharides tend to provide better emulsion stability than proteins, because they are more stable to a wide range of environmental conditions, such as pH, ionic strength and temperature (Chanamai & McClements, 2002).

2.2.1.3 Coalescence
Coalescence is the fusion of two, or more, droplets together to form a single larger droplet. This process occurs when the thin film between the neighbouring droplets is ruptured (McClements, 2005; Walstra & Van Vliet, 2008). In O/W emulsions, coalescence leads to creaming, due to the growth in droplet diameter and eventually it results in the formation of an oil layer on the surface, which is referred to as the ‘oiling off’ of the emulsion. Unlike creaming and flocculation, coalescence is an irreversible process. Dickinson (1992) indicated that the rate, at which coalescence occurs, is influenced by (i) ‘film thinning’ which depends on the colloidal and hydrodynamic interactions between droplets, and (ii) ‘film rupture’ which largely depends on the thickness and mechanical properties of the interfacial membranes (Figure 8). The tendency of emulsion droplets to coalescence is, therefore, dependent on the stability of the liquid film of the aqueous phase separating them. Biopolymer interfacial layers are less susceptible to coalescence, given the strong steric stability provided by the proteins and polysaccharides.
Coalescence can occur: (i) when an emulsion is subjected to mechanical forces (orthokinetic stability), which cause droplet collision in the presence of weak interfacial layers or (ii) when the droplets come into close contact with each other, for an extended period of time. Droplet coalescence can be controlled by ensuring that there is a strong repulsive interaction between droplets, in order to prevent close contact, which may result in the rupture of interfacial films between the droplets. Emulsion droplets stabilised by thick viscoelastic interfacial membranes are resistant to rupture and provide strong steric repulsion, and hence are resistant to coalescence (Dickinson & Matsumura, 1991). Examples of thick interfacial layers have been described for biopolymers, such as proteins and polysaccharides.

### 2.2.1.4 Partial coalescence

In O/W emulsions, a fraction of the oil droplets can crystallise, depending on the degree of fat saturation and temperature. Partial coalescence occurs when partially crystallised droplets, with low steric stability interfacial layers, encounter other droplets; crystals protruding from the surface of partially crystallised droplets can easily rupture the interfacial membranes. Partial crystallisation occurs during flow or agitation, which leads to the formation of irregular shaped droplet aggregates (Rousseau, 2002; Walstra & Van Vliet, 2008).
Partial coalescence is likely to occur in emulsions, which contain oil droplets that remain in close contact for extended periods, that is, flocculated emulsions, concentrated emulsions and creamed layers. Hence, droplet-droplet interactions, such as electrostatic and steric repulsion, which prevent droplets from coming into close contact, can decrease the rate of partial coalescence. Biopolymer emulsifiers are more resistant to penetration by fat crystals, due to their thick interfacial films and the steric effect that they provide (McClements, 2007). Consequently, partial coalescence is less likely to occur in emulsions stabilised by proteins and polysaccharides.

2.2.1.5 Ostwald ripening

Ostwald ripening is a phenomenon in which large droplets grow in size, at the expense of small ones, due to the diffusion of the dispersed phase from one droplet to another, via the aqueous phase. This process occurs because the solubility of the material contained in the droplet increases, as the size of the droplet decreases. As a result, solute concentration in small droplets is higher, than that in larger ones, which leads to the movement of solute from smaller droplets to larger droplets, due to a concentration gradient. Generally, Ostwald ripening is insignificant in O/W emulsions because, most of the oils forming the dispersed phase are not soluble in water (McClements, 2005, 2007).

The rate of Ostwald ripening increases, with increasing interfacial tensions. In order to retard its progress, emulsifiers, which are effective in reducing the interfacial tension, are therefore used. Moreover, the transport of molecules, from one droplet to another, is dependent on the rate at which the molecules diffuse across the interfacial film. It is, therefore, possible to control Ostwald ripening by increasing the thickness of the interfacial membrane. The interfacial layers which are resistant to deformation, may also be able to prevent Ostwald ripening. Consequently, emulsion droplets stabilised by proteins or polysaccharides, which form thick and viscoelastic films, are likely to be stable against shrinkage or growth of droplets, given their strong mechanical resistance to changes (McClements, 2005).

2.2.1.6 Phase inversion

Phase inversion refers to the changes in emulsion systems wherein O/W emulsions are converted to W/O emulsions, or vice versa (Dickinson, 1992). Phase inversion results
from an alteration in the composition of the emulsion system (e.g., emulsifier type, emulsifier concentration and dispersed phase volume fraction), storage conditions (e.g., temperature) and processing conditions (e.g., mechanical agitation). In addition, it involves a series of complex processes, which occur during flocculation, coalescence and emulsion formation. At the ‘balance point’ - a point where phase inversion occurs - the system may contain parts of O/W emulsions and W/O emulsions. Phase inversion may have an adverse effect on the quality and stability of some food emulsions (McClements, 2005; Rousseau, 2002).

Phase inversion in food emulsions can be promoted by either: (i) surfactants or (ii) fat crystallisation (McClements, 2005). Phase inversion, induced by fat crystallisation, takes place when an O/W emulsion containing liquid droplets is cooled to a temperature, where the droplets are partly crystalline and then sheared, resulting in phase inversion into W/O emulsion. Partial coalescence causes this type of phase inversion, which leads to the formation of a continuous fat crystal network that traps water droplets within it. Hence, emulsifiers that form dense and viscoelastic films, such as protein and polysaccharides, may be able to protect the emulsion against this type of phase inversion, due to their ability to control partial coalescence.

2.2.2 Steric and electrostatic stability
An O/W emulsion is said to be ‘stable’, if there is only a minimal detectable flocculation of droplets, over a certain period of time. The emulsion droplets can be prevented from aggregating by a combination of steric and electrostatic mechanisms. These important stabilising mechanisms are discussed in the succeeding sections.

2.2.2.1 Steric stability
The emulsion stability conferred by the physical barrier provided by adsorbed biopolymers is called steric stabilisation (see Figure 9). When two emulsion droplets come close to each other, their adsorbed layers may start to overlap and interact with each other. Steric interactions are a result of the intermingling and/or compression of the interfacial layers (Dickinson, 1992; McClements, 2005). This type of interaction can be divided into two contributions as shown below.

Equation 3  
\[ w_{\text{steric}}(h) = w_{\text{elastic}}(h) + w_{\text{osmotic}}(h) \]
The elastic contribution is attributed to the compression of the interfacial layers, whilst the osmotic contribution is attributed to the intermingling of the biopolymer molecules, within the interfacial layers. At intermediate separations, the steric interaction can either be attractive or repulsive, depending on the quality of the solvent, due to the osmotic contribution. However, at close droplet separations, there exists a strong steric repulsion, due to the elastic contribution.

![Figure 9: Schematic representation of the steric polymeric stabilisations](image)

Moreover, three important conditions are needed for an effective steric stabiliser: (i) the biopolymer interfacial layer must be sufficiently thick; (ii) the adsorbed biopolymers must provide complete coverage to the droplet surface; and (iii) the biopolymer must be strongly attached to the surface. Generally, biopolymers that form thick and loosely packed interfacial films, such as polysaccharides, are efficient at stabilising emulsion droplets against flocculation through a steric repulsion mechanism (Dickinson, 1992, 2003; McClements, 2004).

### 2.2.2.2 Electrostatic stability

Colloidal science explains electrostatic stabilisation in terms of an electrical double-layer (Figure 10). A charge is generated on the droplet surface, and a more diffuse cloud of oppositely charged ions develops around it. As the two droplets approach each other, the charge effectively provides a barrier to closer droplet interactions. Electrostatic stabilisation increases, together with the thickness of this layer (Dickinson, 1992; McClements, 2005; Norde, 2003).
Moreover, the electrostatic interactions are influenced by the electrical properties of the droplet surfaces and the ionic strength (Marinova, et al., 1996; Nakauma, et al., 2008) of the continuous phase. The electrical properties of a surface are characterised by the surface charge density ($\sigma$) and the electrical surface potential ($\psi_0$). The surface charge density is defined as the quantity of electrical charge, per unit surface area, whilst the surface potential is defined as the free energy needed to increase the surface charge density, from zero to $\sigma$ (McClements, 2005). These values depend on the nature and concentration of emulsifiers adsorbed at the interface in addition to the surrounding environmental conditions, such as ionic strength, pH and temperature.

The charged surfaces of emulsion droplets can be characterised by describing their zeta potential (see Figure 11). The zeta potential provides an indication of the electrostatic stability of an emulsion system. The presence of droplet charges attracts the oppositely charged ions (counter ions) towards the surface and repels ions with a similar charge (co-ions). The region wherein the concentrations of the counter ion and co-ion (near the surface) are unequal is called the electrical double layer. This double layer consists of two regions: an inner region (Stern layer) of strongly adsorbed ions and an outer region (diffuse layer), where ions are diffusely distributed and less firmly attached. The electrical potential, which exists between the tightly bound surface liquid layer of the droplet and the bulk phase of the solution, is called the zeta potential ($\zeta$) (Hunter, 1981; L. C. Li & Tian, 2007; Tan, 2004).

Figure 10: Schematic representation of the electrostatic stabilisation
The zeta potential may also serve as a useful parameter to indicate the electrostatic stability of droplets against flocculation, in addition to the flow behaviour of the emulsion system (McClements, 2005; Norde, 2003). For instance, protein-stabilised emulsions have high zeta potential values, at pH above or below their isoelectric point (see Figure 12). This indicates that the charge of emulsion droplets is high enough to prevent flocculation, due to the strong electrostatic repulsion between the droplets. However, at pH close to the isoelectric point, the electrical charge of proteins is screened and the electrostatic repulsion is relatively weak, to prevent droplets from coming together to aggregate. Subsequently, aggregation of droplets results in an apparent increase in the viscosity of an emulsion.
Figure 12: A schematic diagram of zeta potential versus pH showing the position of the isoelectric point and the pH values where the dispersion system is expected to be stable (Source: www.silver-colloids.com/.../Intro/pcs18A.html)

Moreover, the viscosity of the electrostatically stabilised emulsions is sensitive to the ionic strength. At high ionic strength, electrostatic interactions are screened and therefore the biopolymer behaves as an uncharged molecule. At reduced ionic strength however, the charged biopolymer molecules expand due to “intramolecular” electrostatic repulsion. The effect of ionic strength on the intra and intermolecular interactions, is manifested in the viscosity of the aqueous phase known as the electroviscous effect (Norde, 2003).

The electrical properties (and hence the electrostatic stability) of emulsion droplets are determined by the characteristics of the interfacial layer formed. Surface active biopolymers are ionic, or capable of being ionised. The characteristics of the interfaces they form depend on the type and number of ionisable groups present. The $pK$ values of the charged groups are particularly important, when determining the degree of the ionisation, at a certain pH. Proteins contain acidic and basic groups, whose extent of ionisation is dependent on the pH and ionic strength of the continuous phase (Dickinson & Stainsby, 1988). Surface-active polysaccharides may also contain ionisable groups (McClements, 2005). When comparing the electrostatic stability of emulsions stabilised by biopolymers, emulsions stabilised by polysaccharides tend to be more stable than emulsions stabilised by protein, because protein-stabilised
droplets tend to undergo flocculation at pH values closer to the isoelectric point of the proteins.

2.2.3 Factors affecting emulsion properties and stability

The physicochemical, sensory properties and stability of food emulsions are influenced by several factors: Droplet size, droplet charge, interfacial rheology and rheology of the aqueous phase determine the important characteristics of emulsions. The stability of the emulsion is significantly affected by the types of emulsifiers used in the system, in addition to the environmental conditions including ionic strength, pH, and temperature (Kulmyrzaev & Schubert, 2004; McClements, 2007).

2.2.3.1 Droplet size

The size of droplets in an emulsion plays an important role, because it determines the stability, appearance, mouthfeel and rheology of the final product. Typically, the particle size, in a food emulsion is within the range of 0.1-50 µm in diameter. Food emulsions contain droplets of varying sizes and therefore, it is important to characterise both the size distribution and the average size of the droplets. The distribution of particle size is represented by a plot of droplet frequency (volume), against droplet size (diameter) (McClements, 2005, 2007).

The droplet size of an emulsion is influenced by factors such as: type and concentration of an emulsifier; homogenisation process; composition of the dispersed and continuous phases; and temperature. The emulsifier must be sufficient enough to completely cover the surface of the oil droplets and reduce the interfacial tension. The faster the emulsifier adsorbs at the surface, the smaller the droplet size. A high-pressure valve homogeniser is normally used, to further reduce the droplet sizes in pre-homogenised emulsions.

Moreover, the composition of both the oil and continuous phases may alter the viscosity of the emulsion and consequently affect the size of the droplets formed. Increasing the viscosity of the emulsion suppresses the formation of eddies responsible for droplet disruption and thus, large oil droplets are formed. Heating the emulsion, on the other hand, slightly decreases the interfacial tensions between the oil and water phases, which results in the formation of small droplet sizes. Furthermore,
the viscosity of an O/W emulsion tends to decrease with an increase in temperature and this facilitates the break-up of droplets, at elevated temperatures (McClements, 2005).

Surface-active polysaccharides can be used to emulsify emulsion droplets. For instance, hydrophobically modified cellulose derivatives can emulsify and stabilise soya O/W emulsions. However, the droplets formed are larger, in comparison to the droplets produced by small molecule surfactants, or proteins at similar conditions (Darling & Birkett, 1987). The relatively reduced emulsifying capabilities can be attributed to their high molecular mass related to a more inefficient adsorption (Dickinson, 2003).

Furthermore, a study on the micro encapsulation properties of gum Arabic and several food protein emulsifiers (soy protein isolate, whey protein isolate and sodium caseinate) revealed that whey protein isolate and sodium caseinate produced the smallest emulsion droplets, in a range of 0.6-1.4 µm. Gum Arabic-stabilised emulsions contained larger emulsion droplets, ranging from 1-5-1.7 µm. Although the droplets stabilised by gum Arabic were larger than those stabilised by proteins, they remained stable against coalescence or aggregation during storage (Kim, Morr, & Schenz, 1996).

2.2.3.2 Droplet charge
In food emulsions, droplets have an electrical charge that strongly influences their stability (Marinova, et al., 1996). The electrical charge of these droplets is similar and the electrostatic interaction between these charged droplets is repulsive, which prevent droplets from coming together to flocculate. This charge originates from the emulsifiers adsorbed at the interfaces, which are ionised or ionisable. Hence, the electrical charge of emulsion droplets depends on the characteristics of the material forming the interfacial layer. As previously discussed in electrostatic stabilisation, biopolymers, such as proteins and polysaccharides, contain either ionised or ionisable groups. Proteins have acidic (-COOH → COO⁻ + H⁺) and basic (NH₂ + H⁺ → NH₃⁺) groups, whereas many polysaccharides such as pectin and konjac mannan have acidic groups (-COOH) (Du, et al., 2006), which may be ionised. The magnitude and sign of the electrical charge of protein emulsifiers are largely influenced by the pH and ionic
strength of the aqueous phase, whilst the electrical charge of surface-active polysaccharides depends on the type of the functional groups attached to their backbone as well as the medium conditions (McClements, 2007).

### 2.2.3.3 Rheology of the aqueous phase/dispersed phase

Food emulsions are very complex systems, which exhibit different rheological properties. The viscosity of a fluid emulsion is proportional to the viscosity of the aqueous phase. As a result, modifications on the rheology of the aqueous phase may influence the rheological behaviour of the entire emulsion system. In emulsion systems, in which the droplet concentration is below the maximum packing fraction, macromolecular polymers (e.g., polysaccharides and proteins) are added in order to increase their viscosity and improve their stability against creaming (McClements, 2005). In general, the more viscous the continuous phase, the slower is the rate of droplet aggregation and the rate of creaming. Emulsions stabilised by addition of polysaccharides are, therefore, stable against creaming or coalescence due to the increased viscosity of the continuous phase, which restricts the movement of droplets.

The ability of the biopolymers to increase the viscosity of the continuous phase depends on their molecular structure. The effective volume of a biopolymer in solution is higher compared to the volume occupied by the actual polymer chain. This is due to the fact that the biopolymer molecule traps a large amount of solvent as it rotates due to Brownian motion (McClements, 2005; Williams & Phillips, 2003). This phenomenon can be described in terms of volume ratio, $R_v$:

\[
R_v = \frac{V_E}{V_A} \approx \frac{4\pi r_g^3 \rho N_A}{3M}
\]

where $V_E$ = ‘effective’ volume of the biopolymer chain in solution, $V_A$ = the actual volume occupied by the biopolymer, $r_g$ = the radius of gyration of the polymer, $\rho$ = the density of the polymer molecule, $N_A$ = Avogadro’s number, and $M$ = the molecular mass of the biopolymer.

Biopolymers, with extended conformational structures, have greater volume ratios than biopolymers with compact structures. With a similar molecular mass, the volume
ratio of stiff and linear biopolymers is higher, than that for the branched ones. In addition, as the electrostatic repulsion between charged molecules increases, the volume ratio also increases, because the biopolymer molecules are extended (McClements, 2005; Walstra, 2003).

