Characterization of Basil (*Ocimum basilicum* L.) seed flour and its functionality in ice cream

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Abstract

This study explored the functional properties of basil seed flour (BSF) by determining the effect of concentration (0.1-1.5% wt/wt), pH (3-9) and ionic strength (0-1M) on the solution and emulsifying properties of BSF solutions and oil-in-water emulsions. The emulsifying properties of BSF were evaluated in terms of particle size and its distribution (d_{32} and d_{43}), the rheological properties and the droplet charge. The visual phase separation of these emulsions after storage for one month at 20 °C and thermostability studies both at lower pasteurisation temperature (80 °C for 30 minutes) and higher retorting temperatures (121.1 °C for 15 minutes) were carried out. Finally, application trials in ice cream were done at two different test concentrations of 0.6% and 2.3% wt/wt.

The use of flour resulted in higher particle size data which was due to the lesser surface-active material present in the flour components. However, at 1.5% BSF concentration, there was no visual phase separation after one month's storage period due to the plethora of components in the milled fractions of BSF, especially the lignin component acting as a Pickering emulsifier at these elevated levels of BSF. There was increase in the particle size when the pH was reduced due to the aggregation of the polysaccharide fractions. This was reversed as the alkalinity in the system increased showing smaller particle sizes. The protein component contributed to the electrostatic stabilisation of the droplets above the isoelectric point and the polysaccharide fractions contributed to the steric stabilisation, thus resulting in stable emulsions. The salt addition led to instabilities in the systems. The viscoelasticity and yield stress combinedly provided by other components in the BSF were lower than basil seed gum (BSG) and led to phase separation with salt addition especially at 1M concentration.

The pasteurisation of the emulsions resulted in flocculation of the emulsions whereas retorting led to thermal hydrolysis of the proteins at elevated temperatures. However, at 1.5% BSF concentration there was possible network formation and thus showing improved stability for the stored emulsions. Finally, in the ice cream application, higher aeration and smoothness were seen with 2.3% BSF concentration than at 0.6% BSF concentration. There was 27% reduction in the meltdown rate for the highest test sample ice cream compared to the lowest test sample. Overall, BSF showed promising characteristics of a natural emulsifier.

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Table of Contents

List of Figures

List of Tables

Chapter 1 General Introduction

In food processing, hydrocolloids can play an important role in improving the textural and sensory qualities of food products. Plant-based polysaccharides make up most of the gum and stabiliser type additives, and they include seed polysaccharides such as locust bean, guar and fenugreek gums. The functional properties of these polysaccharides include gelling, binding, thickening and emulsifying properties. In the growing trend of clean label products in the markets, consumers are increasingly able to assess terms like 'free from' or 'natural' in different food products (Asioli et al., 2017). These surmounting pressures have driven food industries and researchers alike to find natural ingredients that can be added to food systems and thus creating a clean impact. A strategy is to avoid or minimise the inclusion of additives on ingredient lists numbers. There is therefore a high demand currently to use such food ingredients from natural sources that give the same functionality as additives.

Many of food products are colloidal in nature, and emulsions are a particularly important and widely consumed subset of good colloids. Food emulsions are typically either oil-in-water (O/W) (such as milk and mayonnaise) or water-in-oil (W/O) (such as butter). The food emulsion formation is governed by several factors like raw material composition itself (water, oil, stabilisers and emulsifiers) and the processing conditions (homogenisation, mixing and heating). Since emulsions are thermodynamically unstable, they tend to separate into distinct phases over time, unless kinetically stabilised. Emulsifiers are surface-active materials that protects the droplets created during homogenisation against coalescence, imparting stability by forming an interfacial barrier that serves to repel droplets, and with sufficient mechanical strength to resist coalescence on occasions where droplet contact does occur (Dickinson, 1992). Certain surface-active polysaccharide, such as propylene glycol alginate and hydroxypropyl cellulose can provide emulsion stability through interfacial adsorption and by increasing the viscosity of the continuous phase (as non-adsorbed polymer). This can impart long-term stability for the emulsions against both creaming, coalescence and phase separation. (Dickinson, 2003; Huang, Kakuda, & Cui, 2001). Thus, these surface-active polysaccharides can act as a stabiliser and/or as emulsifier. In addition to biopolymeric emulsifiers and small molecule surfactants, there is a third class of emulsifiers, namely Pickering particles, which resist coalescence and impart high stability by providing highly robust interfaces on adsorption. Naturally occurring Pickering emulsifiers include lignin, chitosan, chitin (Albert et al., 2019).

The use of these mentioned additives in food formulation requires their declaration on food labels, either as an additive number (i.e. INS listing or E-number), or by fully listing the additive. However, the raw materials that are the source of these additives are classed as ingredients, and does not require such labelling as additives, therefore the use of these may allow manufacturers to produce clean label foods without listing the additive component. This builds trust in the consumers especially, when the ingredient labels are shorter and thus perceived as cleaner products (Gelski, 2016).

An example of this is basil seed. Basil is used in culinary applications throughout the world in different food applications, like in sherbet or faloodas as texture modifiers or satiety improvers and as a source of dietary fibre (Mathews, Singhal, & Kulkarni, 1993). It has whole lot of nutritional benefits and thus has been used since ancient times as medicines. Basil seed comprises a polysaccharide fraction which is commonly termed basil seed gum (BSG). This has potential use in food applications as surface active and an emulsifying hydrocolloid (Osano, 2010; Osano, Hosseini-Parvar, Matia-Merino, & Golding, 2014), thickening, foaming (Naji-Tabasi & Razavi, 2016), fat replacing (Javidi, Razavi, Behrouzian, & Alghooneh, 2016; Razavi, Shamsaei, Ataye Salehi, & Emadzadeh, 2012), binding, gelling (Naji-Tabasi & Razavi, 2015) and stabilizing agent (Naji-Tabasi & Razavi, 2017). The purified fractions of BSG must be declared in food packs for it being a food additive. Obtaining clearance from food regulators to use such additives can be a laborious task and currently BSG does not have an additive listing and can therefore not be used in manufactured foods. In contrast, the use of basil seed flour (BSF) in food systems poses no regulatory constraints, as this can be classed as a natural food ingredient. So far, there has not been a study to assess the use of the BSF as a food ingredient in food systems and whether its functionality shows the same benefits as purified basil seed gum. Thus, the purpose of the study was to characterise the functional properties of the BSF by:

1. Determining the effect of concentration, pH and ionic strength on the solution and emulsifying properties of BSF.
2. Determining the thermostability and storage stability of BSF emulsions.
3. Evaluating the functionality of BSF in a model food system, i.e. ice cream.

Chapter 2 Review of Literature

2.1 Clean Label Foods

Healthiness and sustainability are two consumer drivers determining purchasing preference on a daily basis (Euromonitor, 2016). In formulating foods towards “better-for-you” products, manufacturers are left with fewer options that allow their products to be perceived as more natural or less processed. The term “clean label” became familiar in the 1980’s, when consumers started to perceive E-numbers listed on the food labels as having negative health impacts (Joppen, 2006). Besides having several generic descriptions, there is still no clear-cut definition of a ‘clean label’ product (Osborn, 2015). The particular clean label definition by Edwards 2013 indicates list of ingredients should be free from ‘chemicals’ additives and being produced with minimal processing using traditional techniques. A more detailed explanation given by Ingredion (2014) to consumers, suggests products devoid of additives/preservatives or are ‘organic’ and/or ‘natural’ can be accepted as ‘clean label’ products. The manufacturing perspective leans towards ingredients that are normally found in kitchen cupboards, a shorter ingredient list with minimally processed raw materials, and avoidance of additives with chemical sounding names.

In a recent study conducted among 30,000 consumers from more than 30 countries. it was found that consumers considered food products as ‘clean label’ when it displayed ‘free from’ ingredients list, ‘organic’ logos and ‘natural’ labels on the food packets (Gelski, 2016). Apart from other relevant claims like ‘no chemicals’ and ‘minimally processed’, one in ten consumers were willing to pay more than five percent for clean label products (Gelski, 2016). According to Asioli et al. (2017) a concept of clean label in a ‘strict sense’ is the information of ingredients that are found to be clean and is more likely to be central route of processing information (includes back of pack ingredient list, not chemical sounding and kitchen cupboard or familiar ingredients) whereas the concept of clean label in a ‘broad sense’ is expected to be clean and is more likely to be from peripheral route of processing information (includes front of pack claims, ‘free-from’ labels and natural or organic labels).

Food products need to be formulated with ingredients from nature to overcome the challenges of the market. Product developers have a huge task to encounter this keeping in mind the demands of the consumers. The trend to explore natural sources as food ingredients has been taken up by several manufacturers. Some products included the reformulation of Kraft - Mac and Cheese from artificial colours to natural colours sourced from paprika, annatto and turmeric without significant differences (Wollan, 2016). Also, Mars Inc. had decided to phase out the artificial colours voluntarily from their products owing to the demand created by the customers to switch to natural colours (Michail, 2016). Thus, it is imperative to match with the current trends and develop such products to the market.

2.2 Basil and basil seed

Basil is an aromatic herb from the Lamiacea family and from the genus *Ocimum*. Scientifically called as *Ocimum basilicum* L., its seen in various parts of Africa, Asia and South and Central America (Paton, 1999; Simon, 1999) (Figure 1). This pharmaceutical plant is widely used in in culinary applications around the world (Naghibi, 2010). Widely used in seasonings applications, the formats used are either in dried powder, as coarser visual specs, as whole seeds in fruit beverages or as oleoresins plated in salt in a typical seasoning.

Figure 1: A) Basil Plant B) Basil Seed and C) Basil Seed with mucilage

2.2.1 Nutritional Aspect of Basil

It is known that consuming certain plant materials like fruits, seeds, leaves and roots have tendency to diminish certain chronic diseases risk particularly due to the presence of ingredients promoting antioxidant activity (Ramarathnam, 1995). These include plant polyphenols, which have preventative benefits against the onset of certain forms of cancer and cardiovascular disease (Gross, 2004; Neuhouser, 2004). Basil leaves and seed essential oil extracts also have antimicrobial and antioxidant properties (Munir, 2017). Basil is traditionally known for aiding digestion (Kwee & Niemeyer, 2011), as well as having anticarcinogenic (Holm, 1999) and anticonvulsant properties (Freire, Marques, & Costa, 2006). The high antioxidant activity of basil is attributed to the presence of phenolic acids in considerable amounts (Javanmardi, 2002; Lee, 2010), with the particular presence of rosmarinic acid contributing to medicinal properties (Petersen & Simmonds, 2003). Apart from this, chicoric acids are found in relatively high concentrations (Lee, 2010). These phenolic acids act against free radicals and protect cells from damage. The radical scavenging activity of swollen basil seeds was found to be at 14.60% (Hajmohammadi, Pirouzifard, Shahedi, & Alizadeh, 2016). Also, notably the ratio of insoluble dietary fibre over soluble dietary fibre is 1.99 (Table 1) promoting satiety increase and aiding in physical health owing to the greater volume and weight fractions of the faecal mass.

Table 1: Basil seeds Nutrition analysis (Hajmohammadi et al., 2016)

DPPH (%)	**Protein (g/100g dry matter)**	**Ash (g/100g dry matter)**	**Total Dietary Fibre (g/100g dry matter)**	**Insoluble Dietary Fibre (g/100g dry matter)**	**Soluble Dietary Fibre (g/100g dry matter)**
14.60	22.50	5.11	62.85	41.86	20.99

The mineral content of the basil seeds was also evaluated by Munir (2017) additionally indicated a high content of magnesium compared to other determined minerals (Table 2). Magnesium is associated with improved physiological functions of heart, brain and skeletal muscles. It is also estimated that 80-90% of dietary magnesium may be lost during food processing (de Baaij, 2015). Hence value addition of foods with basil seed may aid for the lost magnesium levels during processing.

Table 2: Mineral content in basil seed (Munir, 2017)

Iron (ppm)	**Zinc (ppm)**	**Magnesium (ppm)**	**Manganese (ppm)**
22.74±1.01	15.81±0.93	315.53±2.15	10.11±0.87

2.2.2 Basil Seed

The seeds of basil have been used as a traditional medicine in the treatment of dyspepsia, diarrhoea, ulcer and other ailments (Munir, 2017). In several parts of Asia, these seeds are used in certain drinks like faloodas, a cold dessert (Hosseini-Parvar, Matia-Merino, Goh, Razavi, & Mortazavi, 2010). In the streets of Cochin, India they are served as sharbats called in local language as 'Kulukki sharbat', a very popular drink with different extracts of crushed fruits or vegetables. Basil seed extract has also been used in preparing edible films and, because of rich presence of polysaccharides, as emulsifying solutions (Khazaei, 2014). The global share of basil seed in food formulations is predominantly in juice drinks as seen in Figure 2.

Figure 2: Percentage of global share of basil seed in food drinks from 2013-2018 (Teodoro, 2018).

2.2.2.1 Proximate composition of basil seed

The proximate composition of different types of basil seeds from different areas are listed in Table 3. The results show significant differences among the variants from Iran and India especially in carbohydrate, lipid and protein content. The carbohydrate content among the Iranian varieties ranged from 47-50% whereas the Indian varietal showed significantly higher values at 63%. On the other hand, fat content was the highest in the Iranian varieties whereas the lowest seen in the Indian basil seeds. The moisture content was more in Indian compared to the Iran variants being the lowest. These differences could be due to the geographical differences and the climatic factors that operate in those areas.