The rheology of many O/W emulsion products is determined by the presence of biopolymers in the dispersing medium or continuous phase, which provide increased viscosity, shear-thinning flow behaviour and stability against the creaming of droplets. Many polysaccharides also encourage the formation of a three-dimensional network of aggregated droplets, due to their ability to induce depletion flocculation. This droplet network tends to increase the viscosity and the degree of shear-thinning of the emulsions containing unadsorbed polysaccharides, in the continuous phase (McClements, 2005).

2.2.3.4 Types and properties of emulsifiers
Emulsifiers are surface-active materials, which adsorb to oil and water interfaces and they prevent emulsion droplets from aggregating and coalescing. An effective emulsifier can form and stabilise an O/W emulsion, if it has the ability to rapidly adsorb to the interface during homogenisation: It significantly lowers the interfacial tension and forms a protective interfacial layer, which prevents the droplets from flocculation, under environmental stresses. The important functional properties of an effective emulsifier include: (i) the ability to produce small droplet sizes; (ii) a minimum amount needed to form a small droplet; and (iii) the ability to provide long-term stability to the emulsion system (McClements, 2005).

The commonly used emulsifiers in food emulsions are (i) small-molecule surfactants, and (ii) macro-molecular emulsifiers. Macro-molecular emulsifying agents are generally those naturally-occurring proteins from eggs and milk. They also include some naturally-occurring hydrocolloids, such as gum Arabic and galactomannans. Polysaccharides, which are chemically modified, such as pectin and starch are also considered to be surface-active agents (Dickinson, 2007; Garti & Leser, 2001). These macromolecular polymers have been shown to aid in emulsion formation, due to their surface-active properties. They can strongly adsorb at the interface and lower the
interfacial tension in addition to forming a barrier (electrostatic and/or steric) between emulsion droplets.

Moreover, their solvation properties may increase the interfacial layer thickness and interfacial viscosity and thereby improve the stability of the emulsion system. Normally, biopolymers are not as effective as small molecule surfactants, at forming small emulsion droplets. Nevertheless, biopolymer-stabilised emulsion droplets are more stable, because of the mechanical protection provided by the adsorbed film surrounding them (McClements, 2005).

2.2.3.5 Rheology of the interfacial layer

The rheological properties of the interfacial membrane are significant, in relation to the formation and stability of many emulsion systems. Interfacial rheology deals with the study of deformation and the flow behaviour of adsorbed layers at air/water or oil/water interfaces (Dickinson, 1992; Erni, et al., 2007; Norde, 2003). Similar to the rheology of the bulk phase, the important rheological properties of an interface are its viscosity and elasticity (Norde, 2003).

The surface of the droplets may encounter different deformations, when an emulsion is subjected to mechanical stresses. When stress is applied, the interface may undergo disturbances, without changing the total surface area: This is termed as ‘interfacial shear deformation’. Stresses may also cause the surface area to either expand or shrink, which is known as ‘interfacial dilational deformation’. Moreover, an interface may exhibit solid-like properties described by an ‘interfacial elastic constant’ or liquid-like properties described by an ‘interfacial viscosity’. In general, biopolymer interfaces demonstrate viscoelastic behaviour (McClements, 2005, 2007).

Normally, globular proteins (and some polysaccharides that cross-link and intermingle at the interface) form a highly viscous or elastic interfacial layer. The elasticity of their interfacial layer tends to provide resistance against deformations, which cause an emulsion to break down. In contrast, biopolymers (e.g. casein), which do not intermingle and cross-link, form interfacial layers with low viscosities or elastic modulus (Dickinson, 1992; McClements, 2005). The rheology of the interfacial layer is therefore determined by factors, which may affect the structure and strength of the
interactions between the adsorbed molecules, such as pH, concentration, temperature and ionic strength.

2.2.3.6 Ionic strength and pH

Electrostatically-stabilised (by ionic surfactants or charged biopolymers) emulsions are susceptible to environmental stresses, such as salt (ionic strength) and pH (McClements, 2005). The presence of ions in emulsions may promote droplet flocculation. In general, increasing the ion concentration destabilises the emulsions with charged droplets (Dalgleish, 2004). With the increase in ionic strength, the repulsive interactions between droplets decreases due to the screening of charges and it subsequently leads to the compression of the electrical double layer (Hunter, 1981; Kulmyrzaev & Schubert, 2004). As a result, emulsion droplets become flocculated, because the electrostatic repulsion is not high enough to prevent them from coming together (Morrison & Ross, 2002; Shchukin, et al., 2001).

For instance, protein-stabilised emulsions are susceptible to ionic strength and pH of the continuous phase. This is because varying the pH (Bansal, et al., 1978; Marinova, et al., 1996) and varying the ionic strength (Kulmyrzaev & Schubert, 2004) change the thickness of the electrical double layer. Flocculation occurs at the isoelectric point of proteins, since charges are neutralised and repulsive interactions are too weak to prevent droplet flocculation. Therefore, low ionic strength and a pH away from the isoelectric point, promote electrostatic stability in emulsions with charged droplets (Dickinson & Stainsby, 1988; McClements, 2005).

Furthermore, the degree of ionisation (described by the $pK_a$ value) of acidic polysaccharides is influenced by the pH of the solution. Glucomannans containing carboxylic groups (-COOH) have a $pK_a$ value of 3.0 (Du, et al., 2006). Above this $pK_a$ value, the polysaccharide is negatively charged due to the ionisation of the carboxylic groups. Ionic strength and pH may also influence the adsorption properties of anionic polysaccharides (Dontsova & Bigham, 2005). The rheological behaviour of charged emulsion droplets is also sensitive to salt concentration (Martínez, et al., 2007) and the pH of the aqueous phase (McClements, 2005). Flocculated droplets, as a result of the screening of charges and compression of the double layer, tend to increase the viscosity and extent of shear-thinning of the emulsion system.
Recently, Nakauma et al. (2008) reported the effect of pH and salt on the emulsifying properties of different polysaccharides: sugar beet pectin (SBP), soybean soluble polysaccharide (SSPS) and gum Arabic (GA). Amongst the gums tested, GA was found to be more sensitive to pH, compared to SBP and SSPS, in terms of droplet size \( (d_{32}) \). According to these authors, although the sensitivity to pH was primarily related to the isoelectric point of the protein moiety, the electrostatic interactions, between or within the polysaccharide portions, could be another factor, which might affect the adsorption behaviour of GA, by altering the hydrophobic character of the protein fraction. Moreover, the emulsifying activity of SBP was the most sensitive to salt in terms of droplet size. The salt sensitivity of SBP could probably be attributed to the uronic acid contents and the charge density.

### 2.2.3.7 Temperature

Temperature may also influence the stability of O/W emulsions. According to McClements (2005), heating a sterically-stabilised emulsion, which favours biopolymer-biopolymer interactions, rather than solvent-solvent/solvent-biopolymer interactions, will result in droplet flocculation. In addition, emulsions stabilised by globular proteins are sensitive to temperature. This is due to the unfolding and denaturation of protein molecules, when heated above their critical denaturation temperature, thus exposing the hydrophobic segments. This subsequently promotes interaction between protein molecules, via hydrophobic interactions, which leads to droplet aggregation. Moreover, globular proteins also undergo disulphide interchange resulting in the formation of intermolecular covalent bonds.

A comparison on the thermal stability of beverage O/W emulsions stabilised by gum Arabic, modified starch and whey protein isolate (WPI) was investigated by Chanamai and McClements (2002). They reported that emulsions stabilised by gum Arabic and modified starch were more stable against droplet flocculation during heating (30°C to 90°C), than WPI-stabilised emulsions. This result suggests that polysaccharides (e.g. gum Arabic and modified starch) provide better emulsion stability against environmental changes (e.g. temperature) compared to proteins.

The effect of heating on oil-in-water emulsions stabilised by soybean soluble polysaccharide was reported, recently (Nakamura, Maeda, & Corredig, 2007). The
results revealed that heating the emulsions at 60 or 70 °C, did not significantly affect the average size of the emulsions. However, heating the emulsions at 80 or 90 °C for 30 mins caused the droplets to aggregate (see Figure 13). Moreover, the heating stability of emulsions was affected by varying pH (3.0-7.0) conditions. Emulsions at pH > 4.0 showed aggregation after heat treatment.

Figure 13: Effect of heating on the average particle size of emulsions stabilised by soybean soluble polysaccharides diluted to 0.01% (wt/wt) at pH 4 (○: 60°C, ▲: 70°C, □: 80°C, ■: 90°C). The original emulsion contained 20% oil and 4% SSPS-L (A) or SSPS- M (B). The SSPS types (L and M) varies in sugar and protein compositions

2.3 Macromolecular emulsifiers (proteins and polysaccharides)

2.3.1 Proteins and polysaccharides at emulsion formation

Macromolecular biopolymers, which contain non-polar groups, may exhibit surface activity: that is, they can adsorb to the surface of emulsion droplets and form a protective layer around the droplets, which prevents them from aggregating. As mentioned earlier, biopolymers may stabilise an O/W emulsion through steric and/or electrostatic interactions.

In the food industry, proteins are commonly used as emulsifying agents, due to the large number of hydrophobic groups in their structure. Protein emulsifiers have random coil or globular structures and they also have relatively lower molecular weights, compared to polysaccharides. In contrast, polysaccharides are mostly
hydrophilic and they have high a molecular mass with open structures. Hence, they have weak surface activity, due to their strong hydrophilic character (McClements, 2007). However, there are some polysaccharides, such as gum Arabic, pectin, galactomannans, and modified starches, which can adsorb to the interface, due to their hydrophobic groups (Akhtar, *et al*., 2002; McClements, 2007). For example, hydrocarbon side chains in modified starches, and proteins in gum Arabic. These non-polar groups associated with them can either be (i) attached to their backbone, (ii) chemically-bound to their molecule, or (iii) they may be present as contaminants (McClements, 2004).

When comparing the emulsifying ability of a commonly used protein (casein) emulsifier and a polysaccharides (gum Arabic) emulsifier, it can be seen that proteins have a lower surface load at saturation and a stronger binding affinity, than polysaccharides. Hence, only a small amount of protein is required to produce small emulsion droplets, compared to polysaccharides. To illustrate this point, it can be stated that gum Arabic needs more than 1g per gram of oil to produce O/W emulsions with sub-micron droplets sizes, whilst casein requires only 0.1g per gram of oil (McClements, 2004, 2005). However, several studies have been reported on the ability of other types of polysaccharides, such as sugar beet pectin (Leroux, *et al*., 2003) and fenugreek gum (Huang, *et al*., 2001), to produce small emulsion droplets, at relatively low concentrations.

### 2.3.2 Adsorption of proteins and polysaccharides and emulsion stabilisation

The main driving force, for the adsorption of a biopolymer, is the hydrophobic effect. When a biopolymer is dispersed in the continuous phase, some of its non-polar groups are in contact with water, which thermodynamically, is not a favourable condition. Once the biopolymer becomes adsorbed at the interface, it adopts a re-arrangement, whereby the hydrophobic groups are orientated towards the oil phase and the hydrophilic groups are orientated towards the continuous phase. Hence, the conformation and properties of the interfacial layer formed depend on the molecular structure of the biopolymer (see Figure 14). Linear biopolymers re-arrange in such a way that the non-polar groups protrude at the dispersed (oil) phase, the polar groups protrude at the continuous phase and the neutral groups stretch out at the interface. The interfacial layers formed by these molecules tend to be open and thick with low
viscoelasticity (McClements, 2005). A typical illustration, of the mechanism of the adsorption of polysaccharide emulsifier, is the ‘wattle blossom’ model of *Acacia senegal* gum (Figure 15). The protein fraction of the gum adsorbs at the interface and anchors the hydrophilic molecules (carbohydrates) which are in contact with the continuous phase.

On the other hand, globular biopolymers (mostly proteins) adsorb at the interface, so that most of the non-polar groups are in contact with the oil phase - and therefore they tend to have a fixed orientation at the interface. At the interface, they may undergo conformational re-arrangements in order to maximise the contacts between the hydrophobic segments and the oil phase (Norde, 2003). Hence, globular proteins may undergo unfolding at the interface, which exposes more non-polar amino acids and causes hydrophobic interactions (McClements, 2005).

**Figure 14:** Schematic diagram of the conformation of biopolymers at oil-water interface depending on their molecular structure (McClements, 2005)
Generally, interfacial layers formed by proteins are viscoelastic, compact, thin and electrically charged, whilst polysaccharide interfacial layers are loosely packed, thick and either electrically charged or uncharged. The protein interfacial layers may not be thick enough to prevent emulsion droplets from flocculation, via steric repulsion. Thus, an electrostatic interaction is important in the stability of protein-stabilised emulsion droplets, against flocculation (Dickinson, 1992; McClements, 2004). In contrast, polysaccharides form thick interfacial layers around the droplets, which can effectively prevent droplets from flocculation, via steric interactions. Moreover, emulsions stabilised by polysaccharides are more stable in varied environmental conditions, such as pH, ionic strength and temperature, compared to proteins (Chanamai & McClements, 2002; McClements, 2004).

2.4 Plant polysaccharides with surface activity and emulsifying properties

Plant polysaccharides are composed of monosaccharide units linked together by glycosidic bonds. The structural diversity of polysaccharides determines their functional and physical properties, including flow behaviour, solubility, gelling quality, in addition to surface and interfacial properties. Plant polysaccharides, which are commercially used in the food industry as stabilisers, emulsifiers, thickeners and

Figure 15: The ‘wattle blossom’ model representing the active component of Acacia senegal gum in (a) aqueous solution and (b) adsorbed at the oil-water interface. Hydrophilic carbohydrate (C) blocks (ca. $2 \times 10^5$ Da) are attached to the backbone chain of hydrophobic protein (P) (Dickinson, 2003).
gelling agents, are also termed as hydrocolloids or gums. These hydrocolloids occur in plants, as storage substances, cell wall components and mucilage. Chemically and enzymatically modified starches and celluloses are also additional sources of plant polysaccharides, with improved functionality (Izydorczyk, Cui, & Wang, 2005).

Generally, plant polysaccharides are known as stabilisers for O/W emulsions. They impart low mobility to the emulsion droplets and as mentioned earlier, they prevent droplet aggregation and creaming by a non-adsorbing mechanism, such as restricting the movement of oil droplets (Dickinson & Stainsby, 1988). However, some polysaccharides are found to slowly adsorb to the interfaces and considerably lower the interfacial tension, thus providing steric stability and preventing droplets from aggregating (Garti, Slavin, & Aserin, 1999; Huang, et al., 2001). Dickinson (2003) indicated that a hydrocolloid may exhibit substantial surface activity, only if it contains hydrophobic groups (such as methyl or acetyl) and if it contains (or has been contaminated with) some proteinaceous materials.

Naturally-occurring hydrocolloids, such as gum Arabic and fenugreek, in addition to the modified starches and sugar beet pectin, are found to show surface adsorption and emulsification properties. However, the origin of the interfacial properties of hydrocolloids is still a controversial topic. Therefore, it is important to review and investigate the main driving force of their adsorption mechanism.

2.4.1 Naturally-occurring polysaccharides

2.4.1.1 Gum Arabic

Gum Arabic is the most widely known polysaccharide emulsifier. It is commercially used in the beverage and confectionery industries, as a stabilising, emulsifying and flavour encapsulating agent (Dickinson, 2003; Izydorczyk, et al., 2005). Gum Arabic is a naturally occurring exudate from the bark of Acacia trees, generally from the Acacia senegal species. It is a highly branched polysaccharide composed of 1, 3 – linked β-galactopyranose monomers and 1, 6-galactopyranose side chains, which are terminated by either glucoronic acid or 4-O-methylglucoronic acid residues. Gum Arabic has a highly branched molecular structure and a low molecular weight, which is responsible for its low viscosity and high solubility in water, compared to other
hydrocolloids (Sanchez, et al., 2002. Gum Arabic exhibits Newtonian behaviour with its viscosity being shear rate independent (Erni, et al., 2007; Izydorczyk, et al., 2005; Williams & Phillips, 2000) at 4-50 % (wt/v) concentrations (Mothe & Rao, 1999).

Gum Arabic contains a small amount of protein that is covalently linked with highly branched polysaccharide structures, which are known to be responsible for its emulsifying capabilities. It is believed that the protein fraction adsorbs and anchors the molecules to the surface, whilst the carbohydrate portion contributes to emulsion stability, via electrostatic and steric repulsions, thus preventing droplet aggregation and coalescence (Williams & Phillips, 2000). The ability of gum Arabic to form an interfacial layer has proven that it is a true emulsifier, which confers functionality, by forming a stabilising layer on the oil droplets and not through rheological modification of the continuous phase (Dickinson, 2003). However, its surface activity is relatively low, in comparison to commonly used protein emulsifiers. Hence, the concentration of gum Arabic required to produce a stable emulsion is very high, because only the proteinaceous part of the gum is involved in the emulsification process (Dickinson, 2003; Williams & Phillips, 2000).

2.4.1.2 Fenugreek gum

Fenugreek (Trigonella foenum-graecum) is a leguminous plant found in the Mediterranean, northern Africa, India, Western Asia and Canada. The endosperm of its seeds contains galactomannans with a mannose and galactose ratio of 1:1. The galactomannans in the fenugreek seeds are known to control cholesterol and blood glucose levels. Galactomannans, such as fenugreek, guar and locust bean, are highly soluble in water and they are important thickening, stabilising, and emulsifying agents in food products. They exhibit a non-Newtonian behaviour, in which the viscosity decreases with the shear rate (Brummer, Cui, & Wang, 2003; Garti, et al., 1997; Madar & Shomer, 1990).