Table 3: The proximate composition of variety of Basil seeds from Iran and India (Mathews et al., 1993; Razavi et al., 2009)

Composition	Iranian Basil seeds [a,b]						Indian Basil Seed[c]
	Qom	Kerman	Khoram Abad	Yazd	Mashad	Ishfahan	
Moisture (%)	5.07 ± 0.15	5.11 ± 0.16	6.24 ± 0.18	5.02 ± 0.17	5.32 ± 0.16	5.51 ± 0.16	9.63 ± 0.14
Protein (%)	20.16 ± 0.92	19.44 ± 0.67	18.35 ± 0.83	17.94 ± 0.62	19.75 ± 0.76	18.37 ± 0.63	14.76 ± 1.52
Lipid (%)	23.12 ± 0.97	23.70 ± 1.01	22.92 ± 0.78	24.45 ± 0.91	22.54 ± 0.74	21.97 ± 0.71	13.8 ± 0.29
Ash (%)	5.38 ± 0.10	5.54 ± 0.10	4.72 ± 0.12	5.41 ± 0.11	5.34 ± 0.13	5.04 ± 0.11	7.7 ± 0.2
Carbohydrates (%)	47.27 ± 0.64	47.21 ± 0.73	48.97 ± 0.87	48.38 ± 0.81	48.05 ± 0.32	50.11 ± 0.86	63.8 ± 2.01

[a,b] Razavi et al. (2009)

[c] Mathews et al. (1993)

2.3 Grinding of basil seed

2.3.1 Milling

Milling is a broad terminology applied across multiple industrial applications for comminution of materials not only in food/cereal processing but including other materials like cloth or metals etc. Additional terminology typically includes grinding which is more specifically concerned with the particle size reduction (Meuser, 2003). Grinding operations involve lot of energy dissipation as heat: with 99% is lost as heat relative to only 1% used for actual grinding process (Meghwal & Goswami, 2014). The use of high energy grinding applications and the heat generated can damage the quality and quantity of labile or volatile materials such as spices (Andres, 1976). Thermal effects from grinding can also influence the content of moisture and oil content in food materials (Singh & Goswami, 1999). The main rationale for milling or grinding is to obtain finer material properties and increased surface area. Food materials, including seeds, that contain fat can be problematic in grinding the seeds as it blocks the pores of the filters. The following sections describe the main commercial mills used in food manufacturing.

2.3.1.1 Roller Milling

These mills come under the family of pressure mills (Berk, 2009). Roller milling is an age old technique which was used for wheat fractionation and flour production (Sakhare, Inamdar, Preetham Kumar, & Dharmaraj, 2017). The process involves breaking and gradual reduction of the material through the reduction passages (Sakhare et al., 2017). The materials are also broken down as an action of compressing to the point of fracture between a heavy pair of rollers (Gutsche & Fuerstenau, 2004). The highest particle size that can pass between the rolls can be controlled based on the geometry of the mills (Earle, 1983). Also, if the friction coefficient between the particles and the roll is known, the maximum particle size that would be nipped can be calculated (Earle, 1983). Accordingly, wheat can be fractionated into germ, bran and various other flour streams (Sakhare, Inamdar, & Prabhasankar, 2015). Modern mills (Figure 3) are fitted with corrugated rollers, called ‘breaks’ as they aid in breaking the seeds (Berk, 2009). Some units are attached with smooth rollers, as they function to further refine the wheat fractions. Apart from use in the wheat industry, these mills are also used for the corn dry milling and cracking of soybeans prior to flaking and producing oil (Berk, 2009). Furthermore, mills with smooth rollers are commonly used to refine the chocolate mass (Berk, 2009) and production of the breakfast meals like cornflakes.

Figure 3: Roller mill and pair of rolls (Berk, 2009)

2.3.1.2 Impact Mill

A commonly used example of these type of mills is the hammer mill (Figure 4). The crushing elements are the hammers fitted onto the rotors that revolves at very high speeds and smash the particles inside a cylindrical chamber (Berk, 2009). These chambers can be either smooth or fitted with corrugated break plates. Hammers can be stationary or used in swinging mode. The latter is more effective in cases of hard chunks, as the damage is reduced. For particle rich in fibres, knife shaped hammers are effective in cutting and grinding due to impact in the chambers. The larger particles are crushed to a smaller degree in these types of mills. The exit of these chamber is fitted with screens to allow the passing of the smaller particles that can be collected and removed continuously. Extremely smaller particles may come with the air flow through the dust collector unit.

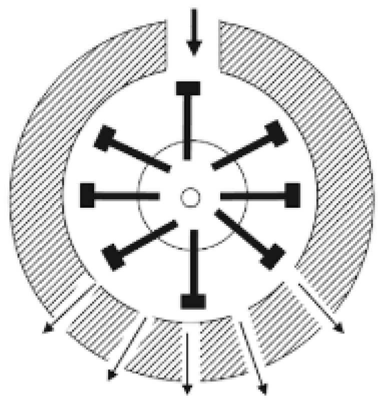


Figure 4: Impact Mill – Basic Structure

2.3.1.3 Ultra-centrifugal Rotor Mill

This high-speed rotor mill (Figure 5) is most commonly used for the size reduction of fibrous, medium-hard or soft materials. These types of mills operate on the principles of impact and shearing in between the rotor and fixed ring sieve. The feed material is passed onto the rotor where the centrifugal action throws the particles with great energy and is pre-crushed on impacting the segmented rotor teeth revolving at great speed (Retsch, 2015). Such compact grinders, with metering mechanisms regulating the feed rate can be used for grinding of spices like fenugreek and black pepper (Meghwal & Goswami, 2014).

Figure 5: Ultracentrifugal Rotor Mill (Retsch, 2015)

2.3.2 Limitations of Conventional Grinding Technologies

The disadvantage of conventional grinding technologies are as follows: -

1. Increased amount of heat generation lead to qualitative and quantitative losses like volatile oil content reduction up to 30-40%, aroma loss, and colour differences (Saxena et al., 2018). Volatile oil content reduction in various spice like caraway, cinnamon, oregano, mace and nutmeg are 32%, 17%, 17%, 37% and 14% respectively (Andres, 1976; Pesek & Wilson, 1986; Pruthi & Misra, 1963; Wolf & Pahl, 1990).
2. The liberation of free oil increased heat and mechanical treatment can hamper continuous working of the grinders as they block the pores and stick to the surface (Saxena et al., 2018).
3. Heat labile materials are not suitable for grinding in these machines.

2.3.3 Cryogenic Grinding

Cryogenics, according to the International Institute of Refrigeration, deals with technological and scientific disciplines involving cryogenic temperatures below -153 °C (Saxena et al., 2018). Cryogenic grinding reduces the temperature of the particles to -195.6 °C (Balasubramanian, Gupta, & Singh, 2012). The particles to be grinded are immersed in a cryogen and as a result the temperature of the product falls below the glass transition temperature of the material and thus particle sizes of 100-200µm or even less is also achieved in further grinding (Retsch, 2015). The application has particular benefits for the processing of high value, sensitive materials such as spices, and is considered advantageous when compared to other grinding techniques, e.g. the original colour of the spices is better retained when a cryogen is used (Pesek & Wilson, 1986). According to Pruthi (1980) additional advantages of cryogenic grinding are as follows :-

1. Reduced oxidation of spice oils and increased stability of the spices.
2. Finer grind because at low temperatures, spices become extremely brittle.
3. Increased uniformity of flavour dispersal throughout the product.
4. Reduced loss of volatiles and increased flavouring power.
5. Lower microbial loads due to sub-zero temperatures.
6. Increased grinding rates as the product does not stick to the surfaces of the grinding equipment's.
7. Lowered costs considering the increased flavour strength in comparison to conventional ground techniques.

2.4 Homogenisation Devices

There are different types of homogenisers used in the food industries for producing food emulsions. When emulsions comprise higher particle size components like the seed flours, care must be taken to avoid wastage of functional ingredients in different components of flour. Some of the homogenisation devices are mentioned below.

2.4.1 High Shear Mixers

These are basic devices (Figure 6) most commonly used in the food industries to homogenize oil and aqueous phases directly (Urban, 2006). This batch process involves oil, water and other ingredients to be homogenized placed in a vessel and agitated by a mixing head with speed up to 3600 rev per minute (Mc Clements, 2016). This rapid mixing generates a combination of rotational, longitudinal and radial velocity gradients which causes the interfacial layer between water and oil phases to be disrupted, and thus making larger droplets into smaller ones. These mixers are suitable for preparing emulsions with viscosities of lower or intermediate ranges. The droplet ranges produced by this type of mixer are in between 1 and 10µm in diameter. These mixers not only produce coarse emulsions but also aids in proper dispersions and ingredient dissolution. Care must be taken to avoid air bubbles incorporation as they may affect adversely on further processing.

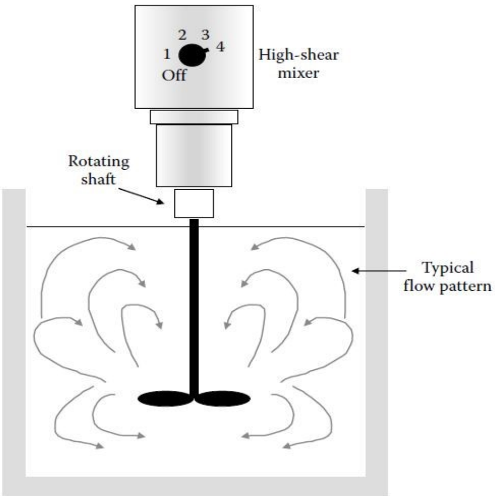


Figure 6: Typical High Speed Shear Device (Mc Clements, 2016)

2.4.2 Colloid Mill

These types of mills (Figure 7) are used for homogenising medium to high viscosity liquids (Walstra, 1983). The device consists of a rotor and a stator. The liquid is fed in the form of coarse emulsions which is homogenised primarily with a high-speed shear mixer. As the emulsion passes through the gap in between the stator and the rotor, a shear stress is generated which breaks the bigger droplets to smaller droplets and through the action of centrifugal force the fluid moves from the centre to the periphery of the disks where it is collected (Mc Clements, 2016). The amount of shear stress to be applied in the liquids can be controlled by varying the gap between the rotor and the stator which ranges from 50 to 1000µm, altering the rotation speed from about 1000 to 2000 revolutions per minute or by using roughened disk surfaces (Mc Clements, 2016). The droplet diameters from these types of homogenisers range from 1 to 5µm in diameter.

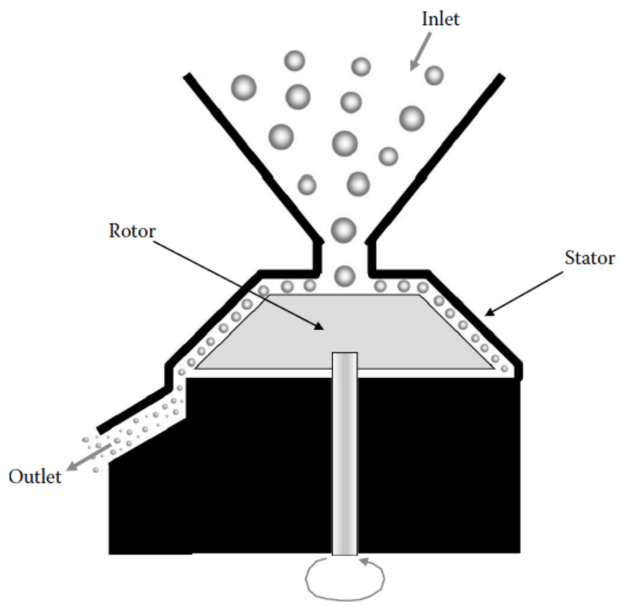


Figure 7: A typical Colloid Mill (Mc Clements, 2016)

2.4.3 Ultrasound assisted emulsification

The concept of ultrasound is defined as the application of sound waves with frequencies beyond the human hearing ability i.e 20 kHz. These waves interact with substances and produce changes in physical and chemical properties. Ultrasound is deemed to be rapid, improved and consistent technique compared to conventional techniques (Silva, Rosa, & Meireles, 2015). Sound waves are considered as non-toxic, safe and environment friendly (Kentish & Ashokkumar, 2011). Most of the earlier studies have shown that it is efficient to use conventional rotor stator devices to produce coarse emulsions and then use ultrasound technology to reduce droplet sizes (Silva et al., 2015). However, high intensity ultrasounds have the capability to produce emulsions with smaller droplet sizes directly with the separate oil and water phases without undergoing a primary homogenisation (Mc Clements, 2016). The drawbacks of generating considerable amounts of heat due to continuous exposure of these sound waves can be eliminated by the application of the ultrasound in short bursts of few seconds. Also, during the application of sound waves it is desirable to have a good agitation as the energy is focussed on a small volume in proximity to the transducer's tip. In smaller vessels containing sample, the need is avoided due to induced fluid flow by the ultrasonic field. However, in the case of larger vessels it is advisable to use an agitator for effective mixing and homogenisation. The basic components of an ultrasonic equipment consist of generator, transducer, amplifier and probe (Figure 8). The generator is responsible for producing high frequency electrical energy above 20kHz ranges. The transducer is responsible for conversion of these into similar frequency based mechanical vibrations. The amplifier multiplies these vibrations and allow it to dissipate through a probe in the form of sound waves (Abbas, Hayat, Karangwa, Bashari, & Zhang, 2013). There are two mechanisms seen in the ultrasonic based emulsification. Initially, the interfacial waves produced due to the applied acoustic field becomes unstable and disrupts the oil phase to its constituents' droplets of the order of 70µm in the water medium (Li & Fogler, 1978a). Next, stages involve the phenomenon of cavitation where the large droplets are broken into sub-micron droplets due to the presence of the impacting shock waves. These shock waves are generated due to cavity collapse by ultrasonic field (Li & Fogler, 1978b). In comparison to conventional rotor stator based dispersions, the ultrasonic based emulsification are found to be more competitive or better in terms of droplet sizes and energy operations (Maa & Hsu, 1999). In comparison to other techniques such as microfluidization, ultrasound is advantageous owing to the drawbacks like higher production costs, equipment contamination and lack of aseptically processing operations in the former one (Abismaïl, 1999). And when compared with mechanical agitation Tadros (2004) found that heat losses were reduced, surfactant amount also was lower and the emulsions were more stable through ultrasound technology.