Several studies have suggested that fenugreek gum exhibits emulsification capabilities. Fenugreek gum has been reported to have the highest emulsification properties, amongst the 14 hydrocolloid gums used in an O/W emulsion system (Huang, et al., 2001). Purified fenugreek gum, containing 0.8% residual proteins, has been shown to lower the surface tension (42 mN/m) and to form a stable emulsion
with small droplet size (2-3μm) and it has an insignificant rheological effect on the continuous phase. The surface activity of the gum was not reduced, after removal of the protein from the crude gum (Garti, et al., 1997). On the contrary, Brummer et al. (2003) reported that purified fenugreek gum, containing 0.57% residual protein, exhibited less surface activity than the un-purified gum. This finding is in agreement with a recent study by Youssef et al. (2009) where crude fenugreek gum (3.74% protein) was purified, using a phenol treatment to obtain a protein-free fenugreek gum (0.16% protein residue). These authors found that removal of the protein in the fenugreek gum significantly reduced its surface activity, which is in contradiction with previous reported studies.

2.4.2. Chemically modified polysaccharides

2.4.2.1 Acetylated sugar beet pectin
Sugar beet pectin is extracted from sugar beet pulp, which is a by-product of beet sugar production (Buchholt, et al., 2004; Funami, et al., 2007; Yapo, et al., 2007). Sugar beet (Beta vulgaris L.) is mostly composed of parenchymal tissue with thin and hydrophilic cell walls, comprised of different types of polysaccharides, such as pectin, hemicelluloses, and cellulose. Most of the structural data of the sugar beet polysaccharides deals with the pectin fraction. Sugar beet pectin is high in galacturonic acid and it contains neutral sugars, including galactose, arabinose and rhamnose. It is also appreciably rich in acetic acid and phenolic esters on its side chains, in addition to having high fraction of ‘hairy regions’ containing high arabinose and less rhamnose fractions (Thibault, Renard, & Guillon, 2001). Michel et al. (1985) confirmed that the high amount of acetyl groups, in sugar beet pectin, is the reason for its relatively poor gelling properties. Deacetylation of pectin by mild acid hydrolysis, results in gel formation, indicating that acetyl groups replace the galacturonic acid residues and this may prevent the alignment and aggregation of chains, due to steric hindrance. The acetyl groups may improve the hydrophobic nature of the pectin, thus creating surface activity and hence, it acts as an emulsifier in emulsion systems (Dea & Madden, 1986). Sugar beet pectin has low viscosity and exhibits Newtonian flow behaviour at 2% concentrations or below (Arslan & Kar, 1998).
Several studies have provided insights into the emulsification properties of sugar beet pectin. Highly acetylated sugar beet pectin was found to be more effective in reducing the surface tension of water than the commercial high-methoxyl pectins producing therefore fine and stable O/W emulsions (Dea & Madden, 1986). Leroux et al. (2003) reported that the molecular weight, protein and acetyl contents of pectin significantly influenced its emulsification properties and the protein moieties attached to it played a major role in stabilising the emulsions. Fractionations of sugar beet pectin by using hydrophobic affinity chromatography revealed that (i) the accessibility of protein at the oil-water interface, (ii) the proportion of ester groups and (iii) the molecular mass distribution of the fractions all influenced the emulsifying capabilities of the polysaccharide (Williams, et al., 2005).

Moreover, enzymatically modified sugar beet pectin, containing 0.13% residual protein, exhibited lower emulsifying activity, than the unmodified pectin (Funami, et al., 2007). This finding indicates the important role of the proteinaceous moiety, in its emulsification properties. It appears that the emulsifying properties of sugar beet pectin are not determined by one single factor and therefore its mechanism of emulsification is still a subject for investigation.

2.4.2.2 Modified starches

Modified starches are chemically modified for a range of functional purposes such as improving acid stability and modifying cooking characteristics of foods. They are also used as emulsifying agents in the food and beverage industry. They provide emulsions with improved shelf-life and they may be used as a substitute for gum Arabic in other industrial applications (Chiu, 1990). Chemical modification of starch is carried out by adding hydrophobic side chains to the originally hydrophilic starch molecule, via oxidation, esterification, and etherification (Prochaska, et al., 2007). The resulting process enables starch to adsorb at the W/O interface, hence stabilising the emulsion system (Tesch, Gerhards, & Schubert, 2002).

Emulsifying starches, such as octenyl-succinate starches, termed ‘OSA’, have long been used for encapsulation and for beverage emulsions. They are colourless and tasteless in solution and they provide protection against oxidation, in the encapsulation process. They also have a high consistency of quality in beverage emulsions, in
comparison with gum Arabic. Moreover, OSA starches increase the viscosity of the aqueous phase and thereby they reduce the cost of the finished product, due to their ability to act both as a surfactant and a stabiliser, at the same time (Tesch, et al., 2002).

Tesch et al. (2002) reported that OSA starches efficiently lowered the interfacial tensions and stabilised emulsion droplets. Emulsification with OSA starches was found to be independent of pH and ion valence and therefore, their main stabilising mechanism is attributed to steric hindrance. Their result also suggests that OSA starches may be used as a substitute for whey protein, with an advantage at low pH values, which is close to the isoelectric point of the protein: where the OSA starches are still very stable. Furthermore, Prochaska et al. (2007) evaluated the functional properties of commercial food grade modified starches and found that starch sodium octenyl-succinate has the highest capability of lowering surface and interfacial tension. Cross-linked starches could also stabilise emulsions at lower pH values, but with less surface activity, than those of other modified starches.

2.5 Basil seed gum (BSG)

2.5.1 Introduction
The origin of the surface adsorption of hydrocolloids is still controversial. Thus, investigating the surface activity and emulsification properties of some novel polysaccharides is worth undertaking. It is of particular interest to study the gum extracted from basil seeds which are found to exhibit excellent functional properties, which are comparable with other commercial hydrocolloids (Razavi, et al., 2009).

2.5.2 Basil
Basil (Ocimum basilicum L.), as shown in Figure 16, comes from the genus Ocimum of the Lamiaceae family consisting of 50 to 150 species of herbs and shrubs (Hiltunen & Holm, 1999; Simon, et al., 1999). It is a popular aromatic and white-purple flowering herb grown in India and Iran (Fekri, et al., 2008; Yousif, et al., 1999). It is also cultivated in the warmer regions of Africa, Central and South America (Simon, et al., 1999).
Aside from its culinary use, basil extracts have been used in food flavourings and in fragrances. Basil contains essential oil, rich in linalool and methylchavicol compounds, which are responsible for its aromatic properties (Vieira & Simon, 2000; Yousif, et al., 1999). The outer layer of basil (*O. basilicum*) seeds contains, a pectinuous matrix, which readily swells when soaked in water (Melo & D'Souza, 2004). The seeds have been used in traditional medicine, for the treatment of coughs, sore throats, diarrhoea, indigestion and kidney malfunctions (Simon, et al., 1999; Vieira & Simon, 2000). In India, these mucilaginous seeds are used in drink preparations, as a source of dietary fibre (Mathews, et al., 1993).

![Figure 16: Basil (*Ocimum basilicum* L) (A) plant (B) seeds (C and D) gelatinous mass after soaking with water](image)

### 2.5.2 Chemical structure and proximate composition

The polysaccharide extracted from the seeds of *Ocimum bacilicum* L. has been reported to contain two major fractions: (i) an acid-stable glucomannan (~43%), with a ratio of glucose to mannose, 10:2; and (ii) a (1→4) linked xylan (~24.3%), with acid side chains at C-2 and C-3 of the xylosyl residues. A minor fraction of glucan (~2.31%) was also reported (Anjaneyalu & Channe Gowda, 1979; Tharanathan & Anjaneyalu, 1975). Figure 17 illustrates the typical structure of a glucomannan. Some glucomannans such as Konjac mannan, contain acetyl groups in its major chain (Li,
Xie, & Kennedy, 2006; Takigami, 2000). Therefore, it is possible that the polysaccharide gum from basil seeds also contain acetyl groups, which may contribute to the hydrophobic character of the polysaccharide.

There have been some studies reported on the proximate chemical composition of basil seeds. Matthews et al. (1993) reported that Indian basil seeds contained 14.8% protein, 13.8% fat, 9.63% moisture, 7.7% ash and 63.8% carbohydrates, with a high amount of crude fibre. Fekri et al. (2008) found the mucilage of Iranian basil seed had ~10.9% protein, 4.86% moisture, and 0.84% ash.

Recently, Razavi et al. (2009) studied the optimal extraction conditions of gum from basil seeds, in terms of pH, temperature and water/seed ratio. Temperature and pH have been shown to significantly influence the quantity and quality of the extracted gum. Chemical analysis of Iranian basil seeds has revealed a higher protein (~18-20%) and fat (~23%) and lower carbohydrate (~47-50%) contents, than the Indian basil seeds reported by Matthews et al. (1993). Furthermore, the purified (alcoholic precipitation) extracted Basil Seed Gum was found to contain a small fraction of (~1.5%) residual protein, ~9.7% fat, ~3.3% ash and ~80% carbohydrate contents.

2.5.3 Functional properties

To date, investigation of the steady shear flow behaviour and viscoelastic properties of BSG, at 0.1-2% (wt/wt) concentrations and at 5-85°C temperatures, have revealed a non-Newtonian pseudoplastic behaviour, a high zero-shear viscosity, and the presence of a yield stress (Hosseini-Parvar, et al., 2009). The Herschel-Bulkley rheological model has been found to best describe the flow behaviour of BSG. It appears that, at
temperatures above 60°C, the elastic modulus ($G'$) of BSG solutions become slightly higher suggesting an enhancement of the gum connectivity under small deformations.

In Figure 18 (above), the shear thinning behaviour of BSG at different concentrations (0.1-2%) are graphically illustrated. The decreasing apparent viscosity with increasing shear rate is an important feature of BSG, with rheological properties comparable to xanthan gum. This concludes this chapter of the thesis – the literature review.
Chapter 3
Materials and Methods

3.1 Introduction
This chapter presents the materials and methods used in the study. The materials and methods for emulsion preparation and chemical analyses, for BSG composition are described. The techniques used to characterise the properties of BSG-stabilised emulsions are also described.

3.2 Materials
Basil Seed Gum (BSG) was obtained from the University of Mashhad, Mashhad, Iran. Soya oil was obtained from AMCO, Goodman Fielder Ltd., New Zealand. Analytical grade reagents, such as sodium dodecyl sulphate, sodium azide, sodium chloride, sodium hydroxide, hydrochloric acid, tetradecane, phenol, ethanol and isopropanol, were used in this study.

3.3 Emulsion preparation
Soya oil-in-water emulsions were prepared by dissolving known amounts of Basil Seed Gum (BSG) in Milli-Q water using an overhead mixer (RW 20.n, IKA Labortechnik) for 2 minutes. The solutions were left overnight, under continuous stirring at room temperature, for complete hydration of the polysaccharide. Soya oil (30% wt/wt) and BSG aqueous solutions were heated in a water bath, at 50°C for 15-20 mins and then pre-homogenised in an Ultra-Turrax (Heidolph DIAX 600), at 9,500 rpm for 1 min. The coarse emulsions were immediately homogenised by five passes through a two-stage high-pressure homogeniser (APV) at 350/80 bar. Sodium azide (0.02%v/v) was added to the freshly homogenised emulsions, as antimicrobial agent.

In order to test the effect of gum concentration, the emulsions were formulated using various concentrations of BSG [0.03 – 1% (wt/wt)] following the method indicated above. In order to determine the effect of pH, 0.3% (wt/wt) BSG-stabilised emulsions were prepared and pH was adjusted between 1-12 before or after emulsification. A solution of 0.1-1 M HCl (or 0.1-1 M NaOH) was used to adjust the final pH of the emulsions. Different concentrations (5-70 mM) of NaCl were added, before or after
the emulsification process, of 0.3% BSG-stabilised oil-in-water emulsions, in order to investigate the effect of ionic strength.

In order to check the origin of the big droplet size population, 1-2% (v/v) Sodium dodecyl sulfate (SDS) was added to emulsions, which were stored for 1 month at 20°C and immediately the droplet size was analysed. In order to test thermostability, emulsions at native pH and at extreme pH values (2 and 10) were prepared and heated in a water bath at 80°C for 30 min, before analysis of the droplet size and rheological properties.

3.4 Droplet size measurements
The droplet size distribution of BSG-stabilised O/W emulsions was determined, by a laser light scattering technique, using Malvern Mastersizer MS 2000 (see Figure 19B). This laser diffraction method is capable of determining droplet sizes ranging from 0.02-2000 µm. The emulsion samples were diluted and then placed in a measurement cell. Deionised water was used as a dispersant and the refractive index used was 1.470 for soya oil.

Figure 19A illustrates the main components of the instrument. A monochromatic beam of light, generated by a helium-neon gas laser with a fixed wavelength (λ) of 633nm, was passed through the measurement cell, where it was scattered by the emulsion droplets. The Mastersizer 2000 also used a monochromatic blue light source (λ = 466 nm) in order to detect small signals generated from sub-micron droplets, thus improving the resolution. It was the dual wavelength detection system which allowed detection of droplet sizes, at lower and higher range of sizes. An array of photosensitive detectors measured the intensity of the scattered light, as a function of a scattering angle. The scattering pattern was recorded by the detectors and sent to a computer. The droplet size distribution, which showed the best fit between the experimental measurements and those predicted by the Mie theory, was then calculated (McClements, 2005).

The size of the emulsion droplets was analysed immediately after preparation and on subsequent days (3rd, 7th, 14th, and 30th) under storage at 20°C. The mean emulsion
droplet size was characterised in terms of $d_{43}$ and $d_{32}$. The $d_{43}$ or the volume mean diameter is expressed as follows:

**Equation 5**

$$d_{43} = \sum \frac{n_i d_i^3}{n_i d_i^3}$$

where $n_1$ is the number of droplets of diameter $d_1$. The $d_{43}$ refers to the sphere of equivalent volume for the real sized droplets. This parameter was used to monitor changes in droplet size distribution given its sensitivity to the appearance of big droplets/aggregates, which greatly contribute to the mean volume. The $d_{32}$ or the surface mean area diameter (*Suater-mean*) is expressed as:

**Equation 6**

$$d_{32} = \sum \frac{n_i d_i^3}{n_i d_i^2}$$

The $d_{32}$ refers to the sphere of equivalent surface area for the real-sized droplets. This parameter is useful for monitoring changes in the average area of droplet surfaces. The measurement of droplet diameters were carried out in triplicates and the results were reported as averages.

![A. Laser diffraction technique (A) Main components of the instrument, (B) Mastersizer 2000 Hydro MU (Malvern Instruments, UK)](image)

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3.5 Rheological measurements

The rheological properties of the fresh BSG-stabilised emulsions were measured in duplicates, within 24 hrs after preparation in a MCR 301 rheometer (Paar Physica, Germany), which was equipped with a cone-and-plate geometry (4°/40mm) as shown in Figure 20. The apparent viscosity was determined at shear rates from 0.01-1000 s\(^{-1}\), at 20°C and viscoelastic properties in terms of \(G'\) (storage modulus) and \(G''\) (loss modulus) were measured at frequencies from 0.01 to 10 Hz at 0.5% strain.

![MCR 301 rheometer (Paar Physica, Germany)](image)

**Figure 20: MCR 301 rheometer (Paar Physica, Germany)**

Rheological properties, such as flow and gel properties, play an important role in food emulsion systems. The viscosity (\(\eta\)) of a liquid indicates its resistance to flow (Macosko, 1994) and it is defined by the following expression:

**Equation 7**

\[
\eta = \frac{\text{shear stress}}{\text{shear rate}} \quad \text{(Pa s)}
\]

Liquid flow behaviour can be categorised into two types: Newtonian and non-Newtonian. Figure 21 (below) illustrates the flow behaviour of different liquids. In ideal liquids (Newtonian), shear stress is proportional to shear rate, and the viscosity is independent of shear rate. Non-ideal liquids (non-Newtonian) can either have pseudoplastic, dilatant or plastic flow behaviour. Non-Newtonian systems are dependent on shear rates, wherein the viscosity changes with the rate of shear applied. The viscosity at a definite shear rate is termed as ‘apparent viscosity’ (McClements, 2005; Rao & Quintero, 2005).
Pseudoplastic liquids exhibit a ‘shear thinning’ behaviour, wherein the apparent viscosity decreases as the shear rate increases. In dilatant behaviour, the apparent viscosity of the liquid increases, as the shear rate increases and is referred to as ‘shear thickening’. Plastic materials (Bingham plastics) show elastic properties, below the yield stress point, but when applied stress is exceeded, they behave like a fluid (Heimenz & Rajagopalan, 1997).