Figure 8: Batch type Ultra-sonicator showing various components (Silva et al., 2015)

2.5 Macromolecular Emulsifiers

2.5.1 Protein

Being amphiphilic in nature, many proteins readily reduce the energy of liquid-liquid and air-liquid interface and thus have been known as emulsion and foam stabilisers (Damodaran, 2005). Proteins are known to form thick steric barriers to prevent coalescence in emulsions and foams (Dickinson, 2009). Most of the protein emulsifiers used in food systems are obtained from milk and eggs. Due to a combination of high functionality and natural perception, proteins are preferred in food industries as emulsifying agents. Milk proteins are typically low in molecular weights and possess either random coil (casein fractions) or globular structures (whey fractions). The interfacial membranes formed by these proteins is negatively charged at neutral pH, thus aiding in electrostatic repulsion between the droplets (Claesson, 2004). Thus, protein stabilised emulsions are very sensitive to pH and ionic strength changes. At pH values close to the isoelectric point of the adsorbed proteins they tend to flocculate, as well as when ionic strength exceeds above particular values. These charge screening effects allow other dominant forces like hydrophobic, Van der Waals or depletion attractive forces overcome the electrostatic repulsion (Mc Clements, 2004). When comparing the emulsifying abilities of proteins and polysaccharides it can be seen that proteins have stronger binding affinity and lower surface load than the generally more hydrophilic polysaccharides, resulting in lower amounts of emulsifier to be used to produce emulsions with sub-micron droplet sizes (Mc Clements, 2005).

2.5.2 Polysaccharide

Seed (mucilage) polysaccharides are very unique category of plant originated materials used in many food processing operations, as they improve the mouthfeel and texture of food products (Cui, 2007). The surface activity of certain hydrocolloids is associated with hydrophobization of the molecular, such as the presence of certain side-groups having non-polar characteristics being chemically attached to the polysaccharide backbone, e.g. propylene glycol alginate or hydroxypropyl cellulose (Dickinson, 2009). These polysaccharides or hydrocolloids can slow down creaming by both assisting in the interfacial stabilisation of the emulsion and by adjusting the rheology of the continuous phase.

The hydrophilic nature of most non-chemically modified polysaccharides renders them relatively non surface active (Mc Clements, 2007). However, the presence of proteinaceous components in certain gums, either directly attached to the polysaccharide, as in the case of gum Arabic, or present as unbound residual fractions during processing, such as in fenugreek, can adsorb to the interface (Akhtar, 2002; Huang et al., 2001; Mc Clements, 2007). These gums have been proven to be effective at sterically stabilising emulsions against flocculation and coalescence, often over a broad range of pH and ionic conditions. It should be noted that there can be significant variations in the viscosifying abilities of surface-active polysaccharide. While gums such as sugar beet pectin and fenugreek can stabilise emulsion as well as acting as viscosifiers, gum arabic has poor viscosification and (in the absence of synergistic interactions with other polysaccharides) is used purely for emulsification (Garti, 1999; Huang et al., 2001; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003). The following sections outline the properties of some commonly and not-so commonly used seed gums and their applications in food products.

2.5.2.1 Fenugreek Gum

Fenugreek (*Trigonella foenum-graecum*) is grown in Mediterranean regions and parts of India and China. Rich in protein and high in insulin stimulating activity, the chemical composition includes fatty acids ranging from 5% to 10% predominantly with linoleic, oleic and palmitic acids. Total carbohydrate content is 45% - 65%, with soluble fibre content of 15% galactomannans (Schryver, 2002). Ground fenugreek has been used in various culinary applications like in spiced curries, and is found to be anti-diabetic, exhibiting hypercholesterolemic properties in humans (Al-Habori, 1998) and showing inhibitory actions on colon carcinogenesis by suppressing the enzymes that affects the protective mucin layer and exposes to carcinogenic toxins (Devasena, 2003). With 1.4 million molecular weight, this gum is extracted from the endosperm or ground seed flour with dilute alkali or water, with yields ranging from 13.6 to 38%. The ratio of galactose to mannose in this gum is approximately 1:1 (Brummer, Cui, & Wang, 2003). Being a random coil polysaccharide, these gums have shown similar rheological profiles to other galactomannan polysaccharides such as guar and locust bean gum, with highly pseudoplastic characteristics (Brummer et al., 2003). The emulsification properties of this are quite different to those of most galactomannans, as indicated by Huang et al. (2001). Fenugreek was shown to possess the greatest emulsification capacity compared to fourteen other hydrocolloid gums. Droplet sizes of 2 -3 µm were produced using this gum even after the removal of protein components, as shown by Garti, Madar, Aserin, and Sternheim (1997). However, separate studies done by Brummer et al. (2003) found that removal of the residual protein content of 0.57% resulted in less surface activity. These findings were found evident in another study done by Youssef, Wang, Cui, and Barbut (2009), where a phenolic treatment of the gums resulting in a protein free fenugreek gum showed significant reduction in surface activity.

2.5.2.2 Psyllium Gum

Psyllium, a common name from plant genus *Plantago*, seed contains high levels of mucilage. Several countries like India and other European countries are the commercial hub of producing these gums. The soluble fibre from its mucilage is used in certain foods and is approved by the Food and Drug Administration (FDA) (Cui, 2007). The mucilage produces a clear and colourless gel and can be extracted by mechanical grinding, yielding about 25% of the seed weight. With a heteroxylan mucilage, it consists of 1,3 and 1,4 mixed linked β-D-xylanopyranosyl backbone having side chains attached to second and third positions of 1,4-linked β-D-xylopyranosyl residues. These side chains are α-L-arabinofuranosyl and β-D-xylopyranosyl residues with additional pectic fractions (Samuelsen, Hanne Cohen, Smestad Paulsen, Brüll, & Thomas-Oates, 1999). This gum does not completely dissolve in water, however, swells up when in contact with water. Freshly prepared dispersions of psyllium gum of 1% show Newtonian behaviour at lower shear rates. Syneresis is evident in solutions kept over time, with gel formation and gel contractions (to about 30%) observed over 3 months storage (this effect can be further accelerated with thawing and freezing cycles). However, this gum is highly stable in enriched salt solutions up to 2.5M NaCl (Cui, 2007). Its use in food systems is as a thickener and stabiliser in ice cream and desserts. According to Haque, Morris, and Richardson (1994), in combination with other polysaccharides such as hydroxypropylmethyl cellulose (HPMC) the loaf volumes of non- gluten wheat breads can be increased to values similar to those for hard wheat control. This network stabilising property based on a synergistic action of psyllium and HPMC assisted in stabilising the foam structures while proofing and avoiding their breakdown during the stages of baking. In United States, convenient breakfast products also have used these gums owing to the cholesterol lowering effect (Childs, 1999).

2.5.2.3 Flaxseed Gum

Flaxseed obtained from the seeds of flax plant is a member of *Linaceae* family. The seeds are oval and flat shaped, with seed coat containing a thick mucilage or epidermis layer (Freeman, 1995). This polysaccharide is soluble in cold water, making it easily extractable compared to other gums, with optimum conditions of pH between 6.5 and 7, 85 to 90°C temperature, extraction time of 2.5 to 3 hours and water:seed ratio of 13:1. The chemical composition of this gum consists of 51 to 75% carbohydrates, 5 to 19% proteins and 4 to 8% ash depending upon extraction conditions, solvent and pH, etc. (Fedeniuk & Biliaderis, 1994; Mazza & Biliaderis, 1989). The main two carbohydrate fractions include a neutral polysaccharide unit consisting of galactose, xylose and arabinose and an acidic polysaccharide unit consisting of D-galactose, D-galacturonic acid and L-rhamnose. The neutral polysaccharide has a (1→4)-β-D-xylosyl backbone to which arabinose and galactose side chains are attached at second and third positions. A backbone of (1→2)-linked α-L-rhamnopyranosyl and (1→4)-linked D-galactopyranosyluronic acid residues with side chains of fucose and galactose are present in the acid polysaccharide. The breakdown of L-fucose, L-galactose, D-galacturonic acid and L-rhamnose is about 1:1.4:1.7:2.6 (Erskine & Jones, 1957; Muralikrishna, Salimath, & Tharanathan, 1987). Flaxseed gum shows Newtonian flow behaviour at lower concentrations and at higher concentrations shear thinning behaviour. Due to broad differences in chemical composition, this gum behaves like gel or as visco-elastic fluid (Cui, 2002). An apparent correlation between rheological properties and structural features is evident when gums containing high levels of arabinoxylan unit were shown to be more viscous due to higher molecular weight with shear thinning flow behaviour. In comparison, gums containing higher levels of acidic polysaccharide showed lower viscosity due to lower molecular weight and exhibited Newtonian flow behaviour (Cui, Kenaschuk, & Mazza, 1996). The pH changes in gum solutions resulted in significant viscosity changes, with higher viscosities at neutral pH and lower viscosities in both lower and higher pH regions (Mazza & Biliaderis, 1989). Like other gums, flaxseed gums have been used as stabilisers and thickeners in ice cream. Ice cream made with 0.25% flaxseed gum, 0.1% carboxymethyl cellulose, 0.1%, alginate and 0.05% glycerine monostearate had good thaw resistance and high overrun (Wang Da-wei, 2003). In bakery goods like breads, these gums had a mellowing effect on gluten allowing greater expansion during baking and fermentation stages, improving the grain texture of the loaf (Garden-Robinson, 1994).

2.5.2.4 Basil Seed Gum (BSG)

This polysaccharide extracted from the seeds of *Ocimum basilicum* L. contains two major fractions of (i) an acid-stable glucomannan (~43%), with mannose to glucose ratio of 2:10; and (ii) a (1→4) linked xylan (~24.3%), with acid side chains at second and third carbon position, and a minor fraction of glucan (~2.31%) (Anjaneyalu & Gowda, 1979; Tharanathan & Anjaneyalu, 1975). A typical glucomannan structure is shown in Figure 9. The presence of acetyl groups in certain glucomannans like Konjac mannan (Takigami, 2000) may also show that the hydrophobic nature of the BSG could be due to these acetyl groups. The optimal extraction conditions of the gum from the seeds was studied by Razavi et al. (2009). The study reported temperatures of 68.1°C and pH 8.09 as significant parameters affecting both the quality and the quantity of the gum and water/ seed ratio 65.98:1. Moreover, the purified gum extract after alcohol precipitation showed a residual protein content (~1.5%), fat (~9.7%), (~3.3%) and carbohydrate (~80%).

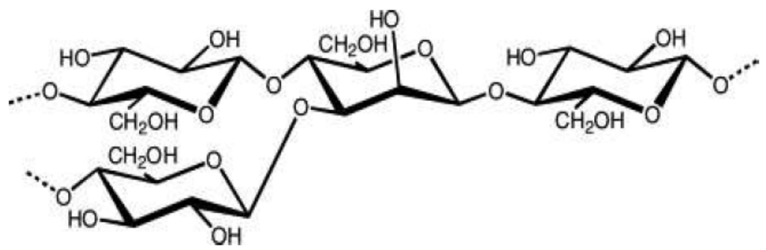


Figure 9: Typical Glucomannan structure (Tharanathan & Anjaneyalu, 1975)

Hosseini-Parvar et al. (2010) have investigated the viscoelastic properties and steady shear flow behaviour of BSG, at 0.1-2% (wt/wt) and at temperatures of 5-85°C showing a higher zero shear viscosity, non-Newtonian pseudoplastic behaviour and presence of yield stress. The apparent viscosity decreased with increased shear rate at all concentrations starting from 0.1 to 2%. The flow behaviour at 1% concentration indicated a higher viscosity at lower shear rates when compared with xanthan, konjac and guar gum indicating a good stabilising action in food formulations like salad dressings and mayonnaise (Hosseini-Parvar, Osano, & Matia-Merino, 2016). A 0.3% concentration of BSG which could form smaller emulsion droplets size less than 1.0μm was shown as the optimal concentration for emulsion stability for one month against flocculation and creaming (Osano et al., 2014). In terms of pH and ionic strength changes, the BSG stabilised emulsions were more sensitive to low pH's (≤6.0), than higher pH's (>7) showing the effects of NaOH during the emulsification process releasing fatty acids and resulting in smaller droplets size at extreme pH like 12. Also, the addition of salts resulted in higher droplet sizes with decreased zeta potential values as the salt concentration progressed (Hosseini-Parvar et al., 2016).

The stability of emulsions for one-month storage showed that native pH remained stable due to maximum adsorption of the gum at the interface, providing long term steric stabilisation. At pH increasingly beyond neutral conditions, the size of droplets increased steadily with time, particularly at lower pH conditions (Hosseini-Parvar et al., 2016). In various food applications BSG has been used in emulsifying, stabilising, thickening, foaming, gelling and fat replacing capacities (Naji-Tabasi & Razavi, 2017). As food stabilisers in mayonnaise, BSG sample showed good spreadability and highest taste score in combination with guar gum at 0.3% concentration and 50:50 proportion (Niknia, 2011). This compares to a study by Razavi, Shamsaei, Ataye, and Emadzadeh (2012), indicating that 0.45% BSG along with 0.6% Xanthan Gum concentration as having good spreadability in low fat mayonnaise. In low fat ice creams, Javidi et al. (2016) found that BSG could function as fat replacer in combination with guar gum in 50:50 ratio, giving a creamy perception when compared against full fat sample. In addition, the combination reduced the coarseness and coldness perception of ice cream with reduction in meltdown rates. BahramParvar, Razavi, and Mazaheri Tehrani (2012) optimised the ice cream formulation using BSG as a stabiliser for improved quality. And in comparison with other commercial ice cream samples containing carboxymethyl cellulose and guar gum combination, BSG with 0.2% concentration reduced ice crystal growth by 30-40% (BahramParvar & Goff, 2013). In low calorie pistachio butter, Emadzadeh, Razavi, Hashemi, Mahallati, and Farhoosh (2011) found that at 0.023% BSG concentration an optimum formulation was achieved as a fat replacer in combination with xanthan gum. In processed cheese, it was also found that cheese with firmer quality, and lower melting profiles with lower costs could be made as BSG could strengthen the protein matrix by making a web network among the casein strands (Hosseini-Parvar, Matia-Merino, & Golding, 2015).

2.5.3 Lignins

Next to cellulose, lignins come second as an abundant biopolymer on the surface of the earth. Among the number of different aromatic molecules, lignin is also the most abundant in nature (Wei, Yang, Yang, & Wang, 2012). The research into the studying of different lignin forms stems from the curiosity of using these renewable molecules as molecular surfactants, emulsifiers and replacement of petroleum-based synthetic polymers (Yang, Wei, Wang, & Tong, 2013). The structure of this aromatic molecule is amorphous and is composed of three monolignols namely coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol. The composition of these monolignols is not the same in any plant sources of lignin and varies accordingly (Rojas, 1994; Sarkanen & Schuerch, 1955).

Lignin macromolecules are known to be surface active as they can adsorb at the liquid-liquid and air-liquid interface to provide kinetic stability of emulsions and foams (Rojas et al., 2007). A recent work which showcases the use of lignin was shown by Wei et al. (2012) where the alkaline lignin extracted from the furfural residues on pH reduction formed particles which could be reversibly used to stabilize pickering emulsions. These particles were used in styrene polymerisation, forming lignin coated polystyrene microparticles. Another study by Sipponen, Smyth, Leskinen, Johansson, and Österberg (2017) have also found that cationic lignins performed better than kraft lignin microparticles to stabilise range of oils in the pH range of 2-6. These cationic lignins were adsorbed on colloidal spherical lignin particles. Apart from the use of lignins as stabilisers, it has also been used as a stabiliser in plastics, in combination with poly lactic acid via pickering emulsions (Li et al., 2019). Lignin forms a significant component in many seed fractions, and its presence may be expected to influence the properties of mucilage seed extracts when present in the final composition. The relative concentration of lignin in these compositions will depend highly on the conditions of extraction, noting that simple creation of seed flours, rather than purified polysaccharide extracts, may contain quite elevated levels of lignin, as well as the presences of other components, such as proteins, phenolics, and other constituent parts of the seed.

2.6 Summary

The purpose of this review is to provide supporting information towards the key research projects of the project, which are to determine the extent by which basil seed gum functionality is retained in the production of basil seed flour, as opposed to a purified basil seed gum. The rationale for this work is based on the fact that the purified gum must be classed as a food additive, and therefore requires clearance for use in food production. In contrast, basil seed flour can be defined as an ingredient, and there are no specific restrictions on the use of this material in food preparation. This consideration extends beyond the particular use of basil seed gum, and thus the comparison of purified gum to milled flour can be made for any potential raw material that can be used as an ingredient but lacks clearance for use as an additive in food. In undertaking this research, the functionality of the polysaccharide in the flour will be determined by consideration such as its relative concentration to the overall composition, and whether it is functionally active in the crude state. The contribution and interactions of other components within the flour on both the polysaccharide, and potentially any other ingredients in a food product also need to be considered in defining the overall properties of the flour and whether it can provide appropriate functionality without requiring excessive concentrations.

Chapter 3 Materials and Methods

3.1 Introduction

This chapter describes the materials and methods used for the study. The method of BSF preparation, emulsion preparation and further analysis are also described. The technique used to characterise the properties of BSF- stabilised emulsions are also described.

3.2 Materials

Basil Seed was obtained from a local market in Iran. Soya Oil was obtained from Goodman Fielder Ltd., New Zealand. Certain analytical grade reagents used for the study were sodium chloride, sodium azide, sodium dihydroxide, hydrochloric acid and sodium dodecyl sulphate. For ice cream preparation, sucrose (Pams, New Zealand), mono and diglycerides (GRINDSTED PS217, Danisco, Australia), guar gum (GRINDSTED GUAR 250), locust bean gum (GRINDSTED LBG 246) and glucose syrup (Avon Glucose Syrup A2151, Penford, Auckland, New Zealand) were obtained. Anhydrous Milk Fat (AMF) and skim milk powder (SMP) were supplied from Fonterra, New Zealand.

3.3 Basil Seed Flour (BSF) preparation

Various size reduction equipment was used to obtain flour size of 600µm. The challenges faced in obtaining the finer flour was the high fat content (table 4) of the basil seed.

3.3.1 Roller Mill

The roller mill was initially used to grind the basil seed into fine powder. Due to increased fat content, much losses were seen, as the grinding would result in paste formation and thus could not be continued further. Furthermore, the obtained powder couldn't pass through the 30 ASTM or 600µm, thus sieve blocking the sieves.

3.3.2 Ultracentrifugal Rotor Mill

This high-speed equipment was used as an alternative to the roller mill. The same issues as seen in the roller mill was experienced. Minimal powder sample was only obtained and much of the material was lost as paste due to increased fat content of basil seeds.

3.3.3 Cryogenic Grinding

Basil seed obtained was ground using liquid nitrogen. Initially, roughly 35-40gms of the whole seed was taken in a mortar. Gently, 50-70ml of liquid nitrogen was poured from the

Dewar flasks to the mortar holding the seeds. Using the pestle, the seeds were initially crushed into coarser grains, followed by transferring the entire contents into a home-based spice grinder. The grinder was operated for 1-2 minutes to obtain finer sizes. These contents were then manually transferred over 30 ASTM sieves to obtain finer flour of 600µm sizes. The overs were collected and kept aside. This process was repeated till a suitable quantity of seed flour was obtained.

3.4 BSF Emulsion Characterisation

Soya oil in water emulsions were prepared by dissolving known amounts of BSF in Milli-Q water using an overhead mixer (RW 20.n, IKA Labortechnik) for 5 minutes. For primary homogenisation, ultrasonication was used to separate the mucilage out of the seeds. Different extracts with 0.5%, 1% and 1.5% BSF in reverse osmosis water was made using an overhead mixer. This was followed by ultrasonication for 15 cycles, each with 60 seconds, 100% amplitude and 250-350W power application depending on the concentration of the flour and the depth of sonotrode was halfway inside the aqueous phase. Temperature was monitored and was ensured to retain it below 50°C. This was followed by heating up the oil phase to 50°C in a water bath for making the emulsion. As oil was gently stirred at 300rpm, the aqueous phase containing BSF was poured gradually till the entire contents was transferred. This was then refrigerated at 4°C and cooled.

For secondary homogenisation, this primary mix was ultrasonicated as per the above-mentioned method in section 3.4. An ultrasonic processor (UIP1000hd, Hielscher Electronics GmbH, Teltow, Germany) was used to prepare the emulsions. During the application of ultrasonic waves, it was ensured that samples were well stirred with a magnetic stirrer at 500rpm for the entire operation. This was for uniform waves application throughout the system. Temperature was monitored for each cycle, as the application of ultrasound waves resulted in 5-10°C increase in temperature for every 60 seconds of operation. The amplitude was set at 100% and the frequency of operation was 20 kHz. Power output was recorded for each cycle of operation. An ice bath was kept aside to reduce the temperature. Sodium azide (0.02% v/v) was added to the emulsions, as an anti-microbial agent.

To test the effect of flour concentration, the emulsions were formulated using different amounts of concentrations of BSF [0.5-1.5%(wt/wt)] as mentioned above. To test the effect of pH, 1% (wt/wt) BSF stabilised emulsions were made and pH 3-9 was adjusted before emulsification. Solution of 0.1-1M HCl (or 0.1-1M NaOH) was used to make changes in the final pH of the emulsions. To test the effect of ionic strength, various concentrations (0-1mM) of NaCl was added, before the emulsification process of 1% (wt/wt) BSF stabilised emulsions.

To check the thermostability the emulsions at native pH and extreme pH values at (3 and 9) were prepared and subjected to heating at 80°C for 30 minutes, before analysing the droplet sizes and the rheological properties.

3.4.1 Droplet Size Measurements

The droplet size distribution of BSF stabilised was done by a laser light scattering technique using Mastersizer (Malvern MS 2000). Droplet sizes of 0.02-2000µm is determined by this laser diffraction method. The Mie Theory is used to accurately predict the light scattering behaviour of the emulsions in this equipment. Sauter diameter, representing surface average diameter is measured as D[3,2] and De Brouckere mean diameter measures the size of the particles constituting the bulk of the sample volume represented as D[4,3]. D[3,2] is sensitive to measurement of fine particles in a size distribution whereas D[4,3] to measurement of large particles. The Equation 1 and Equation 2 representing surface are mean and volume mean are shown as follows:

Equation 1 $$d_{43} = \Sigma \frac{n_i\, d_i^4}{n_i d_i^3}$$

Equation 2 $$d_{32} = \Sigma \frac{n_i\, d_i^3}{n_i d_i^2}$$

where n_l = the droplets no of diameter d_l

The components of the mastersizer is shown in Figure 10. The first component is the optical bench used to calculate the particle size in the sample. One of the main components in the optical bench is the cell, which, serves as an interface between the sample dispersion unit and the optical bench. Here the laser beam analyses the particle when the sample is passed between two windows in the cell. The second component is the dispersion unit that delivers the sample to the optical bench for measurement. These units can handle powder forms of sample or a liquid form. The final component is the computer system that has the Malvern software, analysing the raw data from the optical bench to determine the size of the particles (Mastersizer, 2007). For the measurements, 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 measurement was done in triplicates and the results were reported as averages.

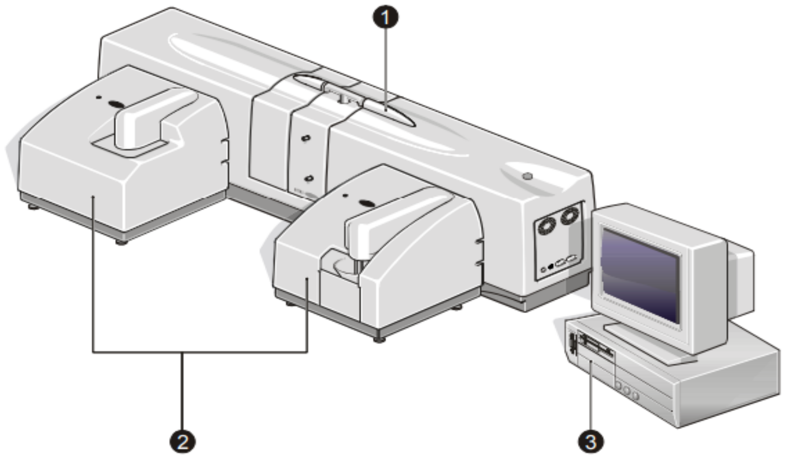


Figure 10: Mastersizer 2000 components

3.4.2 Rheological measurements

The fresh BSF stabilised emulsions were measured in duplicates, within 24hours of preparation in a MCR 301 Rheometer (Paar Physica, Germany) equipped with a vane geometry as shown in the Figure 11. The vane geometry was used for the present study to avoid any wall and slip effects. This also enables a reliable measurement tool for less homogenous samples like our emulsion sample.