![Graph showing shear thinning and shear thickening](image)

**Figure 21: Comparison of the flow behaviour of Newtonian and non-Newtonian (shear-thinning or shear-thickening) liquids**

The rheological properties of a viscoelastic material can be determined by ‘transient’ (e.g. stress relaxation and creep tests) or ‘dynamic’ rheological methods. Dynamic oscillatory rheological test was the chosen method to measure the viscoelasticity of the BSG emulsions. The viscoelastic behaviour of a material is derived from the combination of properties of a solid (elastic deformation) and of a liquid (viscous flow), depending on the magnitude and time-scale of external forces (Barnes, Hutton, & Walters, 1989). An ideal solid material, which responds to an applied load by deforming finitely and recovering that deformation upon removal of the load, is called ‘elastic’. Ideal elastic materials follow Hooke’s law, which describes a direct proportionality between the shear stress ($\tau$) and the shear strain ($\gamma$) via proportionality constant called the ‘shear modulus’ ($G$). The SI units of shear stress are Pa (=N m$^{-2}$) (force/area), whereas shear strain is a dimensionless quantity:

**Equation 8**

$$G = \frac{\tau}{\gamma}$$
An ideal fluid undergoes deformation as long as the load is applied. The material will not recover from its deformation when the load is removed and this response is called ‘viscous’. The flow behaviour of viscous materials is described by Newton’s law, which constitutes a direct proportionality between the shear stress and the rate of deformation or shear rate ($\dot{\gamma}$). The shear rate has dimensions of reciprocal time ($s^{-1}$). The proportionality constant is called the shear viscosity ($\eta$) with units of Pa s:

$$\eta = \frac{\tau}{\dot{\gamma}}$$

From energy considerations, elastic behaviour represents complete recovery of energy expended during deformation, whereas viscous flow behaviour represents complete loss of energy because all the energy supplied during deformation is dissipated as heat (Barnes, et al., 1989). The emulsions characterised in this study are viscoelastic and exhibit some viscous and some elastic behaviour simultaneously.

### 3.6 Surface/interfacial tension measurements

The adsorption properties of BSG were characterised, in terms of surface/interfacial tensions. Surface tension is a measurement of the cohesive energy present at the interface. The surface is the interface of two fluids: if it involves a gaseous phase, it is referred to as ‘surface tension’, and if it involves two liquids, it is termed as ‘interfacial tension’. The denser fluid is referred to as the ‘heavy phase’ and the less dense fluid is termed as the ‘light phase’ (KSV Cam 200 Operation Manual, n.d.).

The surface/interfacial tension of the BSG samples was measured by a pendant drop shape method, using KSV CAM 200 (KSV Instruments, Finland), as shown in Figures 20 and 21, respectively. The droplet shape hanging from the tip of a syringe was determined from the balance of forces, which included the surface tension of that liquid. The interfacial tension at the liquid interface could be related to the drop shape, through the following mathematical expressions:

$$\gamma = \frac{\Delta \rho \times g \times R_0}{\beta}$$
where $\gamma = \text{surface tension}$, $\beta = \text{shape factor}$, $\Delta \rho = \text{difference in density between fluids at the interface}$, $g = \text{gravitational constant}$, and $R_0 = \text{radius of drop curvature at apex}$. The surface/interfacial tension is determined by fitting the shape of the drop (in a captured image) to the Young-Laplace equation, as shown in Figure 22 below.

$$\frac{dx}{ds} = \cos \varphi$$

$$\frac{dz}{ds} = \cos \varphi$$

Figure 22: The pendant drop method can determine the surface tension or interfacial tension of a fluid (Source: reference.findtarget.com/search/goniometer/)

Different concentrations (0.1-1% wt/wt) of BSG solutions were prepared and hydrated overnight, at room temperature. The adsorption of BSG, at air-water interface, was determined, by loading the sample to the syringe, through its attached needle. The needle was passed through a cuvet half-filled with water and covered with parafilm and the sample was dispensed, thus forming a pendant drop of liquid hanging from the syringe. These conditions protected the drop from air currents and controlled the
evaporation (Woodward, n.d.). Measurements were recorded at 5 min intervals for 30 mins at room temperature.

A cuvet filled with \textit{n}-tetradecane (approximately one quarter-full) and covered with parafilm, was used for interfacial tension measurement. The syringe with the sample was punctured through the film-covered cuvet and immersed into the liquid and then the sample was dispensed, thus forming a drop shape within the liquid. Measurements of captured images were carried out at 5 min intervals for 30 mins at room temperature.

3.7 Visual phase separation
The visual phase separation of the emulsions were evaluated, by transferring approximately 25 g of the emulsions into a 15 mL plastic container, which was sealed with a plastic cap and then stored at 4°C and 20°C, for 1 month period. Physical phase separation was monitored during this period.

3.8 Zeta potential measurements
The zeta potential (\(\zeta\)) values of the emulsions was measured, using a Zetasizer Nano ZS (ZEN 3600) instrument (Malvern Instruments, UK), as shown in Figure 24, through a combination of laser Doppler velocimetry (LDV) and phase angle analysis light scattering (M3-PALS) techniques. The zeta potential is related to particle electrophoresis. When an electrical field is applied across the sample, the charged droplets move towards the oppositely charged electrode. The viscous forces exerted by the surrounding liquid tend to prevent this movement and when the equilibrium is reached, between the opposing forces, the droplets move with a constant velocity (\(v\)), which is referred to as 'electrophoretic mobility' (\(U_E\)).

The micro-electrophoresis system is a capillary cell with electrodes at either end, to which a potential is applied. The droplets accelerate towards the electrode, at a certain velocity, which is measured by the frequency shift of the incident laser beam. The measured velocity (\(U_E\)) was converted to zeta potential (\(\zeta\)) by the Henry equation as shown in Equation 11:
Equation 11
\[ U_e = \frac{2\varepsilon f(\kappa a)}{3\eta} \]

where, \( \varepsilon \) = dielectric constant, \( \eta \) = viscosity, and \( f(\kappa a) \) = Henry’s function. The \( f(\kappa a) \) value is 1.5, referred to as the Smoluchowski approximation, which is used for aqueous media.

The samples were prepared by centrifuging emulsions at 19,500 rpm (Sorvall RC-5C) for 1 hr in order to extract the serum phase. The serum used for diluting the emulsions was then filtered using a 0.20µm pore filter. A series of dilutions (0.1 and 0.01) were made and the pH was adjusted to the original pH of the emulsions. One milliletre (mL) of the sample was injected into one of the ports of the folded capillary cell (DTS 1060) and any air bubbles were removed, before inserting the stopper. The zeta potential measurements were undertaken at least twice (at \( 20^\circ C \)) and data were reported as means of duplicates.

![Zetasizer Nano ZS, Model ZEN 3600 (Malvern Instruments, UK)](image)

**Figure 24: Zetasizer Nano ZS, Model ZEN 3600 (Malvern Instruments, UK)**

### 3.9 Preparation of protein-free BSG

Protein-free BSG was prepared, following the method of Youssef *et al.* (2009). This method used the phenol solvent treatment in order to extract proteins from the aqueous polysaccharide solution. At a certain pH, phenol solution has a high dielectric constant, where protein is soluble in phenol and polysaccharide is soluble in water.
Equal amounts of 0.5 %( wt/wt) purified BSG at pH 7 and 90 %( wt/v) phenol at pH 7 were mixed and incubated at room temperature for 30 mins, and then at 10°C for 2 hrs. The mixture was centrifuged at 8,000 x g for 30 mins and the supernatant was decanted. The supernatant was precipitated with 100% ethanol, to a final concentration of 50% (v/v), and the resulting mixture was centrifuged at 9,715 x g for 30 mins. The supernatant was decanted and the residue was stored overnight in isopropyl alcohol, which was changed daily for 5 subsequent days. The residue was air-dried in the fume hood for 6 hrs and the recovered gum was termed ‘protein-free BSG’.

3.10 Chemical composition analysis
The protein content was determined from the nitrogen content (N x 6.25), using the Kjeldhal method (AOAC 968.06). Amino acids profile was determined by hydrochloric acid analysis followed by HPLC separation (AOAC 994.12). Total sugars were estimated using the phenol-sulphuric method (Dubois, et al., 1956).

3.11 Confocal scanning laser microscopy (CSLM) and Light Microscopy
A confocal scanning laser microscope (Leica SP5 DM6000B, Leica Microsystems Germany) (see Figure 25) operated in fluorescence mode, with 10x and 40x objectives of numerical aperture 0.40 and 1.25 respectively, was used, in order visualise the microstructure of the emulsions. This imaging technique has the ability to acquire high resolution optical images, from selected depths of the specimen known as ‘optical sectioning.’ By scanning various thin sections through the sample, a three-dimensional image of the sample can be constructed.

Figure 25: Confocal scanning laser microscope, Model Leica SP5 DM6000B (Leica Microsystems, Germany)
The oil droplets were stained with Nile blue fluorescent dye, which was excited with the 561 nm laser line. The Nile Red contained in the Nile Blue is responsible for staining the oil droplets. Fast green dye, excited with the 405 nm laser line, was used for staining the protein fraction (presumably contained in the polysaccharide). Forty five micro litres (µL) of each fluorescent dye (0.05%) was added to a 2.5 mL emulsion sample, while stirring. An aliquot portion of the stained emulsion sample was placed into the laboratory-made welled microscope slide, using a plastic ring, (see Figure 26 below) and filled it completely. A cover slip was placed on top of the well and was ensured that no air bubbles were trapped inside. The prepared slide was then examined under the microscope and the depth of the scan (z) was 2 µm from the cover slip.

Microstructures of emulsion samples were also obtained using a compound light microscope (CHA, Olympus, Japan) equipped with a camera. A drop of sample was placed on a microscope slide and covered with a cover slip. The images were captured at either 10x or 40x objectives.

Figure 26: Microscope slide with plastic rings containing BSG emulsion samples
Chapter 4
BSG-Emulsifying properties

4.1 Introduction
This chapter reports on the effects of some compositional and processing variables, on the emulsifying and stabilising properties of BSG. Factors such as: gum concentration, pH, addition of salt (NaCl), thermal treatment, and protein content, were investigated. Emulsions were characterised by measuring the droplet size distribution ($d_{32}$ and $d_{43}$) and the zeta potential (mV). The $d_{43}$ value was used to monitor changes in droplet size distribution upon storage. In order to distinguish whether the increase in droplet diameter was due to gradual aggregation of droplets, 1-2% (v/v) SDS (sodium dodecyl sulfate) surfactant was added to stored emulsions. Rheological properties (apparent viscosity and viscoelasticity) of fresh emulsions were also measured. Visual phase separation was monitored, at 20°C for a one month period.

As previously discussed in literature review (section 2.5), BSG exhibited functional properties which could be exploited as a novel food ingredient. To date, there has been no attempt to explore on the potential emulsifying properties of this polysaccharide. Therefore, the aim of this study was to investigate the ability of BSG, to emulsify and stabilise oil-in-water emulsions under various conditions.

4.2 Effect of gum concentration on the emulsifying properties of BSG
30% (wt/wt) soya oil-in-water emulsions were prepared, with different concentrations of BSG [0.03-1% (wt/wt)] as described in Materials and Methods (Chapter 3). Particle size and rheological properties of the emulsions were measured, within 24-hrs after preparation. Visual phase separation and growth in droplet size were monitored, on the 3rd, 7th, 14th and 30th day of storage. SDS (sodium dodecyl sulfate) (1% v/v) was added, in order to check the origin of the growth of droplets, during storage.

4.2.1 Results
The emulsifying ability of BSG (as a function of gum concentration), in terms of particle size distribution, is shown in Figure 27. Increasing the gum concentration systematically decreased the emulsion droplet size. The particle size distributions
became narrow and shifted to the left (smaller droplet size range). At lower gum concentrations (<0.30%), wider droplet size distributions and larger particle sizes >13.0 µm ($d_{43}$) were observed. A monomodal droplet size distribution, with an average droplet size ($d_{32}$) of <1.0 µm, was formed, at a concentration of 0.30%. Further addition of the gum, up to 1%, changed the shape of the droplet size distribution curve; the appearance of smaller peaks at the range of small droplet size (peak at 0.1 µm) was observed in some emulsions.

![Figure 27: Effect of BSG concentration on the particle size distribution of 30% (wt/wt) soya oil-in-water emulsions](image)

The effect of BSG concentration, on apparent viscosity, storage modulus ($G'$) and loss modulus ($G''$), is illustrated in Figure 28 and Figure 29, respectively. With the increase in BSG concentration, the apparent viscosities and viscoelastic properties of the emulsions also increased. Extremely high apparent viscosity (e.g. 170 Pa.s at 0.1 s$^{-1}$ shear rate) and viscoelasticity (e.g. $G'$ = 170 Pa, $G''$ = 23 Pa at 0.1 Hz) were observed at 1% BSG. Generally, all emulsions exhibited shear-thinning behaviour, although, at very low gum concentrations (<0.06%), a Newtonian region was observed at high shear rates (> 100 s$^{-1}$). The elastic modulus ($G'$) was higher than the viscous modulus ($G''$), at all gum concentrations, indicating that the solid-like property of the gum probably dominated the flow behaviour of the emulsion. This is obvious when the rheological behaviour of equivalent amounts of gum in solution is observed.
Figure 28: Effect of BSG concentration on the apparent viscosity of 30% (wt/wt) soya oil-in-water emulsions

The stability of emulsions, upon storage, was assessed from the change in particle size ($d_{43}$) and from the visual phase separation. The droplet size increased rapidly, during storage in emulsions, with very low BSG concentrations (<0.06%), as shown in Figure 30. Although emulsions with gum concentrations, between 0.06% and < 0.30 %, had relatively larger droplets (2.0- 4.5 µm) than at concentrations >0.30%, they remained stable upon storage. Above 0.30%, the average droplet sizes were as small as 1.3µm and they did not significantly change over time.

Figure 29: Effect of BSG concentration on: (a) the storage modulus ($G'$) and (b) the loss modulus ($G''$) of 30% (wt/wt) soya oil-in-water emulsion
The visual phase separation of emulsions, during storage, is shown in Figure 31 (below). It can be seen that phase separation was apparent, in emulsions containing BSG concentration below 0.24%. The height of the serum layer was decreased, with increased gum concentration. This suggests that the physical stability of the emulsions was improved, with increasing gum concentration.
4.2.2 Discussion

Emulsions stabilised by BSG had a decreased average droplet size, with increasing concentration. BSG could form emulsions with monomodal droplet distributions and with average droplet size below 1.0 µm ($d_{32}$), by using as low as 0.30% concentration. At very low gum concentrations, large oil droplets were formed, as manifested by a broad droplet size distribution, which might have been also the result of droplet flocculation, by macromolecular bridging. This is likely to be attributed to the insufficient amount of BSG needed to provide complete coverage to the oil droplets, during homogenisation. As a result, polysaccharide molecules (on one droplet) are being shared, through partial adsorption with the uncovered surface of another droplet as described previously when macromolecular emulsifier is used below a critical concentration for emulsification (Dickinson, 1992). Therefore, bridging flocculation and large droplet sizes may be the causes of the creaming observed in the emulsions at low gum concentrations.

At 0.30% gum concentration, BSG provided sufficient coverage of the droplets and resulted in the formation of small droplets with uniform sizes, which remained stable against flocculation and subsequent creaming. Above 0.30% BSG concentrations, the droplet size distribution was altered, with the appearance of a ‘tail’ which shifted to the right (larger droplet size area) (see Figure 27). This could probably be due to the excess of non-adsorbed polysaccharide, which increased the viscosity of the aqueous phase, resulting in reduced efficiency of droplet disruption, during homogenisation (Huang, et al., 2001). Moreover, a secondary minute peak, at the smaller size area (~0.1µm), was observed. The occurrence of this smaller peak appearing intermittently in some emulsions, suggests that the emulsion exhibit polydisperse behaviour with distinctive droplet size populations. Therefore, high gum concentrations beyond an optimum level led to a worsening of the emulsification process, and polydispersity.

Furthermore, the emulsions exhibited a systematic increase in apparent viscosity, with increasing BSG concentration. The viscosity effect of BSG appears to provide emulsion stabilisation, as confirmed by the absence of creaming, in emulsions above 0.24% gum concentration. It is known that most non-adsorbed polysaccharides provide long term-stability to emulsions by increasing the viscosity of the aqueous phase, consequently restricting the movement of droplets in the system and thereby
preventing flocculation, coalescence and creaming (Dickinson & Stainsby, 1988; Garti, et al., 1999). It is likely that the long-term stability of BSG-stabilized emulsions is the result of the combination of effects; (i) a strong steric stabilization of the oil droplets provided by enough amount of adsorbed BSG at the interface and (ii) an increase in viscosity of the aqueous phase due to an increasing amount of non-adsorbed BSG. Any possible depletion flocculation (Velez, et al., 2003) effect leading to emulsion creaming by an excess of non-adsorbed polysaccharide was probably inhibited at high gum concentrations, due to the high viscosity of the system.