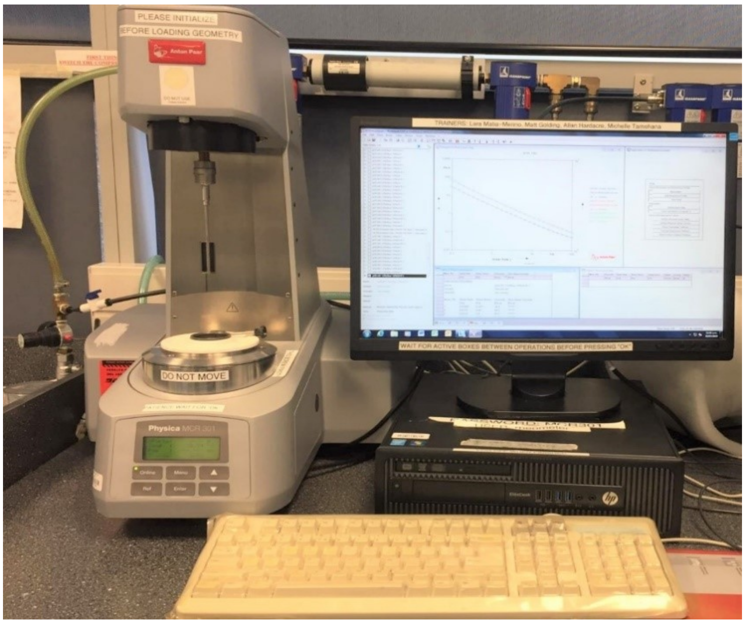


Figure 11: Rheometer MCR301 (Paar Physica, Germany)

The apparent viscosity was determined at shear rates ranging from 0.1 – 100 s^{-1} and viscoelastic properties measuring G' (storage modulus) and G'' (loss modulus) at 20°C. The viscosity of a liquid is its flow resistance calculated as follows (Equation 3)

Equation 3 $$\eta = shear\ stress\ /\ shear\ rate$$

3.4.3 Zeta Potential Measurements

Zetasizer Nano ZS (ZEN 3600) was used to measure the zeta potential (ζ) values of the emulsions. A combination of Laser Doppler Velocimetry (LDV) and phase angle analysis light scattering techniques are used to evaluate it. The method involved preparing the fresh emulsions and then centrifuging the emulsions at 2500 rpm for 10 minutes to obtain the milky emulsion layer. This was then diluted to 1:100 and injected into one of the capillary cells (DTS 1060) and any air bubbles were removed before inserting the stopper. Twice the measurements were taken and then the data were reported as means of duplicates.

3.5 Confocal Laser Scanning Microscopy (CLSM) and Light Microscopy

Confocal Laser Scanning Microscope (Leica SP5 DM6000B, Leica Microsystems, Germany) was used to visualise the microstructure of the emulsions. The oil droplets were stained using Nile red fluorescent dye excited with laser at 488 nm and the Fast Green FCF dye was used to stain the protein fractions in the flour excited with laser at 633 nm. The concentration of the flour ranged from 0.5%, 1% and 1.5% for the emulsions. The milky emulsion layer was used after centrifugation to observe the emulsions under CLSM. For staining, 2.5ml of the emulsion sample was taken and mixed with 45 µl of each of the fluorescent dye. After thorough mixing, a drop was placed in microscopic welled slide. A cover slip was placed on top of the well ensuring no air bubbles were trapped inside. This was followed by examination under the microscopy with different magnifications.

Microstructures were also observed under a compound light microscope (Olympus BX53, Tokyo, Japan) which was equipped with an Olympus XC50 camera. The images were recorded at 10x, 40x or 100x objectives.

3.6 Retorting of emulsions

The emulsions were prepared as mentioned in section 3.4 and then retorted in cans at 121.1 °C for 15min and then immediately cooled with cold water to room temperature in an autoclave. Temperature sensors were mounted in the cans to monitor the temperature changes throughout the operation. 0.5% - 1% (wt/wt) BSF was used at all pH to check the effects of retorting. The emulsions were also made in duplicates.

3.7 Ice Cream Preparation

Four different mixes were prepared from the base formulation consisting of 10 % AMF, 4% glucose syrup, 11% SMP was constant in all the mixes. Three different levels of BSF at 0%, 0.6% and 2.6% were used in the three different mixes with the first mix used as zero control. Total solids were adjusted to 34.6% for these three levels. Actual control sample had other emulsifiers and stabilisers with the absence of BSF. Water was used to mix the final balance of the mixes. The amount of the ingredients used in the formulation of different mixes is shown in Table 4. In the final mix, to incorporate more quantity of BSF, sucrose and SMP levels were adjusted in the formulation, though the entire mixes were maintained at same formulations.

Table 4: Ice Cream Formulation for different mixes

	Mix 1 (%)	**Mix 2 (%)**	**Mix 3 (%)**	**Mix 4 (%)**
	Zero Control	**Control**	**BSF 0.6%**	**BSF 2.6%**
Fat (AMF)	10	10	10	10
Skim Milk Powder (SMP)	11	11	11	10
Sucrose	9	9	9	8
Glucose Syrup	4	4	4	4
Guar Gum	0	0.1	0	0
Locust Bean Gum (LBG)	0	0.2	0	0
Mono-diglycerides	0	0.3	0	0
Basil Seed Flour (BSF)	0	0	0.6	2.6
Water	66	65.4	65.4	65.4
Total Solids	34	34.6	34.6	34.6

For the preparation of the ice cream, all the ingredients were weighed and added in the sequence as shown in Figure 12. A high shear mixer (Silverson L4RT, Waterside, England) was used at 6000rpm for the mixing of the ingredients. SMP was added in the beginning stage, followed by addition of powdered sucrose, glucose syrup and melted fat. When these ingredients were thoroughly mixed the dry ingredients mix consisting of mono-diglycerides, LBG and guar gum or BSF was added and mixed well. This resulting premix was heated above 50°C prior to homogenisation with a two-stage high pressure homogeniser (APV 2000, Rannie/Gaulin, Alberstlund, Denmark) operating at 22MPa (220 bar) and 2 MPa (20 bar) for the first and second stages respectively. This premix was cooled and allowed for ageing at 4 °C overnight. The next day, these mixes were frozen in ice cream machine (Tetra Pak KF80) and the ice cream was transferred into test containers and immediately transferred to freezer at -20 °C.

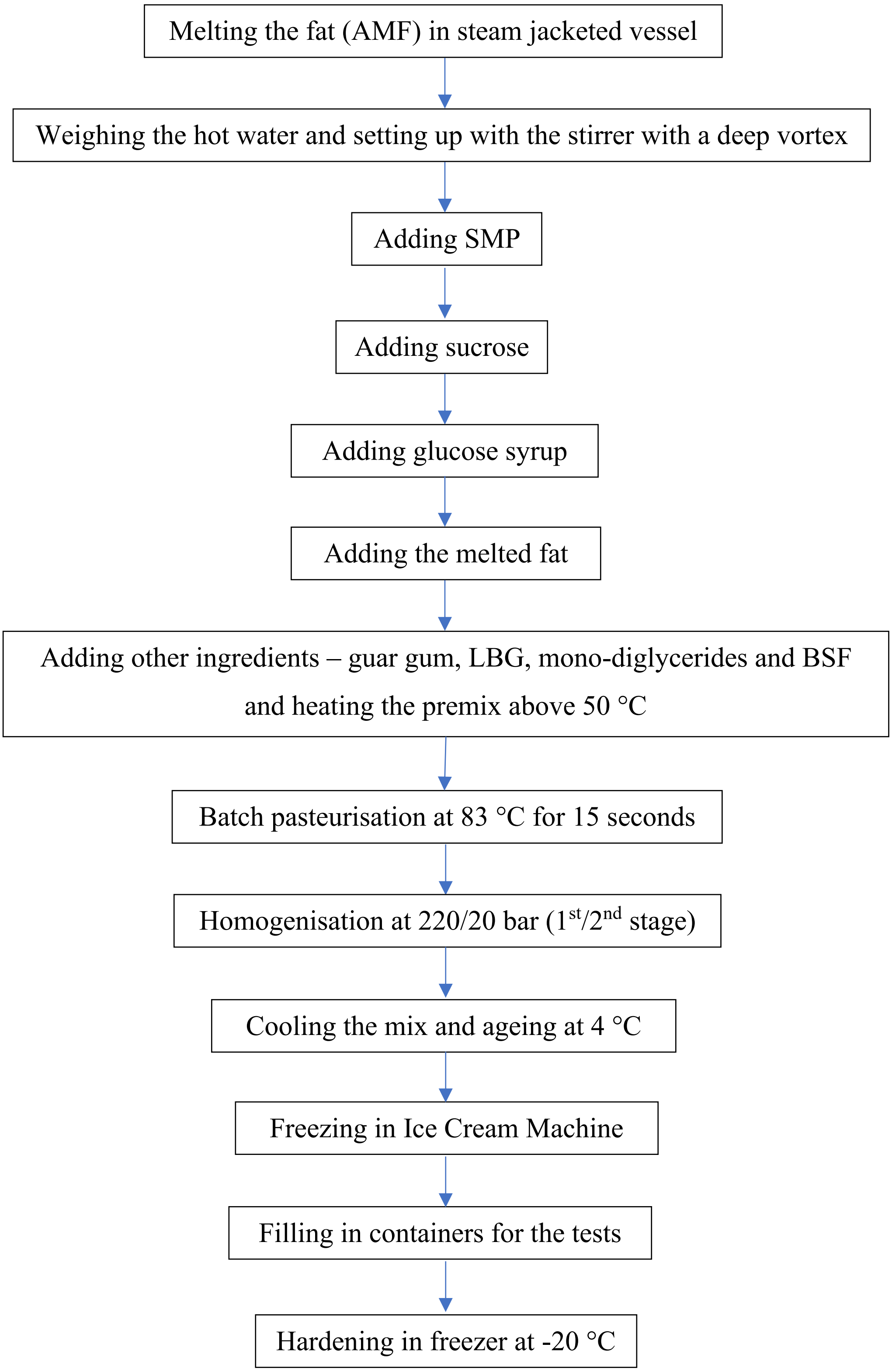


Figure 12: Flow chart of the batch scale ice cream production

3.8 Ice Cream Characterisation

3.8.1 Overrun in Ice cream

The measurement of the amount of air that is incorporated into the ice cream after freezing and aeration of the ice cream mix. This measurement was calculated by comparing the weight of the ice cream corresponding to the ice cream mix at the same volume using the below equation.

Equation 4

$$Overrun = \frac{Weight\ of\ mix(g) - Weight\ of\ ice\ cream\ (g)}{Weight\ of\ ice\ cream(g)} x\ 100$$

3.8.2 Meltdown rate of Ice cream

The meltdown rate of the ice cream was measured by putting the ice cream on a certain mesh screen of 1x1 cm squares as shown in Figure 13 and leaving it to melt in a controlled room temperature of 20 °C for 120 minutes. The balance was kept on the bottom of the samples and the weight was recorded for every 10minutes interval. The meltdown curves were then plotted as a function of the weight against time.

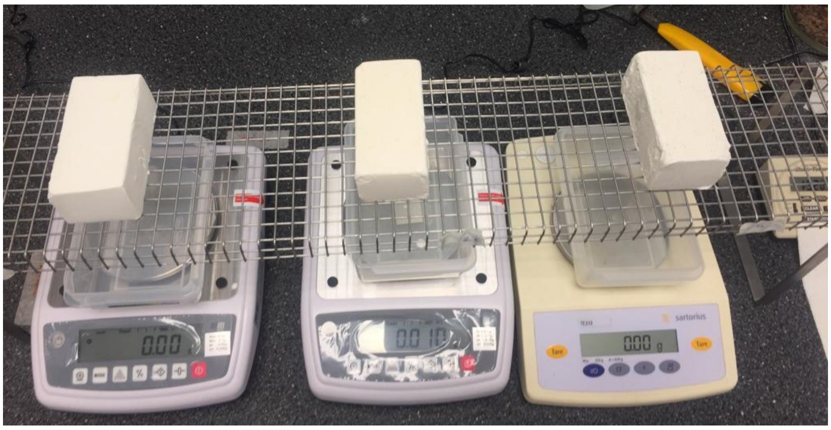


Figure 13: Meltdown rate of ice cream in mesh screen

3.8.3 Firmness Tests of ice cream

The ice cream firmness was analysed using a texture analyser (TA.XT.Plus Texture Analyzer, UK) as seen in Figure 14 using a 5mm stainless steel cylindrical probe, whose penetration speed was 5.0 mm/s. The pre-test speed and the post-test speed was set at 3.0 mm/s and 5.0 mm/s respectively. The probe was inserted to a depth of 10mm. All these measurements were carried at 20 °C.

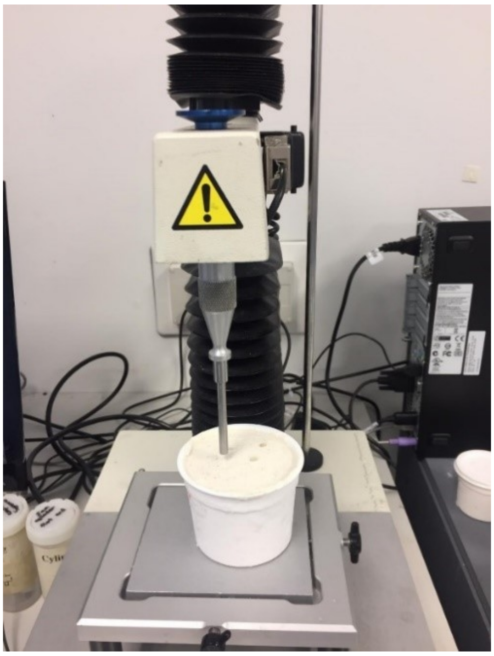


Figure 14: Firmness tests performed in a Texture analyser

3.8.4 Particle size distribution

The particle sizes of the fresh and melted ice cream mixes were analysed in the particle size analyser (Malvern MS 2000). The diluted samples (1:1000) with MiliQ water were put in the sample chamber at 16-18°C. The measurements were done in triplicates and the mean d_{43} and d_{32} was recorded.

Chapter 4 Rheological and Emulsification properties of BSF

4.1 Introduction

This chapter details the effects of pH and salt content on the viscosifying, emulsifying and stabilising properties of the BSF. Emulsion characterisation was done by measuring the droplet sizes distribution (d_{32} and d_{43}) and zeta potential (mV). Droplet size changes during storage were also measured. The rheological properties of the emulsions were determined. Visual phase separation was also monitored for a month's period at 20°C. Finally, the microstructures were visualised using CLSM and/or light microscopy.

BSG is known to exhibit functional properties, providing the capacity for both emulsification and thickening, as discussed in literature review (section 2.5.2.4). BSF is a milled composition of the basil seed, composed of BSG, protein , lignins and fat that could be used a natural food ingredient in food systems, whereas BSG is yet to obtain clearance for use as an additive in foods. Therefore, this effort was made to investigate BSF properties in solution, as a viscosifying agent, and its ability to emulsify and stabilise oil-in-water emulsions under various conditions.

4.1.1 Proximate composition of BSF used for this study

The proximate composition of the Iran variant used for the study is shown in Table 5 below. This was carried out at the Nutrition Lab, Massey University, Palmerston North, New Zealand. Earlier trials of BSG extraction were done with the Iran variant, and thus it became imperative to use the Iran variant itself for the present study.

Table 5 : The proximate composition of basil seeds from two different areas

Constituents	Dry matter (%)	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Neutral Detergent Fibre (%)	Acid Detergent Fibre (%)	Lignin (%)
Iran variant	94.9	5.1	6	19.2	22.1	33.6	20.9	7.8
Vietnam variant	91.6	8.4	5.7	14.8	18.6	43.6	27	9.7

4.2 Viscosity of BSF solutions

The increasing concentration of BSF, on viscosity, storage modulus (G') and loss modulus (G'') is illustrated in Figure 15 and Figure 16 respectively. With the increase in BSF concentration, the viscosities and the viscoelastic properties of the extracts also increased. High viscosity (*e.g.* 111 Pa.s at $0.1s^{-1}$ shear rate) and viscoelasticity (*e.g.* *G'* =15.2 Pa, *G''* =6.19 Pa at 0.1 strain) were observed at 1.5% BSF. All the extracts at all concentrations showed shear thinning behaviour. The storage modulus (*G'*) was higher than the loss modulus (*G''*), at all extracts concentrations. This indicates that viscoelastic behaviour dominates the rheological properties of the flour, although the very low modulus values suggest very weakly gelling characteristics. On a comparative weight basis, a 1%BSG solution showed a viscosity of 170Pa.s at $0.1s^{-1}$ shear rate and viscoelasticity as *G'* =170 Pa, *G"*= 23 Pa at 0.1Hz whereas BSF sample showed a viscosity value of 57Pa.s at $0.1s^{-1}$ shear rate and viscoelasticity as *G'* =7.8 Pa, *G"*= 3.7 Pa at 0.1Hz (Hosseini-Parvar et al., 2016).

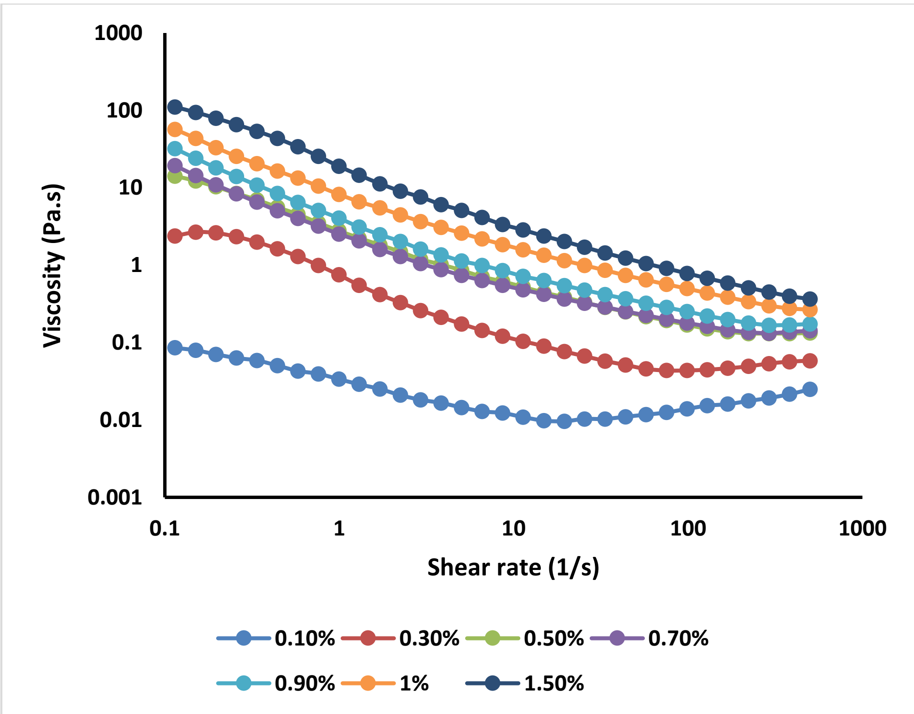
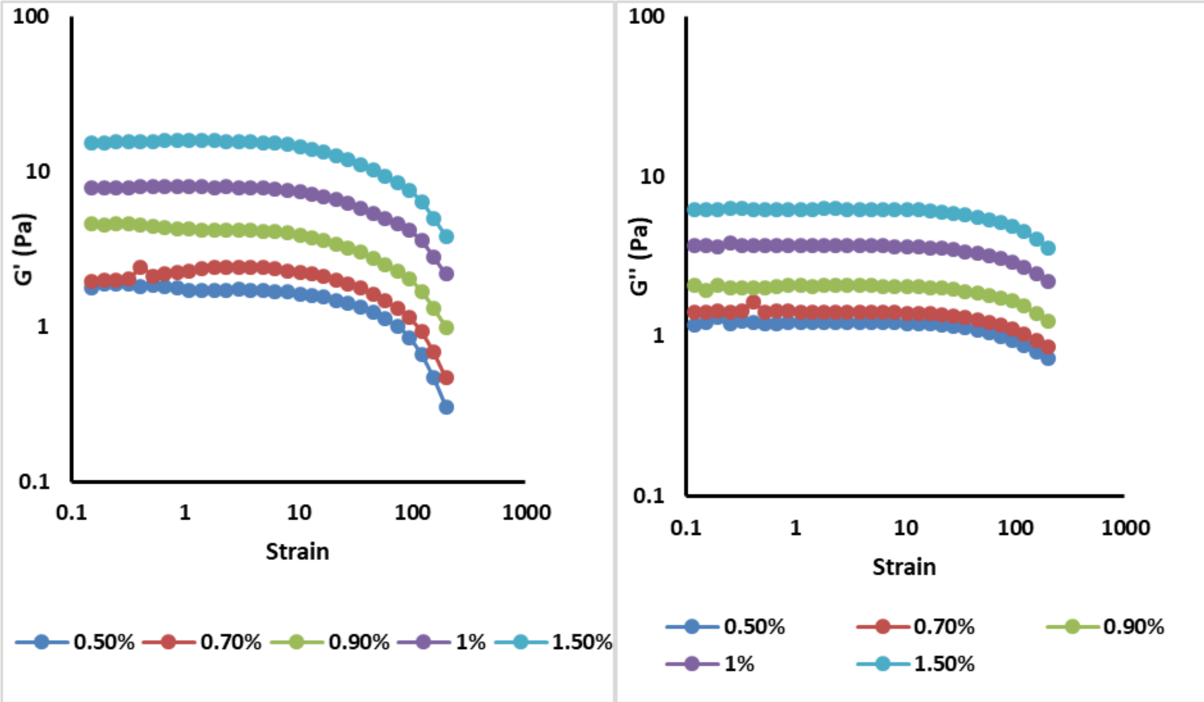


Figure 15: Effect of increasing BSF concentration on the viscosity of the extracts



a. b.

Figure 16: Effect of flour concentration on: (a) the storage modulus (G') and (b) the loss modulus (G'') of extracts

4.3 Effect of flour concentration on the emulsifying properties of BSF

10% (wt/wt) soya oil-in-water emulsions were made with different concentrations of BSF [0.5-1.5%(wt/wt)] as described in materials and methods (Chapter 3). Rheological properties and particle sizes were also measured within 24 hours post preparation. On 3rd, 7th, 14th and 30th day of storage, both the droplet sizes and the visual phase separation were measured.

4.3.1 Results

Particle size distribution of the emulsions as a function of flour concentration is shown in Figure 17. There was an apparent increase in the droplet sizes when concentration was increased from 0.5% to 1.5%. Wider particle sizes were seen across all the concentrations of the flour used. The d_{43} values rose from 34.72 µm at 0.5% concentration to 55.6 µm at 1.5% concentration. A bi modal distribution was seen in all the extracts.

The effect of BSF concentration, on viscosity and storage modulus (G') and loss modulus (G''), is shown in Figure 18 and Figure 19 respectively. With the increase in BSF concentration, the viscosity and viscoelastic properties of the emulsions also increased. Higher viscosity (*e.g.* 50.5 Pa.s at 0.1s^{-1} shear rate) and viscoelasticity (*e.g.* *G'*= 8.83 Pa, *G''*= 1.93 Pa at 0.1Hz) were observed at 1.5% BSF. Shear thinning behaviour was seen in all the emulsions with storage modulus (*G'*) higher than the loss modulus (*G''*) at all the flour concentration indicating a solid like property of the flour dominating the flow behaviour of the emulsions.

The phase separation of the emulsions after storage for 1 month at 20 °C is shown in Figure 20. Phase separation was seen in the emulsions with 0.5% BSF concentration and a fine top oil layer in 1% BSF concentration emulsions. With increased BSF concentration at 1.5%, there was no phase separation suggesting improved physical stability.

Further, the microstructures of the emulsions at three different BSF concentrations is shown in Figure 21. Observed droplet sizes were in reasonable agreement with particle size distribution data, with droplets of approximately 25 µm observed across all concentrations, and with increasing numbers of smaller droplets (<10 µm) appearing as the BSF concentration is increased. A few particulate structures were observed at the interface of some droplets, possibly proteins or lignin or other insoluble materials that were receptive to staining with the Fast-Green dye.

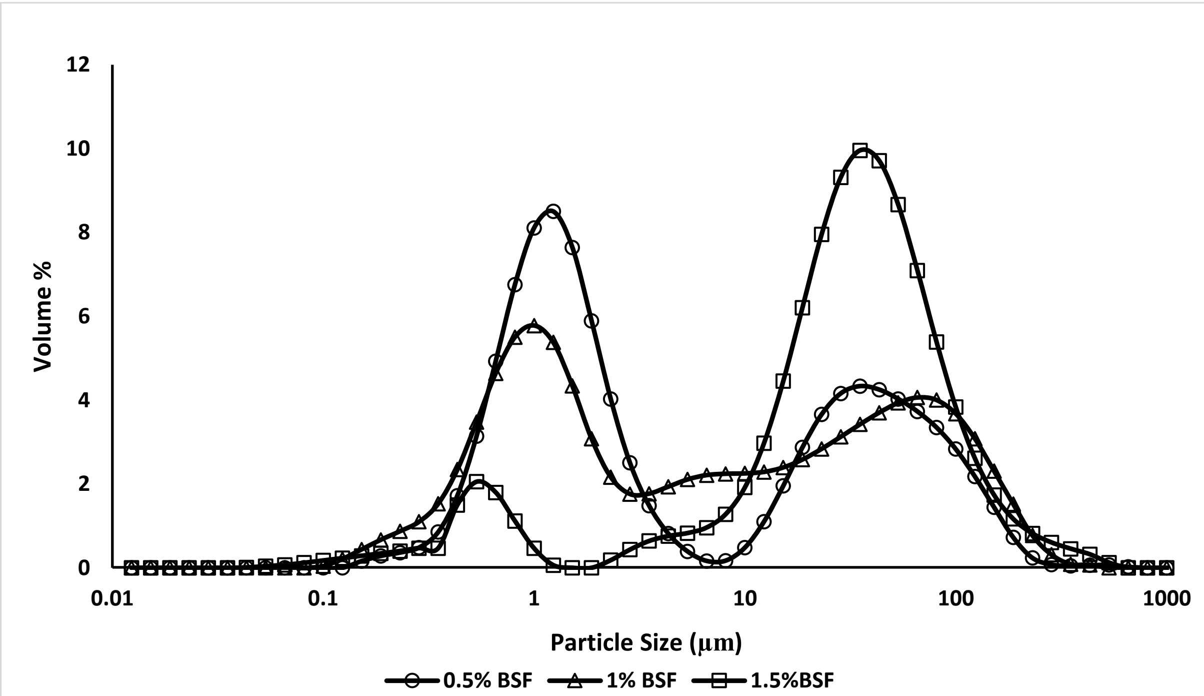


Figure 17: Effect of BSF concentration on the particle size distribution of 10% (wt/wt) soya oil-in-water emulsions