BSG-stabilised emulsions also exhibited shear-thinning flow behaviour at all gum concentrations, where viscosity decreased with increased shear rate (0.01-1000 s⁻¹). It is possible that the origin of the shear-thinning behaviour at low BSG concentrations (<0.18%) is the result of the breakage of bridged-droplets, achieving Newtonian profile at high shear rates. When further amounts of BSG were used for emulsification, a systematic increase in thinning behaviour and viscosity occurred, instead of obtaining a Newtonian behaviour over the whole shear rate studied, which normally represents emulsion stability. This may indicate that the non-adsorbed BSG is likely to dominate the rheological properties of the emulsion, and therefore only a thinning effect is observed under shear. This can be supported by the findings of Hosseini-Parvar et al. (2009), who reported that solutions of BSG (0.02-2.0 % wt/wt) exhibited all pseudoplastic flow behaviour. It was also found that pseudoplasticity of BSG solutions significantly increased, with increasing gum concentration. Just for comparison, at the same gum concentration (1% wt/wt) and at low shear rate (0.1s⁻¹), the apparent viscosity of BSG in the emulsion was 170 Pa.s, while BSG in the solution was 38 (Pa.s). It can be inferred that the shear-thinning behaviour of the “stable” emulsions is the thinning of BSG present in the aqueous phase.

Droplet aggregation, through a bridging mechanism (at lower BSG concentrations), or through an increasing amount of non-adsorbed polysaccharides (at higher BSG concentration) tends to increase the viscosity and extent of shear-thinning of o/w emulsions (McClements, 2005). Regarding the non-adsorbed polymer, and its impact on the aqueous phase, polysaccharides with stiff and linear molecules, have large hydrodynamic volume, which relates to their high viscosity and shear-thinning behaviour. This is likely to be the case in BSG, with very high viscosity at low shear
rate. Shear-thinning is the result of the molecular realignment of the polymer chains, towards the direction of the flow, as shearing is increased, thus resulting in lesser interactions between adjacent molecules. It is worthwhile to notice that even at high shear rates (~10³ s⁻¹), BSG did not show Newtonian region (or complete alignment with the flow) at high concentration of the gum. According to Vardhanabhuti and Ikeda (2006), the shear-thinning behaviour of hydrocolloids allows liquid foods to be pumped easily and it provides ease of swallowing, due to a thinner consistency in the mouth. BSG-stabilised emulsions showed increased viscoelasticity, with increasing gum concentration. The elastic behaviour was higher than the viscous behaviour at all gum concentrations tested, suggesting that the elasticity of BSG could significantly contribute to the stability of the emulsion by providing more of a solid-like structure (G’>G’’) and a yield stress which restrict droplet movement.

The emulsion stability, as measured by the increase in droplet size and phase separation upon storage, improved with increasing BSG concentration. At lower amounts of BSG, phase separation was observed and oil droplet size rapidly increased upon storage, especially at concentrations <0.06%. No phase separation and no significant change in the mean droplet size were observed in the emulsions containing >0.24% BSG, during one month of storage.

As a summary, droplet flocculation, at very low BSG concentrations, initially led to rapid phase separation and eventually to coalescence (over time) as manifested in the growth of droplet size. As gum concentration increased, oil droplets were presumably fully covered and stabilised against droplet aggregation. The increase of viscosity, at the continuous phase, and the likely thick steric layers provided by the gum at the interface resulted in the formation of very stable emulsions. Only a small amount of BSG is therefore, required to emulsify and form small emulsion droplets, with improved storage stability. Subsequent studies on the emulsions were performed using 0.3% wt/wt BSG based on the findings described above.

4.3 Effect of pH on the emulsifying properties of BSG
The effects of pH, on the emulsifying properties of BSG, were evaluated at a fixed gum concentration of 0.30% (wt/wt), at various pH (1-12) conditions. In preliminary experiments, the pH of BSG was adjusted before and after emulsification. The droplet
size measured was much bigger in pH adjustment after emulsification compared to pH adjustment before emulsification (see Figure 32 below, as an example at pH 4.0). The pH adjustment after emulsification had probably altered the already formed emulsion droplets which lead to the bigger droplet sizes (probably by promoting flocculation of the emulsion). Hence, the method adopted to study the effect of pH, was the adjustment of pH before emulsification. Fresh emulsions were characterised, in terms of droplet size distribution, droplet charge (zeta potential), rheological properties and microstructure images (CLSM and/or light microscope). The increase in droplet size \( (d_{43}) \) and visual phase separation were monitored, on the 3\(^{rd} \), 7\(^{th} \), 14\(^{th} \) and 30\(^{th} \) day of storage, at 20\(^{o}C\). 1-2% of SDS was added to stored emulsions and the droplet size was measured to confirm the origin of large emulsion droplets.

![Figure 32: Effect of pH adjustment (before vs. after emulsification) on the particle size distribution of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt)-soya oil-in-water emulsions (BE=before emulsification; AE=after emulsification)](image)

**4.3.1 Results**

The emulsifying ability of BSG (as a function of pH), in terms of droplet size distribution, is shown in Figure 33 (below). Increasing the pH markedly reduced the average droplet size of the emulsions. This was indicated by the shift of the size distribution curves, to the smaller droplet size area. Emulsions at native pH (≈7.0) exhibited monomodal particle size distribution, with small \( d_{32} \) of <1.0 \( \mu \)m. At more alkaline conditions (pH >7.0), particle size distributions were almost independent of pH, having \( d_{32} \) values below 1.0 \( \mu \)m. On the other hand, emulsions at lower pH
conditions (<6.0) had larger droplet sizes, as manifested by the shift of the size distribution, to the larger droplet population. Addition of 1-2% (v/v) SDS on the emulsion (which displaces the polysaccharide at the interface) did not reduce the droplet size (Figure 34 below) suggesting that big droplets were initially present when the emulsion was formed.

The effect of pH, on the apparent viscosity of BSG-stabilised emulsions, is shown in Figure 35 (below). At very low shear rates (<0.1s⁻¹), emulsions at low pH (<4.0) had higher apparent viscosities, than emulsions at higher pH conditions (>5.0). However, as the shear rate increased, the apparent viscosity was almost independent of pH and all emulsions exhibited shear-thinning flow behaviour.

![Figure 33: Effect of pH on the particle size distribution of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt)-soya oil-in-water emulsions](image-url)
Figure 34: Effect of SDS addition (1-2% v/v) on the droplet size distribution of fresh 0.3% (wt/wt) BSG-stabilised 30% (wt/wt) soya oil-in-water emulsions at pH 2, day 1

Figure 35: Effect of pH on the apparent viscosity of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt)-soya oil-in-water emulsions

The viscoelastic properties of BSG-stabilised emulsions, as affected by different pH conditions, are shown in Figure 36. Emulsions at extremely low pH conditions (<2.0), exhibited high viscoelasticity. In all other emulsions, viscoelastic properties did not significantly change, when varying the pH of the BSG solutions. It was observed that storage modulus ($G'$) was higher, than the loss modulus ($G''$), indicating that elastic property dominated the viscoelastic behaviour of the emulsions at all pH studies.
Figure 36: Effect of pH on: (a) the storage modulus ($G'$) and (b) the loss modulus ($G''$) of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt) soya oil-in-water emulsions

Figure 37: Effect of pH on the zeta potential of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt)-soya oil-in-water emulsions

Figure 37 illustrates the effect of pH, on the zeta potential of BSG-stabilised emulsions. Zeta potential values were reduced, with decreasing pH values. This was very apparent in emulsions at pH 2.0, where the zeta potential value dropped from -44.5 (pH 4.0) to -2.6 mV (pH 2.0). In emulsions above pH 4.0, the zeta potential...
values increased with pH, indicating that the BSG-stabilized droplets became more negatively charged. Above pH 6.0, zeta potential measurements did not significantly varied (>−50mV), although a maximum value was found at −57.8 mV at the native pH of BSG ~7.0.

The effect of pH, on the stability of emulsions during storage, was evaluated, in terms of the growth in droplet size ($d_{43}$) and visual phase separation. The average droplet size of all emulsions steadily increased, except for emulsions at native pH, which remained constant, during storage (Figure 38 below). At very acidic conditions (pH <2.0), emulsion droplet sizes were extremely large and markedly increased from ca. 20 µm at day 1 to ca. 33 µm after one week storage. Addition of an excess amount of SDS (1-2% wt/wt) to emulsions stored for 1 month did not change the droplet size (Figure 39). This means that droplet coalescence is likely to be the cause of the growth in droplet size. If the increasing droplet size over time was caused by flocculation of droplets, the sizes measured in the presence of small molecule surfactants such as SDS (which would displace the adsorbed polymer) would revert to the original sizes (day 1). No visual phase separation was apparent, in the emulsions across all pH conditions during storage.

![Figure 38: Effect of pH on the average particle size ($d_{43}$) of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt) - soya oil-in-water emulsions stored for 1 month at 20°C](image-url)
Figure 39: Effect of SDS addition (1-2% v/v) on the particle size distribution of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt)-soya oil-in-water emulsions at varying pH (2-4) at 1 month storage.

Figure 40: Microstructures of fresh 0.3% (wt/wt) BSG-stabilised 30% (wt/wt)-soya oil-in-water emulsions at pH 2. (A) control emulsion (pH ~7) at 40 x magnification CLSM, (B) pH 2 emulsion at 40 x magnification CLSM, and (C) pH 2 emulsion at 40 x magnification light microscopy.
Furthermore, the microstructures of 0.3% BSG-stabilised emulsions as influenced by pH (see Figure 40) revealed that lowering the pH (<2.0) resulted in bigger initial droplet sizes (Figure 40 B and C) compared to the emulsions at pH ~7.0 (Figure 40A). Also, emulsion flocculation/aggregation was detected at pH 2.0 (Figure 40B). It was also observed that some inclusions of fluorescent material were trapped with the emulsions droplets. In order to confirm that the “inclusions” were inside the droplets, a 3D vertical cross-sectioning of emulsion droplets was carried out (Figure 41D). The right hand side depicts a vertical section of the “y” axis, whereas the bottom section corresponds to the vertical section of the “x” axis as shown in the micrograph.
In the image of Figure 41A, the red fluorescence from the Nile Red present in the Nile blue dye, represents the oil droplets. In image B (Fast Green channel), the green fluorescence was observed at the droplet interface and within the droplet itself. The images C and D (Figure 41) show the combined fluorescence of dyes. Clearly, the Fast Green dyed the polysaccharide fraction. Theoretically, Fast Green dyes the proteins and the interaction is known to be hydrophobic (Tromp, et al., 2001). The fact that the fluorescence was detected strongly at the interface suggests that Fast Green had interacted with the polysaccharide adsorbed onto the oil droplets, most likely through hydrophobic interactions. The 3D image of a single cross-sectioned oil droplet also showed, that polysaccharide inclusions were indeed present as indicated by the green fluorescing dyes detected inside the droplets. This indicates that BSG must contain a large proportion of hydrophobic segments on its structure. It is unlikely that all fluorescence detected came from protein since this was only 0.36% (0.3 x 1.2) of the BSG present in the emulsion.

4.3.2 Discussion
The emulsifying ability of BSG, in terms of droplet size and droplet size distribution was sensitive to pH changes. The BSG-stabilised emulsions were more sensitive to low pH (<4.0), than at alkaline conditions (>pH 6.0). A drop in pH rapidly increased the particle size ($d_{32}$) and decreased the zeta potential values (especially at pH < 4.0). Varying the pH may affect the ability of the BSG to adsorb at the interface, hence the formation of initially larger droplets. At acidic pH’s the polysaccharide is more likely to be in a more aggregated state before emulsification, resulting in the formation of bigger droplets as observed at pH ≤ 5.0. This is particularly emphasised between pH 2.0-3.0, where the dramatic change in zeta potential occurs. It is likely that the pKa of the acidic fractions of BSG is around these values. Under alkaline conditions (>pH 6.0), however, the particle size of BSG emulsions decreased below 1.0 μm ($d_{32}$) although the zeta potential values hardly became more negative.

The reduction in droplet size at very alkaline conditions may also reflect the effect of the increasing amount of added NaOH on the oil phase itself, helping the emulsification process (Bansal, et al., 1978; Marinova, et al., 1996). The high negative charge of the BSG-stabilized emulsions droplets above pH 4.0 (zeta potential above -40mV) would appear to indicate that BSG-emulsions are electrostatically
stable. The big droplets sizes still detected between pH 4.0 and pH 6.0 may also correspond to big droplets initially present when the emulsion was formed. The micrographs taken at very acidic pH (~2.0) certainly confirm the big droplets present initially in the emulsion, in addition to flocs formed. BSG is a complex gum composed of a major glucomannan fraction, which is acid stable and a minor xylan fraction, which contains acid side chains. It would seem more likely that the xylan fraction would be more sensitive to pH and salt, which may contribute to the net charges of the polysaccharide. A pH close to 2.0 would appear to be the pH where the charges on BSG have almost disappeared.

A similar result was found by Nakauma et al. (2008) in gum Arabic, which was used to stabilise 15% (wt/wt) o/w emulsions. The $d_{32}$ increased rapidly, due to emulsion breakdown and the zeta potential decreased, at pH below 3.0. According to these authors, although the sensitivity to pH was primarily related to the isoelectric point of protein moiety, the electrostatic interactions, between or within the polysaccharide portions, could be another factor which might affect the adsorption behaviour of the gum, by altering the hydrophobic character of the polysaccharide fraction. Consequently, emulsions stabilised by polysaccharides will undergo flocculation, or sometimes coalescence at the pH where the carbohydrate fraction has lost all the net charge (not the protein moiety). This would decrease the electrostatic repulsion, which contributes to the stabilisation of the emulsion system. In our case, coalescence was detected at pH 1.0-2.0 over time (Figure 38), corresponding to the loss of charge on the polysaccharide. Very monomodal large droplets were obtained at this pH.

Furthermore, the apparent viscosities of BSG emulsions were higher at acidic conditions (pH <4.0), than at alkaline conditions, at very low shear rates (0.01-0.1 s$^{-1}$) reflecting possible enhanced aggregation of “bigger” droplets. However, apparent viscosities became pH independent at high shearing rates and all emulsions exhibited shear-thinning behaviour. The viscoelasticity of the emulsions was also resistant to pH changes apart from very extreme conditions (pH < 2.0) where higher $G'$ and $G''$ were detected. Overall, the rheological properties of the emulsions did not significantly vary irrespective of the size of the emulsion droplets, which indicates that the rheology is controlled by the BSG present. The viscosity of BSG itself is very resistant to pH changes (Hosseini-Parvar, 2009).
The average droplet size \(d_{43}\) of BSG emulsions increased during storage. A marked increase was observed in emulsions at pH < 2.0 and a slight increase above pH 4.0. This growth in droplet size indicates that oil droplets coalesced (as demonstrated at pH 2.0 after the addition of SDS) and/or underwent flocculation over time (most likely between pH 4.0-6.0). However, no change in \(d_{43}\) at native pH (~7.0) was observed, suggesting that the emulsions remained stable, in terms of droplet size. This may be related to (i) a maximum adsorption of BSG at the interface and/or to (ii) a maximum droplet charge at this particular pH value. Furthermore, no visual phase separation was apparent in any of the emulsions, upon storage. Although droplet flocculation, by the addition of acid, resulted in coalescence, the emulsions remained stable against phase separation, during storage. This may again be attributed to the viscoelasticity provided by the BSG in the aqueous phase. The yield stress keeps the big droplets suspended so that no creaming occurs.

Microstructure images further confirmed that strong aggregation of droplets and coalescence occur at low pH (<6.0). Interestingly, some inclusions were found in large emulsion droplets at pH 2.0. This may suggest that BSG strongly adsorbed at the interface and some of the polysaccharides were trapped within coalesced droplets during the homogenisation process. These fluorescent polysaccharide inclusions were present in the initial droplets after homogenisation in day 1. It seems likely that BSG is strongly hydrophobic, especially when the charge is almost lost (at pH 2.0). During the breakdown and coalescence process taking place during homogenisation, the BSG is easily trapped within the hydrophobic oil droplets. The probable explanation for the unique behaviour of BSG can be attributed to its molecular features. BSG is mainly composed of glucomannans (~43%), a minor xylan fraction (~24%) and a small protein fraction (1.2%). Some glucomannans (e.g. konjac mannan) are known to contain hydrophobic acetyl groups (Li, et al., 2006; Takigami, 2000). Therefore, it is possible that BSG may contain a large proportion of acetyl groups, which may be responsible for its strong hydrophobic character, as confirmed by the fact that it is easily dyed by the Fast Green (normally used for proteins).

### 4.4 Effect of salt on the emulsifying properties of BSG

The effects of salt (NaCl), or ionic strength, on the emulsifying and stabilising properties, were evaluated at a fixed BSG concentration of 0.30% (wt/wt), with
varying salt concentrations (5-70 mM). Preliminary tests were carried out with the addition of salt before and after the emulsification process. The droplets formed were larger in emulsions where the salt was added after emulsification than before emulsification (Figure 42) suggesting strong flocculation of the already formed droplets. Accordingly, addition of salt prior to emulsification was chosen to determine the impact of ionic strength on BSG emulsions, since this seemed to improve significantly the droplet size obtained. The emulsions were characterised, in terms of particle size, droplet charge and rheological properties. Microstructures of the emulsions were also obtained using CLSM and/or light microscope. Visual phase separation and any increase in particle size were monitored, on the 3\textsuperscript{rd}, 7\textsuperscript{th}, 14\textsuperscript{th} and 30\textsuperscript{th} day of storage, at 20°C. In order to confirm the origin of big emulsion droplets 1-2% SDS was added.