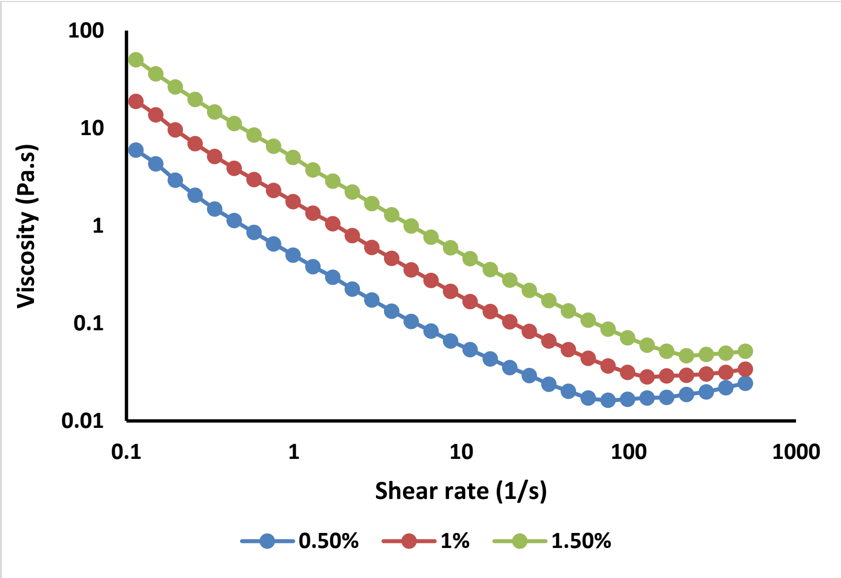
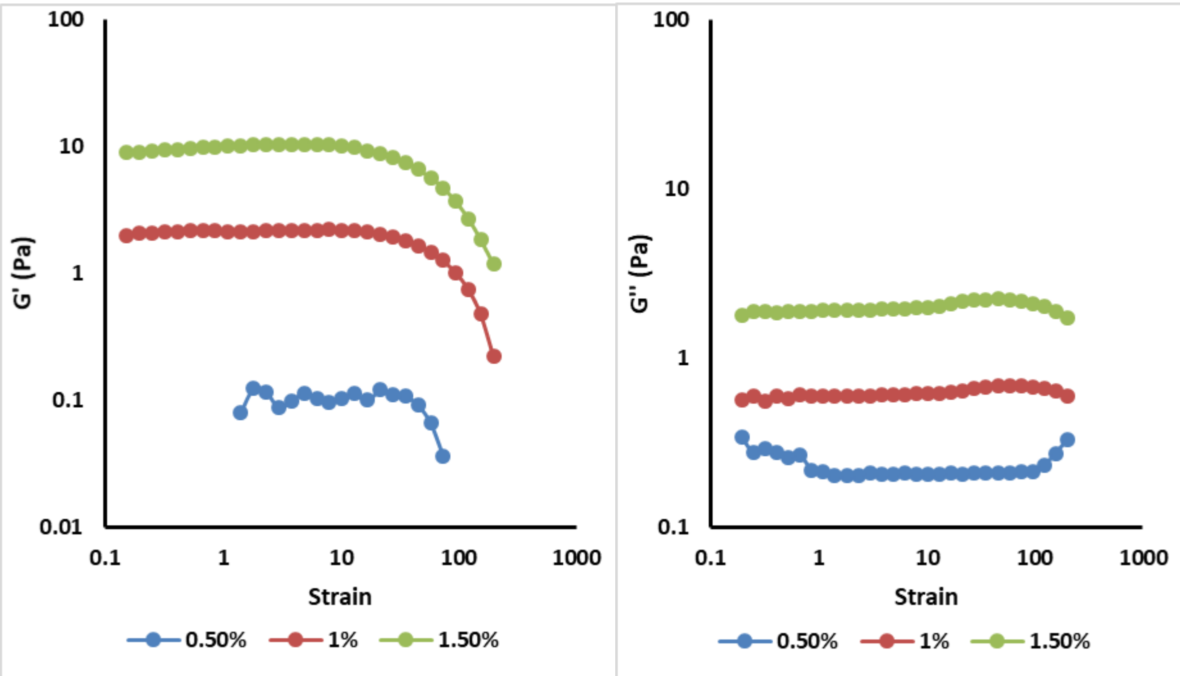


Figure 18: Effect of increasing BSF concentration on the viscosity of 10% soya oil-in-water emulsions



a. **b.**

Figure 19: Effect of flour concentration on:(a) the storage modulus (*G'*) and (b) the loss modulus (*G''*) of the emulsions containing 10% soya oil-in-water emulsions

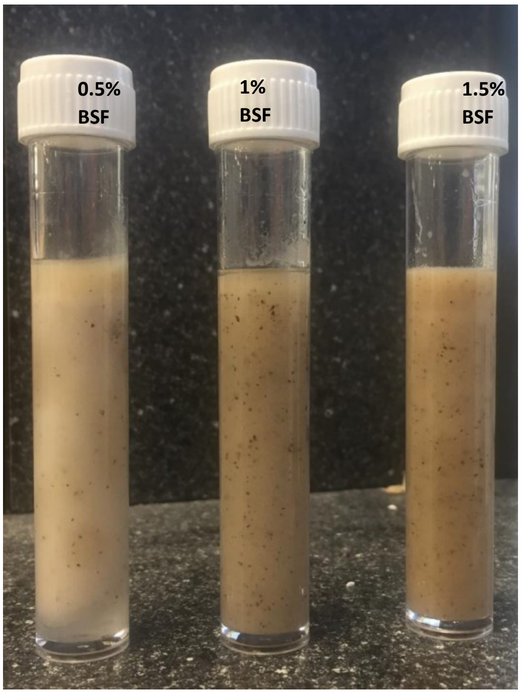


Figure 20: BSF stabilised soya oil-in-water emulsions stored for 1 month at 20°C

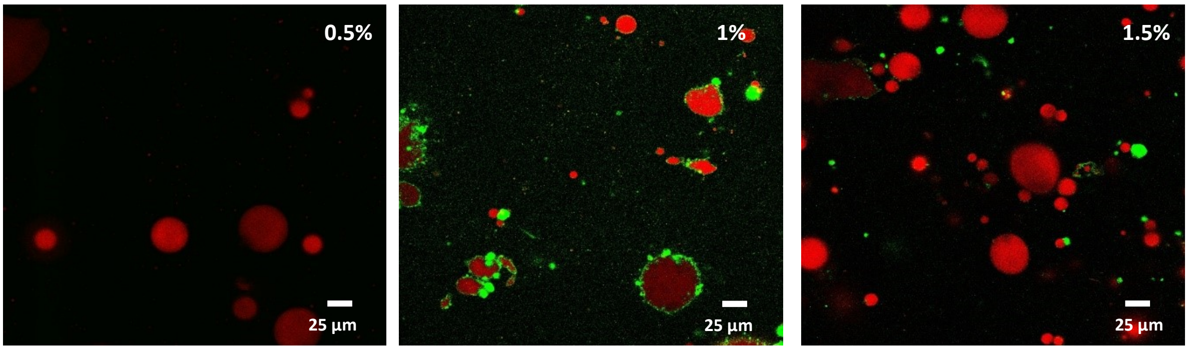


Figure 21: Microstructures of 0.5%, 1% and 1.5% BSF concentrations stabilised soya oil-in-water emulsions

4.3.2 Discussion

The flour concentration increase resulted in an increase in the emulsion droplet sizes from 0.5% BSF to 1.5% BSF. All the BSF emulsions formed bi-modal distributions with average droplet sizes below 3µm. At lower flour concentrations of 0.5% and 1%, smaller droplet sizes were formed (d_{32} = ~1.5 µm), whereas at higher concentration of 1.5% higher droplet sizes were formed (d_{32} = 2.8 µm). However, at lower droplet size area, the 1.5% BSF emulsion sample provided the smallest droplet size area followed by 1% and 0.5% BSF concentration. This could indicate that the emulsions are more stable when the flour concentration was increased. Observed droplet sizes are noticeably larger in comparison to previous research on emulsions stabilised with BSG, even for elevated concentration of flour. It is possible that the higher droplet size as measured by light scattering is partly a consequence of the presence of insoluble particulate materials in the flour; however, confocal microscopy does confirm a population of fat droplets of approximate diameter of 25 μm, in agreement with mastersizer data. One possible cause for the increase in droplet size relative to gum-stabilised emulsions is a reduced concentration of surface-active material available in the flour to provide adequate surface coverage for stabilisation. A study conducted by Aluko, McIntosh, and Reaney (2001) have shown the stabilising action of purified coriander protein concentrate to be better than the whole flour usage resulting in smaller size droplets due to better emulsion forming properties of the purified fractions. Alternatively, other stabilising fractions may be present in the flour, and the relative levels of these for any given concentration of flour may be exerting an influence on the interfacial composition formed during homogenisation. Of particular interest, is the role of the lignin component in the flour, noting that previous studies have shown lignin to be an effective Pickering stabiliser of emulsions (Stewart, Golding, Matia-Merino, Archer, & Davies, 2016; Wei et al., 2012). Preferential stabilisation of droplets by lignin when using BSF may lead to formation of large, but highly stable droplets, due to the large size of the adsorbing species relative to soluble, molecular surface-active components.

The emulsions also showed an increase in the viscosity with increasing BSF concentration. The increase in the viscosity could also provide emulsion stabilisation as confirmed by the absence of creaming and phase separation in the highest BSF concentration at 1.5%. It is known that by increasing the viscosity of the continuous phase most non-adsorbed polysaccharides provide a long-term stability. This helps in preventing coalescence, flocculation and creaming by stopping the movement of the droplets during the storage of the emulsions (Dickinson & Stainsby, 1988). It could be also said that the long term stability of the BSF emulsions is a result of several combined factors such as (i) due to the steric stabilisation provided by the BSG adsorbed at the interface, (ii) viscosity increase of the system due to un-adsorbed BSG at the interface, (iii) surface-active lignin molecules that can adsorb at the oil-water interface and thus provide kinetic stability of the emulsions (Stewart et al., 2016). The work carried out by Stewart et al. (2016) have found that the lignin microparticles were able to stabilise oil-in-water emulsions for at least five months in chilled storage conditions and (iv) the protein content in BSF is approx. 19%. This could also influence the maximum emulsion stability at the native pH of 7 which was the case here. Khalid, Babiker, and El Tinay (2003) have found in their study that protein extracted from sesame flour at native conditions had maximum emulsion stability at 70% than at other lower or higher pH conditions.

A shear thinning flow behaviour was seen in BSF stabilised emulsions at all the flour concentrations, showing decrease in viscosities as the shear rates increased. The shear thinning behaviour could be either due to the continuous phase rheology of the emulsion as influenced by the relative concentration of flour, and/or a consequence of flocculation of the emulsion. A systematic increase in viscosity and thinning behaviour was seen when the flour concentration increased from 0.5% to 1.5%. It could be due to the plethora of components present in the system contributing to the increase in viscosity of the continuous phase like the non-adsorbed BSG, protein component and lignins. The findings by Hosseini-Parvar et al. (2010) have found that the solutions of BSG (0.02-2% (wt/wt)) exhibiting pseudoplastic behaviour with increase of the gum concentrations.

Polysaccharides have a large hydrodynamic volume, thus showcasing higher viscosity and shear thinning nature as was the case with BSG at high viscosities and low shear rates. Noticeably, at higher shear rates there was not seen a Newtonian region in the case of these BSF emulsions. Thus in food applications, this shear thinning behaviour allows foods to be swallowed with ease (Vardhanabhuti & Ikeda, 2006). Moreover, the elastic nature of these BSF emulsions were higher than the viscous behaviour ($G' > G''$) at all the three concentrations tested. This elastic nature could also contribute to the stability of the emulsions by providing a solid like structure and a yield stress thus restricting the droplet movement especially at the higher concentrations.

There was an improved emulsion stability seen when the concentration was increased to 1.5% BSF. At lower concentrations, especially at 0.5% phase separation was seen, and an oil layer at the top was evident in 1% concentration after 30 days of storage.

As a summary, at very low flour concentrations there was phase separation which lead to coalescence over time. As flour concentration increased up to 1.5%, the oil droplets were covered fully and stabilised against droplet aggregation. An increase in the viscosity of the continuous phase at the highest concentration, is likely due to the thick steric layer provided by the BSG at the interface along with lignins providing kinetic stability. The proteins at the interface could also have provided the steric and electrostatic interactions that would have stabilized the droplets against flocculation and coalescence during storage.

4.4 Effect of pH on the emulsifying properties of BSF

The emulsifying properties of BSF, by changing the pH (3-9) conditions at a constant flour concentration of 1% (wt/wt) was evaluated. The pH of the BSF emulsions was adjusted before the emulsification as followed by Hosseini-Parvar et al. (2016) due to improvement in particle sizes when adopting this method rather than after the emulsification. These emulsions were characterised, in relation to droplet size distribution, rheological properties, droplet charges (zeta potential) and microstructural images (CLSM and/or light microscope). Increase in the droplet size (d_{43}) and visual phase separation was also monitored on the 3rd, 7th, 14th and 30th day of the storage at 20 °C.

4.4.1 Results

The emulsifying ability of BSF in terms of droplet size distribution with varying pH conditions is shown in Figure 22. The pH increase reduced the average droplet size of the emulsions. This was shown by shifting of the size distribution curves to the smaller droplet size area. Emulsions at pH 5 and pH 9 showed smaller d_{32} of <1.0µm whereas emulsions at pH 3 and pH 7 showed higher d_{32} values. Notably, at pH 9 the d_{32} values were the least below 1.0µm.