![Figure 42: Effect of the addition of NaCl (before vs after emulsification) on the particle size distribution of 0.3% (wt/wt) BSG –stabilised 30% (wt/wt)-soya oil-in-water emulsions (BE = before emulsification; AE= after emulsification)](image)

4.4.1 Results

The emulsifying ability of BSG (as a function of ionic strength), in terms of particle size distribution is shown in Figure 43. The addition of salt drastically changed the particle size and particle size distribution of all the emulsions. Increasing the salt concentration shifted the size distribution to the right (large droplet population area) obtaining more monomodal emulsions with narrow distributions. The addition of NaCl, up to 50mM, increased the $d_{32}$, from 0.99 to 16.1 µm. However, upon further
addition of salt, up to 70 mM, the average particle size slightly decreased to 14.7 µm.

It can also be seen that the control emulsion (no NaCl) had monomodal size distribution, with the smallest mean droplet size ($d_{32} = 0.99$ µm).

The effects of salt, on the rheological properties of BSG-stabilised emulsions, are shown in Figure 44. At lower shear rates, the apparent viscosity dropped, upon the addition of <10mM NaCl. However, as NaCl concentration was further increased up to 70mM, the apparent viscosity also increased. At increasing shear rates, the viscosity of all the emulsions appeared to be independent of salt concentration. Strong shear-thinning flow behaviour was also observed, in all the emulsions. The same trend was observed for the viscoelastic properties, with the elastic modulus (e.g. 70.2 Pa at 0.1 Hz, 35mM NaCl) dominating once again the viscous (e.g. 15.4 Pa at 0.1Hz, 35mM NaCl) one for all the emulsions (see Figure 45).
Figure 44: Effect of salt (5-70 mM NaCl) on the apparent viscosity of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt)-soya oil-in-water emulsions

The effects of salt, on the zeta potential values of the BSG-stabilised emulsions, are illustrated in Figure 46 (below). Increasing the ionic strength steadily decreased the zeta potential values of the emulsions. A marked decrease, from -58.1mV to -14.6 mV, was observed, upon increasing NaCl concentrations, from 5 to 50 mM. Upon
further increase in salt concentration, up to 70mM, the zeta potential values levelled out at -14.6 mV.

![Graph showing the effect of salt on zeta potential](image)

**Figure 46**: Effect of salt (5-70mM NaCl) on the zeta potential of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt)-soya oil-in-water emulsions

The effect of salt, on the storage stability of the BSG-stabilised emulsions, in terms of $d_{43}$, is shown in Figure 47. The mean droplet size ($d_{43}$) of the emulsions steadily increased over time, across all concentrations of NaCl. It was observed that, at 25mM NaCl, mean droplet sizes were larger, than at 35mM NaCl (e.g. 15.5µm vs. 14.0µm at day 7), and the same trend was observed, at 50mM compared to 70mM NaCl, with droplet sizes of 17.3µm vs. 16.1 µm at day 7, respectively. The droplet size distribution of emulsions stored for 1 month did not revert back to the original size at day 1 after the addition of 1-2% SDS (see Figure 48). As explained in section 4.3.2, this indicates that droplets probably coalesced over time. There was no apparent phase separation observed in any emulsions, during the storage period.
Figure 47: Effect of salt (5-70 mM NaCl) on the average particle size ($d_{43}$) of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt) soya oil-in-water emulsions stored for 1 month at 20°C.

Figure 48: Effect of SDS addition (1-2% v/v) on the particle size distribution of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt)-soya oil-in-water emulsions at varying concentrations of salt (35-70mM NaCl) at 1 month storage.
Furthermore, the microstructures of emulsions (Figure 49 above) showed that the addition of salt resulted in the formation of large initial emulsion droplets, showing some coalescence happening over time as detected clearly in Figure 49 B and C, where two droplets are coalescing. Some polysaccharide inclusions trapped inside the bigger droplets were also observed, similar to emulsions at low pH, although not as emphasized at this ionic strength.

4.4.2 Discussion

The emulsifying activity of BSG was sensitive to salt, in terms of particle size and particle size distribution. Increasing ionic strength above 25mM NaCl resulted in monomodal and narrow size distributions, of larger initial droplets. The increase in droplet size was accompanied by a substantial decrease in zeta potential, upon the addition of salt. This phenomenon was probably the result of droplet coalescence occurring during homogenisation, as well as posterior flocculation and coalescence,
via the screening of the electrostatic repulsion, which consequently reduced the
surface potential of BSG and therefore the potential on the emulsion droplets. In
electrostatically stabilised emulsions, the thickness of the electrical double layer tends
to decrease, as the concentration of ions increases (Kulmyrzaev & Schubert, 2004)
thus, resulting in decreased zeta potential. The salt sensitivity of BSG suggests that
electrostatic repulsion contributes to its stabilising mechanism. Because the salt was
added prior to emulsification, it is very likely, that BSG at higher ionic strength would
tend to be more aggregated, before emulsification resulting in bigger droplet sizes
during the initial homogenization process. The formation of initial bigger emulsion
droplet size, upon addition of salt was further confirmed by the CLSM and light
microscopy images.

The salt sensitivity of BSG could be similar to that of sugar beet pectin. The addition
of salt (NaCl and CaCl$_2$), to emulsions stabilised by sugar beet pectin (SBP), caused
droplet flocculation. The effect of CaCl$_2$ is greater compared to NaCl, suggesting that
CaCl$_2$ causes bridging flocculation more effectively than NaCl (Nakauma, et al.,
2008). These authors explained the flocculation of droplets, through the screening of
charges and through bridging via hydrogen bonds (e.g. polyanion-cation-water-
polyanion linkages), resulting in a growth in droplet size.

Furthermore, the apparent viscosity and viscoelasticity of the emulsions initially
dropped, upon increasing salt concentration (5-10mM NaCl), at very low shear rates.
However, a further increase in the ionic strength progressively increased the values of
these rheological parameters. At higher shear rates, rheological properties were salt
independent. The rheological properties of electrostatically stabilised emulsions, such
as proteins, are sensitive to pH and ionic strength (Martínez, et al., 2007;
McClements, 2005). Initially, the viscosity decreases, with increasing ionic strength,
due to screening of charges. Above a certain salt concentration, attractive interactions
between droplets overcome the repulsive interactions, which leads to droplet
flocculation and this causes an increased emulsion viscosity, with the addition of salt.
No major rheological changes in the emulsions stabilised by the polysaccharide used
in this study, were observed, perhaps because the rheological behaviour of the gum
itself has been shown to be quite resistant to changes in ionic strength (Hosseini-
Parvar, 2009).
The addition of salt decreased the stability of BSG emulsions, in terms of sizes \(d_{43}\), as indicated by the increase in average droplet size, during storage. As explained earlier, the growth of mean droplet size is likely to be due to droplet flocculation, by the addition of salt, probably leading to coalescence over time, which is an irreversible process as confirmed by the addition of SDS (see Figure 48). It is probable that incomplete surface coverage of the droplets formed under increasing amount of salt, leads to coalescence. Steric stability maybe compromised as in the case of very low pH (~2.0) emulsions, where coalescence rapidly occurred. However, physical phase separation was not observed, in any emulsions during storage, again probably due to the viscoelasticity and yield stress provided by non-adsorbed BSG.

4.5 Thermostability of BSG-stabilised O/W emulsions

The effects of heating, on the emulsifying and stabilising properties of BSG, were evaluated at a fixed concentration of 0.30% (wt/wt). Emulsions at native pH ≈7 (control) and at extreme pH’s (2 and 10) were prepared, as per Materials and Methods (Chapter 3) and heated at 80°C for 30 mins. The particle size \(d_{32}\), particle size distribution and rheology were measured, within 24 hrs after the emulsion preparation. The visual phase separation and increase in mean droplet size \(d_{43}\) were monitored, during the storage period of one month, at 20°C.

4.5.1 Results

The droplet size distribution of BSG emulsions affected by heating is shown in Figure 50. The size distribution of droplets drastically changed, after heating the emulsions, irrespective of pH conditions. Heating substantially increased the mean droplet size \(d_{32}\) of the emulsions at pH 2, which increased from 20.4 to 85.2 µm. In the case of the control emulsions (pH ≈ 7) and the emulsions at pH 10, their mean droplet size increased from 0.99 to 1.5 µm and 0.36 to 0.99µm, respectively. Only the extreme pH conditions were plotted as these represent the conditions where the effect of heating was very apparent.

The effects of heating, on the apparent viscosity and viscoelasticity of the BSG emulsions, are shown in Figure 51 and Figure 52 respectively. At very low shear rates, heated emulsions, at extremely low pH (2), had slightly higher apparent viscosities, than the emulsions at
more alkaline pH’s. As the shearing rate increased, all emulsions showed strong shear-thinning flow behaviour. The control emulsions (native pH) showed no changes in apparent viscosity, after heating.

![Graph showing particle size distribution](image)

**Figure 50**: Effect of heating on the particle size distribution of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt) - soya oil-in-water emulsions (BH - before heating; AH – after heating)

In terms of viscoelasticity, the storage modulus ($G'$) and loss modulus ($G''$) of the emulsions increased after heating, except for pH 10. Generally, the storage or the elastic modulus ($G'$) dominated the viscoelastic behaviour of unheated and heated emulsions. At pH ~7.0, there was no significant change observed.
The effects of heating, on the mean particle size ($d_{43}$) of the emulsions, during storage, are shown in Figure 53. A growth in the mean droplet sizes was observed, in both the heated and unheated emulsions at acidic pH. For example, at pH 2, the droplet size of the unheated emulsions increased from 20.4 to 29.8 µm, whilst the heated emulsions increased from 85.2 to 89.6 µm, upon storage from day 1 to 7th, respectively. No significant changes with time were observed at the native and more alkaline pH’s for
the unheated emulsions as also described in section 4.3.1. However, the droplet size slightly increased after heat treatment (i.e. from 2.9 to 12.8µm over one week period for the control emulsions). The droplet size of heated alkaline emulsions (pH 10) appeared to be quite “plateau” with no apparent change overtime on the heated emulsions. In general, the mean droplet sizes of the heated emulsions were significantly larger, than the unheated emulsions, regardless of pH conditions. Furthermore, no visual phase separation was apparent in any emulsion, upon storage.

![Graph](image)

**Figure 53:** Effect of heating on the average particle size ($d_{43}$) of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt) -soya oil-in-water emulsions stored for 1 month at 20°C

### 4.5.2 Discussion

BSG-stabilized emulsions, at extreme pH (2 and 10) conditions, were sensitive to heating, in terms of droplet size ($d_{32}$) and droplet size distribution. Heating at 80°C, 30 mins caused the droplets to aggregate, thus resulting in a marked increase in the size of the emulsion droplets. Irrespective of pH, the heated emulsions had markedly larger droplets, than the unheated ones. The large droplet size, in the heated emulsions, may be due to droplet flocculation induced by heating. Heating favours hydrophobic interactions which lead to droplet aggregation (McClements, 2005). Possible
hydrophobic interactions via adsorbed BSG are likely to be responsible for the aggregation in the present case. Unfortunately, no SDS treatment was carried out in order to confirm the origin of large emulsion droplets.

This observation is similar to the findings by Nakamura et al. (2007) in heat-treated oil-in-water emulsions stabilised with soybean soluble polysaccharide (SSPS) containing protein impurities, which included proline-rich and glutamic acid-rich peptides. Droplet aggregation was shown after heating the emulsions at 80-90°C for 30 mins. Additionally, the heat stability of the emulsions stabilised by SSPS was also influenced by pH (3.0-7.0). In this case, emulsions at pH 7.0 had larger droplet sizes after heating than at lower pH (3.0-4.0) conditions. The heat-induced aggregation of emulsions stabilised by SSPS would seem to be likely related to the presence of proteinaceous moieties, emphasizing the important role of hydrophobic fractions present in the polymer fraction in influencing the functional properties of the polysaccharide.

In terms of rheological properties, the apparent viscosity of the emulsions was not significantly affected by the thermal treatment. Only a slight increase in apparent viscosity at low shear rate at very acidic pH was detected, maybe reflecting the emulsion droplet aggregation after heat treatment, easily broken under shear. In general, the flow behaviour of the emulsions did not significantly vary. This can be correlated to the study of Hosseini-Parvar et al. (2009). These authors reported that the apparent viscosity of 1% BSG solution was not significantly affected by temperature, thus suggesting the heat-resistant nature of BSG in terms of viscosity properties.

The viscoelastic properties of the emulsions (native and low pH) were slightly increased, after heating. Conversely, the viscoelasticity of the extremely alkaline emulsions substantially decreased. Based on the polydispersity (3 distinctive droplet populations) of the aggregate size formed after heating at pH 10 (Figure 50), this was probably due to the formation of a more inhomogeneous system which resulted in a decrease in viscoelasticity. On the other hand, at acidic pH, it appears that heating improved the viscoelasticity of the emulsions. It is inferred that droplet flocculation, due to heating and change in pH (towards high acidity), probably contributed to the
creation of a stronger droplet network, which resulted in higher viscoelastic parameters, especially the elastic modulus. Hosseini-Parvar et al. (2009) also found that both the storage and loss modulus of 1% BSG solution gradually increased, with increasing temperature (60-85°C).

The heated and unheated emulsions, at extreme pH (2 and 10) conditions, showed a moderate increase in mean droplet size ($d_{43}$), thus suggesting that the emulsion droplets slightly coalesced or were subjected to flocculation over time. Although the mean droplet sizes of the heated emulsions were larger than the unheated emulsions, they did not phase separate over time. Again, the absence of phase separation may be attributed to the high apparent viscosity and viscoelasticity of the aqueous phase, which controlled the movement of droplets conferring physical stability to the emulsions.

4.6 Effect of protein content on the emulsifying properties of BSG
In order to determine the impact of protein, on the emulsifying properties of BSG, different gum preparations, with varying protein contents, were used at a fixed concentration of 0.30% (wt/wt) BSG, based on polysaccharide content. The prepared gums, based on their purification process, were termed “crude” BSG, “purified” BSG and “protein-free” BSG. The crude and purified (alcohol treatment) BSG, used in this experiment, were prepared following the method described by Hosseini-Parvar et al. (2009). The protein-free (phenol treatment) BSG was prepared, following the method by Youssef et al. (2009). The proteins were extracted from BSG by treatment with phenol solution as proteins are soluble in phenol and polysaccharides are soluble in water at certain pH conditions. Although not all proteins were entirely removed from the gum during purification, the term “protein-free” was used to distinguish it from the purified one. Furthermore, the protein contents of each gum preparation were determined, as per Materials and Methods (Chapter 3).

The emulsifying properties of BSG, as a function of protein content, were characterised in terms of droplet size ($d_{32}$) and droplet size distribution. The flow behaviour and viscoelasticity of the fresh emulsions were also evaluated. The stability of the emulsions, in terms of growth in particle size ($d_{43}$) and phase separation were monitored, during one month of storage, at 20°C. Addition of 1-2% (v/v) SDS was
carried out in order to confirm the origin of the growth of droplets. Microstructures of the fresh emulsions were also obtained using the CLSM and/or light microscope.

4.6.1 Results

The protein contents of the gums were determined using two methods: (i) determination of total nitrogen content (N x 6.25) and (ii) quantification of the amino acid composition. The results obtained from these two methods were compared. Table 1 (below) presents the results on the protein contents of the different gum preparations.

Table 1: Protein composition of crude, purified and protein-free BSG

<table>
<thead>
<tr>
<th>Gum preparation</th>
<th>Protein content (%)</th>
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<tbody>
<tr>
<td></td>
<td>By Nitrogen</td>
</tr>
<tr>
<td>Crude BSG</td>
<td>1.4</td>
</tr>
<tr>
<td>Purified BSG</td>
<td>1.0</td>
</tr>
<tr>
<td>Protein-free BSG</td>
<td>1.1</td>
</tr>
</tbody>
</table>

From Table 1, it can be seen that protein contents varied significantly, between nitrogen and amino acid analyses. Protein quantities obtained based on nitrogen were higher, than those obtained based on amino acids analysis. Furthermore, protein contents did not seem to decrease after further purification of the gum, based on nitrogen analysis, which shows that Kjeldhal analysis is not sensitive enough for this low protein levels. On the other hand, protein content based on the amino acid analysis was reduced from 1.2% (crude BSG) to 0.65% (protein-free BSG), after gum purification. It appears that protein content determination, using amino acid analysis, is more accurate than the nitrogen method. The latter can include non-protein nitrogen, and therefore the protein quantity may appear higher compared to Amino acid analysis related only to protein.

The emulsifying properties of BSG (as a function of protein content), in terms of particle size distribution, are shown in Figure 54. The emulsions stabilised by crude, purified and protein-free BSG had all monomodal droplet distributions. Crude-BSG produced fine emulsion droplets, with \( d_{32} \) of 1.0 \( \mu \)m. Purified-BSG formed the finest droplets (\( d_{32} = 0.72 \mu \)m) and the protein-free BSG formed the most coarse emulsion
Although purified-BSG had the smallest $d_{32}$, its span value—indicating the width of the distribution—was higher (2.4) than crude BSG (1.62), but smaller than protein-free BSG (7.5).

Figure 54: Effect of protein content on the particle size distribution of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt)-soya oil-in-water emulsions

Figure 55: Effect of protein content on the apparent viscosity of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt) - soya oil-in-water emulsions
The apparent viscosity of protein-free BSG emulsions was higher, in comparison with the crude and purified BSG emulsions (Figure 55). This indicates that further attempt of purification (removal of protein impurities) of the gum contributed to the increase in viscosity of the emulsions. A similar trend was observed for the viscoelastic properties of the emulsions, as shown in Figure 56 below. Protein-free BSG had higher viscoelasticity, than crude and purified BSG. Furthermore, storage or elastic modulus (\(G'\)) was higher, than loss or viscous modulus (\(G''\)), in all the emulsions. This can be related to the droplet sizes observed in Figure 54 or to a more aggregated state of BSG after the phenol treatment, which increased the viscoelasticity of the emulsion system.

Figure 57 shows the effect of protein content on the growth of droplet size, during storage. The mean droplet size \((d_{43})\) of protein-free BSG dramatically changed, during storage, increasing from 4.0 to 22.3 µm, whilst for crude and purified BSG, no significant change (e.g. 1.31-1.38 µm) was observed. Addition of SDS to protein-free BSG emulsions (at day 7) did not revert back to the original droplet size distribution in day 1 (see Figure 58), which again suggests, that droplets had coalesced over time. Moreover, no visual phase separation was apparent in any emulsions, during storage.

Figure 56: Effect of protein content on the storage (\(G'\)) and loss (\(G''\)) modulus of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt) - soya oil-in-water emulsions
Figure 57: Effect of protein content on the average particle size ($d_{43}$) of 0.3% (wt/wt) BSG-stabilised 30% (wt/wt)-soya oil-in-water emulsions

The microstructures (see Figure 59) show that fresh emulsions stabilised by crude and protein-free BSG are homogenous. However, it is observed that protein-free BSG rapidly coalesced (Figure 59B) when compared to crude BSG (Figure 59A). This suggests that further purification of the gum may have altered the adsorption properties of the polysaccharide.
4.6.2 Discussion

Polysaccharides are generally not considered to be surface active agents, due to their strong hydrophilic character. In contrast, proteins are used as emulsifiers, due to their hydrophilic and hydrophobic side chains, which make them effective surface active agents. Hence, small fractions of proteins, present in some hydrocolloids, are believed to be responsible for the observed emulsification properties (Huang, et al., 2001).

In order to determine the effect of protein content, on the emulsifying properties of BSG, the crude gum, containing <1.2% (wt/wt) protein, was further purified, in order to remove protein impurities. The proteins calculated from the nitrogen content showed no marked difference, between the three gum preparations. The accuracy of this nitrogen method was thought to be limited, in terms of measuring small quantities (<1%) of proteins. Also, some nitrogenous compounds, detected by this method, could not have been totally originated from proteins. Accordingly, amino acid profiling was undertaken and it was found to show a more accurate result, than the previous method. The results of the amino acid analysis show that protein impurities were substantially removed, by further purification of BSG. Still, protein could not be totally removed and 0.65% was present after the purification based on phenol treatment. This may indicate that this residual protein is part of the BSG structure and is not an ‘impurity’.

The emulsifying properties of BSG, in terms of particle size ($d_{32}$) and size distribution, were altered, upon removal of protein impurities. Amongst gum preparations, protein-
free BSG produced the largest emulsion droplets of 2.8 µm ($d_{32}$). There may be different explanations for this: (i) the purification process itself could have modified the rheological properties of BSG resulting in an increased viscosity which reduced the efficiency of the homogenisation, manifested in the formation of larger emulsion droplets; (ii) the purification process could have changed the functionality of BSG in terms of hydrophobicity and conformational structure, changing its adsorption properties and resulting in bigger emulsions droplets and/or a higher degree of droplet aggregation/coalescence; (iii) the reduction of almost 0.5% in the protein fraction may have been detrimental in the adsorption properties of the gum resulting in bigger sizes due to bridging flocculation and/or coalescence. Nevertheless, at the same gum concentration of 0.30% (wt/wt), protein-free BSG, containing as low as 0.65% (wt/wt) protein, had comparable droplet sizes (>2 µm) with native fenugreek gum that contained 3% (wt/wt) protein (Garti & Leser, 2001).

Conversely, crude BSG produced finer droplets, with monomodal size distribution. The purified BSG had smaller $d_{32}$ than crude BSG, but its span value indicated a wider size distribution, wherein the peak was shifting to the left (small size area), resulting in a decreased mean surface area. Upon storage, the mean droplet sizes of both gums were comparable and did not change significantly. It can be inferred that a purification process based on alcohol treatment only, did not influence significantly the behaviour of the gum; if anything improved the emulsification process, probably aided by the decrease in viscosity detected, as opposed to further purification based on the phenol solvent method.

Protein-free BSG-stabilised emulsions had the highest apparent viscosity, amongst the BSG preparations tested. This result was in agreement with the report of Youssef et al. (2009), which indicated that further purification (phenol solvent treatment) of purified fenugreek gum increased its intrinsic viscosity. It can be implied that the purification of BSG modified its intrinsic viscosity resulting in higher apparent viscosities of the protein-free gum. Moreover, the increased apparent viscosity of the protein-free BSG could also be responsible for the stability of the emulsion against phase separation. It is also possible that the BSG maybe in a more aggregated state, resulting in an increased viscosity.
The stability of the emulsions, in terms of growth in droplet size \( (d_{43}) \), was reduced, upon removal of proteins based on the phenol solvent treatment. This was manifested by a dramatic increase in the droplet size of protein-free BSG emulsions, during storage, implying a strong coalescence process happening over time (see Figure 58). This could support the idea of the polysaccharide not being strongly attached to the interface after being modified by the purification process and therefore, not preventing coalescence. However, despite the growth in droplet size, the emulsions remained stable, with no indication of phase separation.

Moreover, the results of this study were almost similar to the report of Huang et al. (2001), although their investigation was focused on different gums. The emulsifying properties of 14 hydrocolloid gums, containing different amounts of protein impurities, were investigated. Fenugreek gum, having the second highest protein content (14%) amongst the gum samples, produced the smallest and most uniform emulsion droplets, with a narrow size distribution. It also formed the most stable emulsion, after 90 days of storage. It was inferred that protein impurities contributed to the high emulsifying activity of the gum. However, PGA and methylcellulose gums, containing as low as 0.1% protein, also produced stable emulsions. This indicates that the emulsion stabilising properties of gums could not only be attributed to the surface-active protein impurities, but it could also be associated with the structural features of the polysaccharides. As in the case of BSG, it may be possible that acetyl groups may be present in its glucomannan fraction. Although Dickinson (2003) indicated that the emulsifying ability of polysaccharides was dependent on the surface-active protein impurities attached to the gum, it appears that it is not an absolute requirement to produce still small oil droplets.

### 4.7 Summary and Conclusion

This study investigated the emulsifying and stabilizing properties of BSG, in terms of particle size, particle size distribution, rheology and visual phase separation. Factors such as BSG concentration; pH; ionic strength; heating; and protein content were evaluated. The results of this study revealed that BSG could form small oil droplet sizes (<1.0 µm) and stabilise emulsions against phase separation by using as low as 0.30% (wt/wt).
The emulsifying properties of BSG in terms of droplet size were sensitive to changes in pH and ionic strength (salt) as confirmed by the formation of larger droplet size and decrease in zeta potential values at low pH and high salt concentration. In general, emulsions at alkaline conditions (>pH6) and low ionic strength had small droplet size with uniform droplet distribution. Additionally, heating and purification by phenol treatment (removal of protein from the gum) appear to reduce the emulsifying capabilities of BSG.

The rheological properties of BSG stabilised-emulsions appear to be dependent on gum concentration and purification. On the other hand, these properties are relatively pH, salt and heat-independent. Increasing the gum concentration and removal of gum proteins resulted in high apparent viscosity and viscoelastic properties of the emulsions. Typically, at low shear rates, the apparent viscosity and viscoelasticities of emulsions increased after heating and at low pH conditions. Conversely, addition of salt initially decreased the rheological properties of emulsions at low shear rates. At higher shear rates, however, the flow behaviour and viscoelasticities of the emulsions tend to be independent of processing conditions. The resistance of BSG to processing conditions suggests that it could provide strong emulsion stability. This was confirmed by the stability of emulsions against phase separation at concentrations as low as 0.24 % (wt/wt), or in any emulsions tested at different processing conditions (pH, salt and heat).

Although removal of proteins reduced the emulsifying properties of BSG, a homogeneous emulsion was still formed. This suggests that the emulsion formation mechanism of BSG is not solely attributed to the surface-active protein impurities but could also be associated with the hydrophobic character of the gum itself, since this could have been modified by the purification process itself. Also, the emulsions remained stable against phase separation.

Overall, BSG represents a very promising gum that can be categorised as a novel hydrocolloid emulsifier. An effective emulsifying hydrocolloid has the ability to adsorb at the air/water and oil/water interfaces. In order to shed more light on the origin of the emulsifying properties of BSG, adsorption properties of the gum were investigated.
Chapter 5
Adsorption properties of BSG

5.1 Introduction
This chapter reports on the adsorption (surface/interfacial tensions) properties of BSG. In the earlier study, it was shown that BSG, containing small amounts of protein impurities, could emulsify and stabilise O/W emulsions (refer Chapter 4). According to Dickinson (2003), a hydrocolloid can be an effective emulsifying agent, if it is surface-active. That is, it must have the ability to rapidly and substantially adsorb at the interface, thereby reducing the surface/interfacial tensions. Hence, it is claimed that the known emulsifying capability of gums is mainly attributed to the presence of proteinaceous moiety, being this the driving force for the adsorption.

It has been indicated by Garti and Lesser (2001) that the protein fraction is not an obligatory requirement, for the active adsorption of gum. Conversely, recent studies of other gums indicated that the removal of protein in the gum substantially reduced its surface activity (Brummer, et al., 2003; Youssef, et al., 2009) This is why it is worthwhile to investigate the adsorption properties of BSG and evaluate the effect of the protein content, on its surface activity. Therefore, this study aimed at investigating the adsorption properties of BSG, in order to shed more light on the ‘controversial’ driving force of gum adsorption.

5.2 Results and discussion
The adsorption of BSG, at air-water/oil-water interfaces were measured, using the pendant drop shape method, described in Materials and Methods (Chapter 3). Aqueous solutions of crude, purified and protein-free BSG, at varying concentrations (0.1-1% wt/wt), were prepared. The protein composition of each gum preparation is presented in Table 1 (Chapter 4). The surface/interfacial tension measurements were carried out at five minute intervals, for 30 min at 20°C. The results were plotted against time and BSG concentrations.
5.2.1 *Surface tension of BSG*

The effect of crude, purified, and protein-free BSG (as a function of gum concentration), on the surface tension, is shown in Table 2 and Figure 60. When compared with the surface tension at the air/water interface (72.5 mN/m, 20°C), all the gum preparations exhibited the ability to reduce the surface tension of pure water. Increasing the gum concentration substantially reduced the surface tension of the air and water interface to as low as 47.8 mN/m for crude BSG, 51.3 mN/m for purified BSG and 52.5 mN/m for protein-free BSG, at 1% (wt/wt) gum concentration. However, it appears that purification (removal of protein impurities) markedly influenced the adsorption properties of BSG. This observation is in agreement with Youssef *et al.* (2009) and Brummer *et al.* (2003) who both indicated that reducing the level of protein in the fenugreek gum significantly lowered its surface active properties. In contrast, Garti *et al.* (1997) reported that the physical separation of the attached proteins, from crude fenugreek gum, did not affect its adsorption ability. It is, however, noted that their purified gum still contained a considerable amount of protein.

<table>
<thead>
<tr>
<th>BSG (%)</th>
<th>Crude</th>
<th>Purified</th>
<th>Protein-free</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>72.5</td>
<td>72.5</td>
<td>72.5</td>
</tr>
<tr>
<td>0.1</td>
<td>72.3</td>
<td>72.3</td>
<td>72.1</td>
</tr>
<tr>
<td>0.3</td>
<td>68.7</td>
<td>70.7</td>
<td>-</td>
</tr>
<tr>
<td>0.5</td>
<td>58.4</td>
<td>69.1</td>
<td>71.7</td>
</tr>
<tr>
<td>0.7</td>
<td>55.5</td>
<td>65.4</td>
<td>66.9</td>
</tr>
<tr>
<td>1</td>
<td>47.8</td>
<td>51.3</td>
<td>52.5</td>
</tr>
</tbody>
</table>

Moreover, the surface tensions of crude, purified, and protein-free BSG, at 0.5% (wt/wt) gum concentration, as a function of time, are illustrated in Figure 61. BSG appeared to rapidly adsorb and equilibrate the tension at the air/water interface, within 30 mins. It was observed that the surface tension for crude BSG systematically
decreased for a period of time (0-20 min) and equilibrium was attained, after 30 min. On the contrary, the surface tension values of the purified and protein-free BSG slightly increased, over time, until equilibrium was reached. This observation suggests that purification of BSG probably altered its adsorption behaviour by changing its hydrophobic character and thereby reduced its ability to lower the surface tension. Possible desorption occurred, since the surface measurements appeared to be even increased slightly over time.

Figure 60: Changes of the surface tensions of crude, purified, and protein-free BSG as a function of gum concentration at 20°C, 30 min

Figure 61: Changes of the surface tensions of 0.5% (wt/wt) crude, purified, and protein-free BSG as a function of time at 20°C
5.2.2  **Interfacial tension of BSG**

The effect of crude, purified, and protein-free BSG (as a function of gum concentration), on the interfacial tensions of oil and water interface, is shown in Table 3 and Figure 62. When compared to the initial interfacial tension, at the oil/water interface (54 mN/m, 20°C), all the gum preparations significantly lowered the interfacial tension. The degree of tension reduction was dependent on gum concentration. With the increase in gum concentration, the interfacial tension was substantially reduced and values, as low as 12.6 mN/m for crude BSG, 22.3 mN/m for purified BSG and 27.7 mN/m for protein-free BSG, were measured, at 1% (wt/wt) gum concentration.

**Table 3: Interfacial tensions of crude, purified, and protein-free BSG at various concentrations (20°C, 30 min)**

<table>
<thead>
<tr>
<th>BSG (%)</th>
<th>Crude (mN/m)</th>
<th>Purified (mN/m)</th>
<th>Protein-free (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>54</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>0.1</td>
<td>41.1</td>
<td>47</td>
<td>48.9</td>
</tr>
<tr>
<td>0.3</td>
<td>36</td>
<td>46.4</td>
<td>-</td>
</tr>
<tr>
<td>0.5</td>
<td>28.8</td>
<td>40.2</td>
<td>45.5</td>
</tr>
<tr>
<td>0.7</td>
<td>22</td>
<td>32.9</td>
<td>37.5</td>
</tr>
<tr>
<td>1</td>
<td>12.6</td>
<td>22.3</td>
<td>27.7</td>
</tr>
</tbody>
</table>

The behaviour of BSG, at the oil/water interface, was similar to the behaviour observed at the air/water interface. It appears that the ability of the BSG to lower the interfacial tension was reduced, upon the treatment to remove protein content. The time dependence of interfacial tensions, for crude, purified and protein-free BSG at
Figure 63. The interfacial behaviour of BSG, over time, was similar to its observed surface tension behaviour. As expected, the time at which crude BSG adsorbed at the interface and reached the equilibrium interfacial tensions was relatively short. It was also observed that purified BSG progressively lowered the interfacial tension, over time, which was in contrast with the behaviour of protein-free BSG. This observation suggests that the purification of BSG to obtain “protein-free” BSG had probably altered its interfacial properties and some desorption processes may be taking place over time with the resulting increase in interfacial tension.
Figure 62: Changes of the interfacial tensions of crude, purified, and protein-free BSG as a function of gum concentration at 20°C, 30 min

Figure 63: Changes of the interfacial tensions of 0.5% (gum purity) of crude, purified, and protein-free BSG as a function of time at 20°C
Based on the results above it appears that the ability of BSG to lower the interfacial tension was slightly reduced, upon the treatment to remove protein content. This result may suggest two different things:

(i) the proteinaceous moiety in the gum plays a significant role, in decreasing the tension at the oil/water interface. This finding is in accordance with the results obtained by Youssef et al. (2009) and Brummer et al. (2003) and it contradicts the report of Garti et al. (1997); Moreover, in a recent study (Funami, et al., 2007), sugar beet pectin, containing 1.56% protein, was enzymatically modified resulting in polysaccharide with only 0.6% residual protein. It was found that the enzyme modification significantly reduced the interfacial activity of the sugar beet pectin. These findings also suggest that proteinaceous moiety, bound to the carbohydrate moiety of the pectin, plays an important role in its adsorption properties. In the case of BSG, with a minimum residual protein of 0.65% (probably part of the structure of the gum), still surface activity is detected, suggesting that protein may not be the only driving force for adsorption, but also the polysaccharide itself may play a significant role.

(ii) the hydrophobicity and/or conformational structure of the polysaccharide itself have been modified by the purification process, changing the adsorption properties (especially when treated with phenol). Even when both extracts, purified and protein-free, were less surface active than the crude one (at the same polysaccharide concentration), the “purified” (ethanol treated) BSG produced smaller droplet sizes than the “crude” BSG whereas the “protein-free” (phenol + ethanol treated) BSG led to bigger sizes. Therefore, it seems there is no definite relationship between the surface activity values and the droplet sizes obtained.

5.3 Conclusion
The present study confirmed that BSG exhibited adsorption capabilities. All three gum preparations (crude, purified, and protein-free) demonstrated an ability to decrease surface/interfacial tensions. The treatment to remove protein impurities from BSG, however, reduced its ability to lower tensions at the interface. It can therefore be inferred that: (i) protein plays an important role in gum adsorption but it is not the only driving force for adsorption and (ii) polysaccharide itself maybe modified by the purification process. At this juncture, it would be more interesting to compare BSG with other hydrocolloid gums which are known to exhibit emulsification properties.
Chapter 6

Comparison of the emulsifying properties of BSG, Fenugreek gum, gum Arabic, and sugar beet pectin

6.1 Introduction

This chapter reports on the comparison of the emulsifying properties of BSG, with other commercial gums. BSG was shown in the previous chapters to produce a very stable O/W emulsion, when assessed by average droplet size, rheology and storage time procedures. Surface and interfacial tension measurements also confirmed that BSG exhibited gum adsorption capabilities. There is only a limited amount of information available, in relation to novel gums as food emulsifiers and therefore, it is also of interest to evaluate their ability to emulsify and stabilise O/W emulsions. The objective of this study was to compare the emulsifying properties of BSG with some known surface active commercial gums to verify BSG efficiency and confirm where it stands amongst them.

The commercial gums used in this study were: Fenugreek gum (FenuLife®; Frutarom Switzerland Ltd. Reinach, Switzerland), gum Arabic (Sigma-Aldrich, USA.) and sugar beet pectin (GENU® BETA pectin; CP Kelco, Denmark). The crude and protein-free BSG were used as reference gums. BSG exhibited excellent emulsification properties at a concentration of 0.17% wt/wt, based on gum purity (or 0.3% wt/wt BSG extract) and hence this fixed gum concentration was used in all other gums, for comparison. Furthermore, the protein and carbohydrate contents of the various gums were determined, as described in Materials and Methods (see Chapter 3), except for the sugar beet pectin, in which the composition was specified by the manufacturer. The emulsifying and stabilising properties of the various gums were evaluated, in terms of droplet size, rheology and phase separation during storage.
6.2 Results

6.2.1 Protein and carbohydrate composition of various gums

The protein and carbohydrate contents of various gums are presented in Table 4. Sugar beet pectin and fenugreek gum had higher amounts of protein and carbohydrate, compared to gum Arabic and BSG. As expected the purification of crude BSG, into protein-free BSG, substantially increased the carbohydrate content from 58 to 84%.

Table 4: The protein and carbohydrate contents of different powder extracts (% wt/wt)

<table>
<thead>
<tr>
<th>Gum</th>
<th>Protein content (%)</th>
<th>Carbohydrate content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude BSG</td>
<td>1.2</td>
<td>58</td>
</tr>
<tr>
<td>Protein-free BSG</td>
<td>0.6</td>
<td>84</td>
</tr>
<tr>
<td>Fenugreek</td>
<td>3.9</td>
<td>86</td>
</tr>
<tr>
<td>Gum Arabic</td>
<td>2.2</td>
<td>82</td>
</tr>
<tr>
<td>Sugar beet pectin</td>
<td>5.0</td>
<td>85</td>
</tr>
</tbody>
</table>

6.2.2 Particle size distribution

The particle size distribution of the emulsion droplets, for the various gums, is presented in Figure 64. A difference was found in the size distribution pattern of emulsion droplets. The emulsions, which exhibited monomodal distribution with sharp peaks, were found in crude BSG and sugar beet pectin. On the other hand, enlarged size distribution, which shifted to the larger droplet population area, was observed in protein-free BSG, gum Arabic and fenugreek gum.
Table 5: The average particle sizes of 30% (wt/wt) soya oil-in-water emulsions in the presence of various gums at 0.17% wt/wt gum (based on similar carbohydrate content)

<table>
<thead>
<tr>
<th>Gum</th>
<th>Average droplet size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>($d_{32}$)</td>
</tr>
<tr>
<td>Crude BSG</td>
<td>0.99</td>
</tr>
<tr>
<td>Protein-free BSG</td>
<td>1.8</td>
</tr>
<tr>
<td>Fenugreek</td>
<td>4.1</td>
</tr>
<tr>
<td>Gum Arabic</td>
<td>1.7</td>
</tr>
<tr>
<td>Sugar beet pectin</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Table 5 contains the tabulated results for the average droplet size ($d_{32}$ and $d_{43}$), for the emulsions prepared with different gums. Amongst the gums, crude BSG had the smallest $d_{32}$ and $d_{43}$ of 0.99 µm and 1.3 µm, respectively. Protein-free BSG had comparable $d_{32}$, but it had higher $d_{43}$, than the values obtained with gum Arabic and sugar beet pectin. Fenugreek gum produced the largest emulsion droplets, in terms of $d_{32}$ and $d_{43}$.

Figure 64: Comparison of the particle size distributions of the different gum emulsions at 0.17% gum purity
6.2.3 Apparent viscosity

The apparent viscosity of the emulsions prepared with different gums is shown in Figure 65. Crude and protein-free BSG gums had comparable apparent viscosities. Overall, BSG gums exhibited higher apparent viscosity (~170 Pa.s at 0.1 s\(^{-1}\) shear rates) compared to the other gums tested. Gum Arabic, on the other hand, showed the lowest (~0.24 Pa.s at 0.1 s\(^{-1}\) shear rates) apparent viscosity. It was also observed that crude and protein-free BSG demonstrated shear-thinning behaviour at higher shear rates (>10s\(^{-1}\)), whilst the other gums exhibited Newtonian flow behaviour, which reinforced the unique properties of BSG.

![Figure 65: Comparison of the apparent viscosity of the different gum emulsions at 0.17% gum purity](image)

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115
Figure 66: Comparison of the average particle sizes ($d_{43}$) of the different gum emulsions at 0.17% gum purity during 1 month storage at 20°C

6.2.4 Storage stability

The growth in droplet size ($d_{43}$) of the emulsions prepared with different gums (refer to Figure 66) was monitored for a period of one month, at 20°C. Emulsions stabilised with Fenugreek gum appeared to be the least stable, in terms of $d_{43}$, which drastically increased during storage. The $d_{43}$ of protein-free BSG also increased with storage. The smallest $d_{43}$ which remained stable over time was found in emulsions stabilised with crude BSG, followed by sugar beet pectin and gum Arabic. Particle size measured after addition of SDS confirmed that coalescence occurred with Fenugreek and protein-free BSG.

In terms of visual phase separation, the crude BSG emulsions did not show apparent phase separation during storage, by using as low as 0.14 % wt/wt (based on gum purity), which was equivalent to $\approx 0.24 \%$ (wt/wt) gum, (see Figure 31, Chapter 4). In the emulsions stabilised by protein-free BSG, no phase separation was observed at concentrations above 0.15% (based on purity), as shown in Figure 67. Moreover, the emulsions stabilised by fenugreek gum exhibited phase separation, at all gum concentrations (0.07-1%) tested. Gum Arabic emulsions (data not shown) also showed phase separation, by using up to 1.5% gum concentration. Sugar beet pectin was found to stabilise emulsions against serum phase separation, at gum concentrations above
0.20%. Generally, the extent of serum phase separation was dependent on gum concentration; with the increase in gum concentration, the height of the serum layer was reduced.

6.3 Discussion
The emulsifying properties of the different gums, at a fixed concentration of 0.17% (purity), in terms of particle size \(d_{32}\) and \(d_{43}\) and particle size distribution, showed that crude BSG, containing as low as 1.2% protein, produced the smallest emulsion droplets with monomodal distribution. Protein-free BSG, where protein levels were only 0.6% (wt/wt), also formed small emulsion droplets, with \(d_{32}\) values (<2.0 µm) similar to commercial gum Arabic (2.2% protein) and sugar beet pectin (5% protein). It was interesting to note that Fenugreek gum, which contained the second highest protein content (3.9%), produced the largest emulsion droplets amongst the gums tested. These results suggest that the emulsifying properties of these gums could not only be attributed to their protein fractions, but it could also be associated with the structural features of the extracted gum.

Furthermore, the emulsions prepared with various gums, at 0.17% (purity) concentration, had different apparent viscosities. BSG-stabilised emulsions had the highest apparent viscosity, amongst the emulsions tested. Crude and protein-free BSG emulsions had similar apparent viscosity. The emulsions stabilised by gum Arabic, sugar beet pectin and fenugreek gum, exhibited very low apparent viscosities with Newtonian flow behaviour at higher shear rates (>10s\(^{-1}\)). This observation is in agreement with the reported flow behaviour of the gums tested. Gum Arabic has a highly branched compact structure and low molecular weight which account for its high water solubility and low viscosity in comparison to other polysaccharides of the same molecular weight (Sanchez, et al., 2002). The flow behaviour of Gum Arabic is considered to be Newtonian at concentrations as high as 50% (w/v) at shear rates >100 s\(^{-1}\) (Mothe & Rao, 1999). Similar to other random coil polysaccharides, fenugreek gum exhibits shear thinning flow behaviour at high concentrations (>1%), while Newtonian flow behaviour is observed at low concentrations (Brummer, et al., 2003).
Moreover, sugar beet pectin has been found to exhibit low viscosity and Newtonian flow behaviour at 2% concentrations or lower (Arslan & Kar, 1998). Conversely, shear thinning flow behaviour was observed in BSG emulsions, where apparent viscosity decreased with increasing shear rates. This observation suggests that the flow behaviour of the emulsions appeared to be influenced by the rheological behaviours of the gums tested. Additionally, the amount of non-adsorbed...
polysaccharide may be very different amongst the gums, for example in the case of BSG, the non-adsorbed BSG may dominate again the rheological properties, whereas not enough polysaccharide is present in the aqueous phase to show any shear thinning behaviour in the other cases (i.e. fenugreek gum).

In terms of stability during storage, Fenugreek gum emulsions appeared to be the least stable, as confirmed by the apparent phase separation at all gum concentrations (0.07-1% purity) tested and by the dramatic growth in average droplet size ($d_{43}$) during storage showing coalescence. In contrast, a study by Huang et al. (2001) indicated that 0.5% Fenugreek gum produced a very stable emulsion, with less than 1 µm average droplet size ($d_{32}$) when compared to the other 13 gums used in the study. It is worth noting, however, that their Fenugreek gum contained high levels of protein (13.9%), which probably contributed to the formation of a stable emulsion. Also, variations in the methods used in the purification process and in the emulsion preparation and possible modifications of the Fenugreek gum itself used in this study (FenuLife®) could probably explain the difference in the results.

When compared to the other gums used in the study, crude BSG produced the most stable emulsion, in terms of average droplet size ($d_{32}$), rheology and visual phase separation. Also, the protein-free BSG containing only a trace amount of protein was able to form a stable emulsion, comparable to sugar beet pectin. In the emulsion stabilised by 0.17-1.5% (purity) gum Arabic, although the droplet size did not change over time, the emulsions phase separated, probably due to the big sizes (>1 µm) and the low viscosity of the aqueous phase, which was insufficient to prevent creaming. Huang et al. (2001) reported that it required 12.5% gum Arabic, containing 2% protein, to produce stable 40% (wt/wt) O/W emulsions. This suggests that BSG is probably advantageous over gum Arabic and the other gums, in that only a small amount is required to emulsify and stabilise O/W emulsions.

6.4 Conclusion
The present study compared the emulsifying properties of BSG with some food hydrocolloids, by focusing on the particle size and particle size distribution and the stability against phase separation, during storage. By using as low as 0.17% (purity) gum, crude BSG appeared to demonstrate the best emulsifying and stabilising
properties, amongst the gums used in the study. Although protein-free BSG formed larger oil droplets, than crude BSG, it still produced stable emulsions, comparable to other gums, such as sugar beet pectin. Moreover, the emulsifying properties of the different gums seem to be not only associated with the proteinaceous fraction attached to the hydrocolloid molecule, but possibly to the hydrophobic character of the polysaccharide itself.
Chapter 7
Conclusions and Recommendations

This study aimed at investigating the emulsifying properties of Basil Seed Gum (BSG), in terms of droplet size ($d_{43}$ and $d_{32}$), rheological properties (apparent viscosity and viscoelasticity), droplet charge (zeta potential), phase separation, and adsorption properties (surface and interfacial tensions). Soya oil-in-water emulsions (30% wt/wt) were formulated and stabilised by BSG containing < 1.2% (wt/wt) protein and a major glucomannan fraction. Different concentrations (0.03-1.0% wt/wt) of BSG were tested, in addition to the effect of pH (1-12), effect of salt (5-70mM NaCl), effect of heating (80°C, 30 mins) and effect of purification (removal of protein from the gum) on 0.3% (wt/wt) BSG-stabilised soya oil-in-water emulsions.

The particle size, rheological measurements, zeta potential and microstructures of the emulsions were determined in addition to the visual phase separation monitored during storage (at 20°C for 1 month period). The surface/interfacial tensions of different BSG preparations (crude, purified and protein-free, at varying gum concentrations (0.1-1% wt/wt), were also measured at 20°C. In addition, comparisons of the emulsifying properties of BSG with some hydrocolloids (gum Arabic, fenugreek gum and sugar beet pectin), at 0.17% (gum purity) were investigated.

- The results of the study showed that BSG could form small emulsion droplets (<1.0 µm) and stabilise emulsions against phase separation by using as low as 0.24% (wt/wt). Emulsions stabilised with 0.3% (wt/wt) BSG appear to be stable at all pH tested with the potential of forming very small droplet sizes at alkaline conditions (>pH 6). The emulsifying and stabilising properties of BSG especially above its pH 3.0 are probably attributed to electrostatic repulsion and steric mechanisms. The electrostatic stabilisation is manifested by the high zeta potential values. A strong viscoelastic interfacial layer providing steric stability at the interface is highly probable in this type of biopolymer at interfaces. The apparent viscosity and viscoelasticity of BSG emulsions appear to be dependent on concentration but independent on pH, except from extremely low pH (~2.0). Additionally, stability is likely to be improved by the viscoelasticity provided by
the non-adsorbed polysaccharide in the aqueous phase. On the other hand, BSG emulsions at acidic pH, present bigger initial droplet sizes with a remarkable physical stability of one single emulsion phase, even though coalescence was detected at pH 2.0. Growth in droplet size during storage was also apparent, indicating droplet coalescence as confirmed in emulsion microstructures.

✓ The emulsifying and stabilising properties of BSG were sensitive to salt, in terms of particle size. This was indicated by the increase in droplet size and the decrease in zeta potential, with increasing salt concentration. At very low shear rates, the apparent viscosity and viscoelasticity initially dropped with increasing ionic strength; however, these rheological properties progressively increased with a further increase in ionic strength. At higher shear rates, the rheological properties tend to be salt independent. Additionally, growth in droplet size was apparent during storage, thus indicating droplet coalescence, as confirmed in the microstructures of the emulsions. However, the occurrence of phase separation was not observed, suggesting that the emulsions remained stable, irrespective of droplet size. This suggests that BSG could provide physical emulsion stability against salt probably via an increase in viscoelasticity and yield stress provided by the non-adsorbed polysaccharide.

✓ BSG-stabilised emulsions (at extremely alkaline and acidic pH) were affected by heating, in terms of droplet size and droplet size distribution. The flow behaviour of the emulsions was heat–independent, whilst the viscoelasticity was moderately changed after heating. Although, the droplet sizes of the heated emulsions were large and increased upon storage, they remained stable against phase separation. This again indicates that BSG confers emulsion stability under extreme physico-chemical conditions.

✓ Surface and interfacial measurements confirmed the adsorption of BSG at air/water and oil/water interfaces. All three gum preparations (crude, purified and protein-free) exhibited an ability to reduce the surface/interfacial tensions. However, the removal of proteins from the gum reduced its ability to lower the interfacial tension and hence, its emulsifying ability. Although BSG with minimum protein content had reduced emulsifying properties, an emulsion with
monomodal droplet distribution was still formed. It can be inferred, that (i) protein plays a significant role in gum adsorption, but it is not the sole driving force for adsorption and (ii) functionality of the polysaccharide itself maybe altered by the purification process.

BSG appeared to exhibit a strong hydrophobic character as demonstrated by the fluorescing Fast Green dye (normally used for proteins) detected at the interface. At low pH emulsions (~2.0), some inclusions were trapped within coalesced droplets during homogenisation process. The presence of these fluorescent polysaccharide inclusions in the coalesced droplets again suggests that BSG is strongly hydrophobic.

When comparing BSG with some hydrocolloids, crude BSG appeared to demonstrate the best emulsifying and stabilising properties by using as low as 0.17% (purity) gum. Although protein-free BSG formed larger emulsion droplets than crude BSG, it still produced stable emulsions comparable to other gums, such as sugar beet pectin. Over-all, BSG represents a very promising gum, which can be categorised as a novel hydrocolloid emulsifier.

Further work is recommended to be done on the characterisation of the molecular structure of BSG in order to confirm the origin of its emulsifying properties. Surface conformation including adsorbed layer density and thickness could be studied by using neutron and/or x-ray reflectivity at the oil-water interfaces. Given the highly viscoelastic properties of BSG, additional experimental work in low fat products, such as salad dressings could be of interest, as well as the incorporation of BSG on many other food systems, where emulsification is part of the process.
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