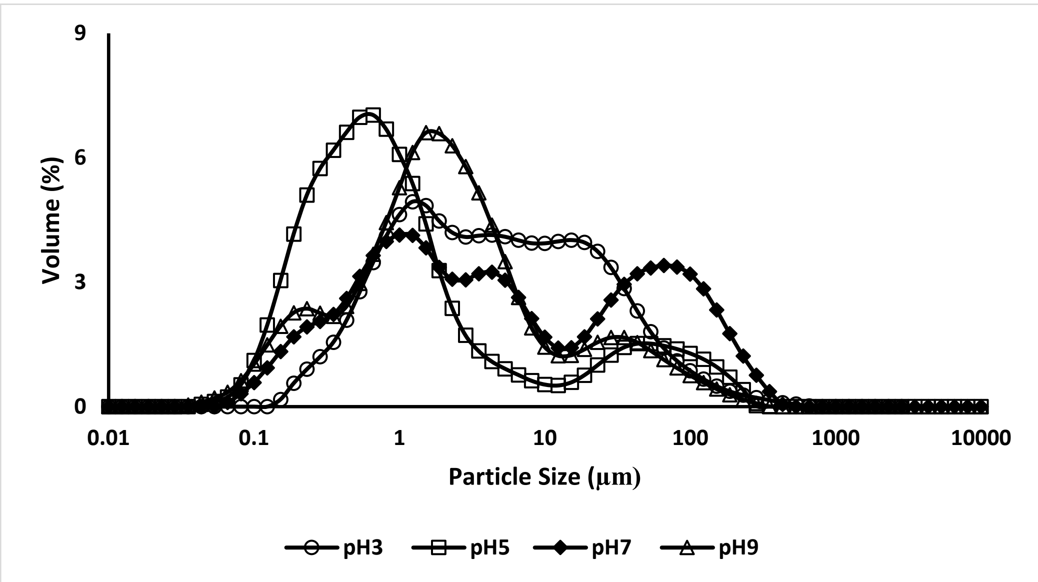


Figure 22: Effect of pH on the particle size distribution of 1% (wt/wt) BSF-stabilised 10% soya oil-in-water emulsions

The effect of pH on the viscosity of the BSF stabilised emulsions is shown in the following Figure 23. All the emulsions showed shear thinning behaviour at all pH conditions. At very low shear rates (<0.1s^{-1}), emulsions at lower pH had the lowest apparent viscosities than emulsions at other pH conditions. This trend continued even at higher shear rates for the lowest pH sample. However, as the shear rate increased, emulsions at pH 9 had higher viscosity (e.g. 2.2 Pa.s at 1s^{-1} shear rate) compared to other pH conditions at all increasing shear rates. The viscoelastic properties of BSF-stabilised emulsions, with varying pH conditions is shown in Figure 24. Emulsions at native pH exhibited higher viscoelastic properties compared to other pH conditions. The viscoelastic properties were the lowest for the pH 3 condition. Storage modulus (*G'*) was higher than the loss modulus (*G''*) in all the emulsions at all the at all the pH conditions indicating the dominance of elastic property across.

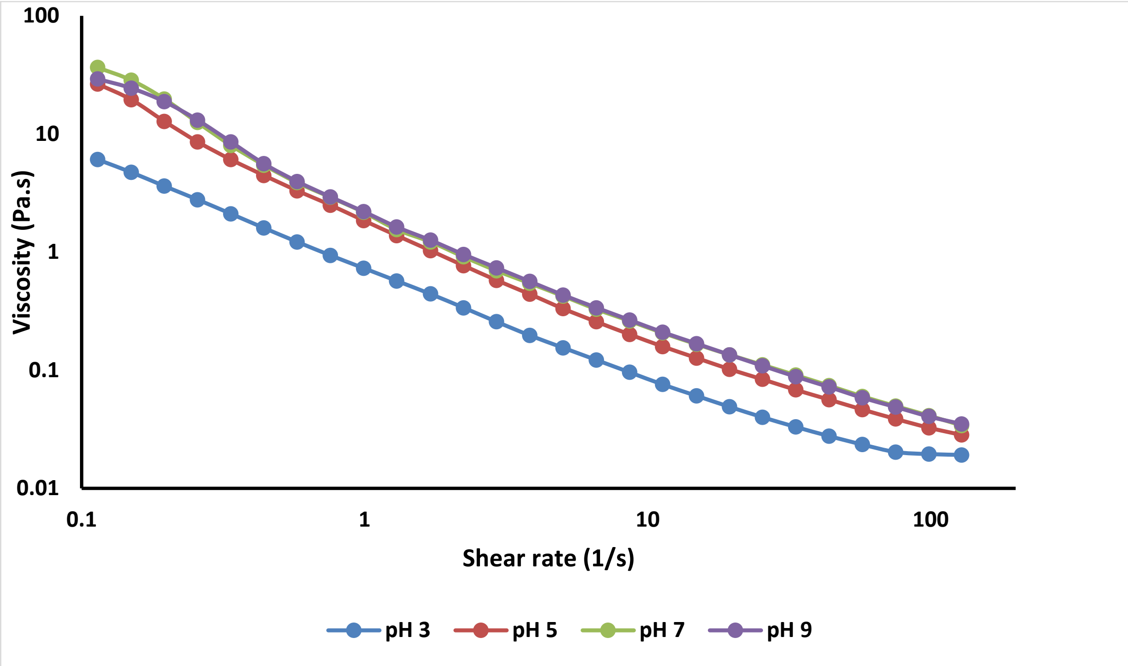
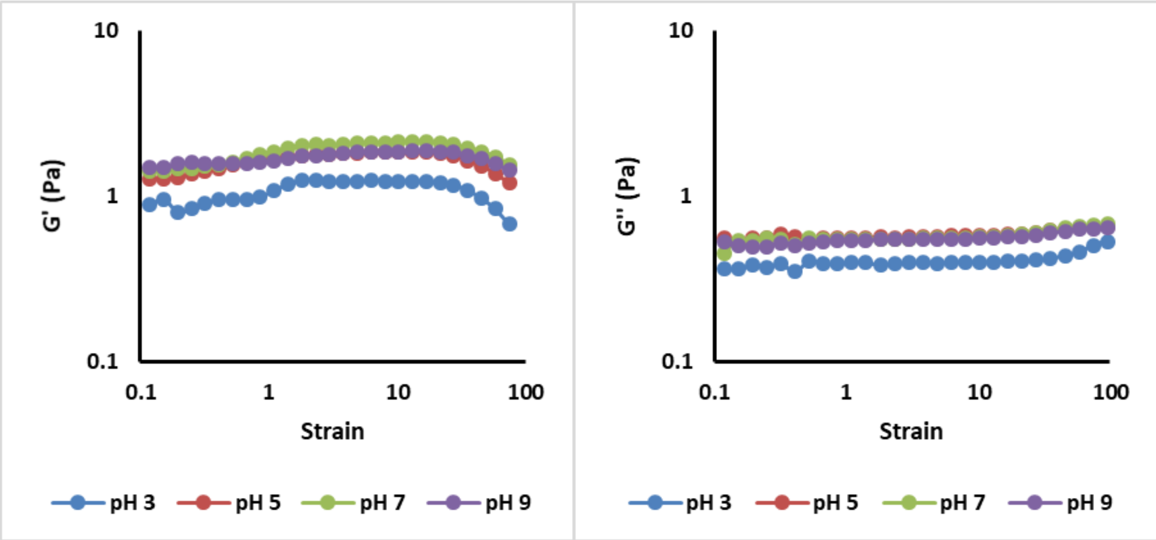


Figure 23: Effect of pH on the viscosity of 1% (wt/wt) BSF- stabilised 10%(wt/wt) soya oil-in-water emulsions



a. **b.**

Figure 24: Effect of pH on: (a) the storage modulus (*G'*) and (b) the loss modulus (*G''*) of the emulsions containing 10% soya oil-in-water emulsions

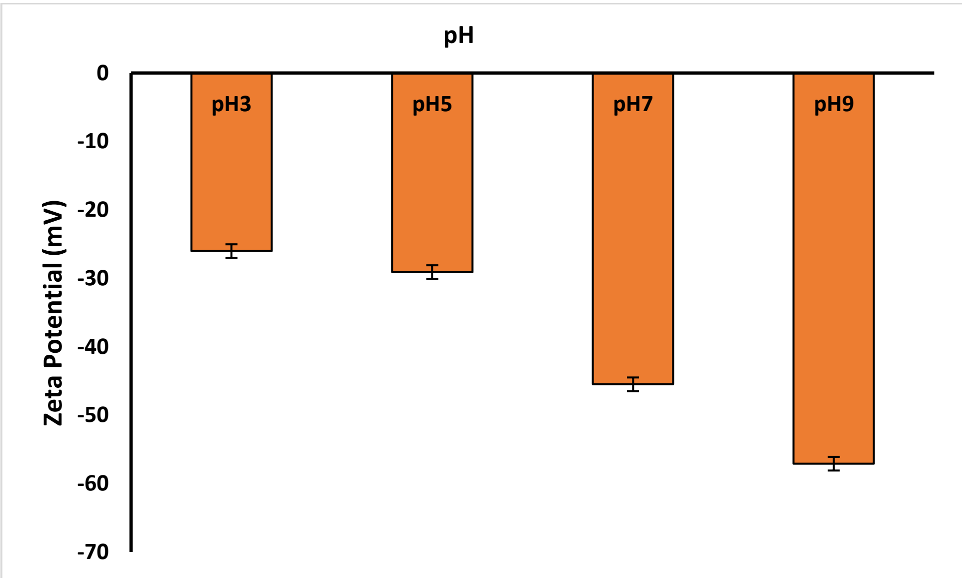


Figure 25: Effect of pH on the zeta potential of 1% (wt/wt) BSF-stabilised 10% soya oil-in-water emulsions

The effect of pH on the zeta potential of BSF stabilised emulsions is shown in Figure 25. As pH rose from acidic to alkaline conditions the zeta potential values increased. Apparent shift in zeta potential values were seen at native pH 7, where the zeta potential value rose from -29.1 mV (pH 5) to -45.5 mV (pH 7). There was no significant change in zeta potential values at pH 3 and pH 5 (-26 mV and -29.1 mV respectively) whereas from native pH 7 to alkaline pH 9 there was a significant difference (-45.5 mV and -57.1 mV respectively). The increase in zeta potential values with increase in pH indicates that BSF-stabilised emulsion droplets become more negatively charged. The maximum value was found at -57.1 mV at pH 9 of BSF emulsions.

The growth in the droplet sizes (d_{43}) and visual phase separation during storage was evaluated for different pH conditions. The average droplet size of the emulsions increased steadily except at pH7, which increased significantly during storage as seen in Figure 26 below. At pH conditions (pH 3 and pH 5), the increase in particle sizes were not significant after 1-month storage. However, at pH 9, the emulsion droplet sizes doubled from 9 µm on day 1 to 18 µm (approx.) on day 30. Visual phase separation in terms of oil at the top was seen in all the pH conditions except at pH 9 during storage for 1 month as seen in Figure 27 below. In all the three emulsions at pH 3, pH 5 and pH 7 there was an oil layer at the top except at pH 9 where there was no oil separation at the top showing more stability after a month's storage time.

Furthermore, the microstructures of 1% BSF stabilised emulsions as influenced by various pH is shown in Figure 28. The lowering of the pH resulted in increased droplet sizes especially seen at pH 3. A degree of flocculation/ aggregation was seen at pH 5 and pH 7 (Figure 28) compared to other pH conditions. Both the micrographs and the size distribution data showed similar results at pH9.

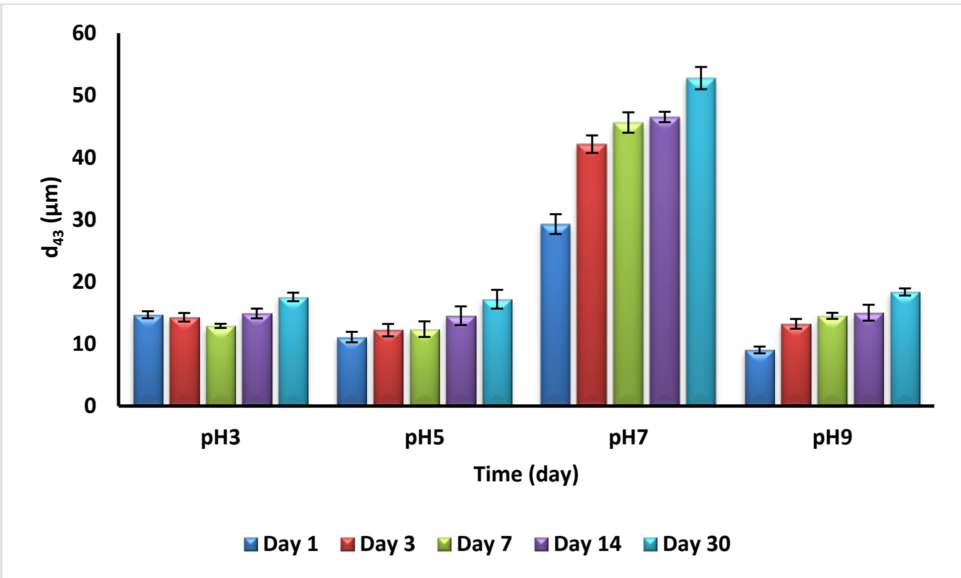


Figure 26: Effect of pH on the average particle size (d_{43}) of 1% (wt/wt) BSF-stabilised 10% (wt/wt) soya oil-in-water emulsions stored for a month at 20°C

Figure 27: BSF stabilised whole emulsions at different pH stored for 1 month at 20°C

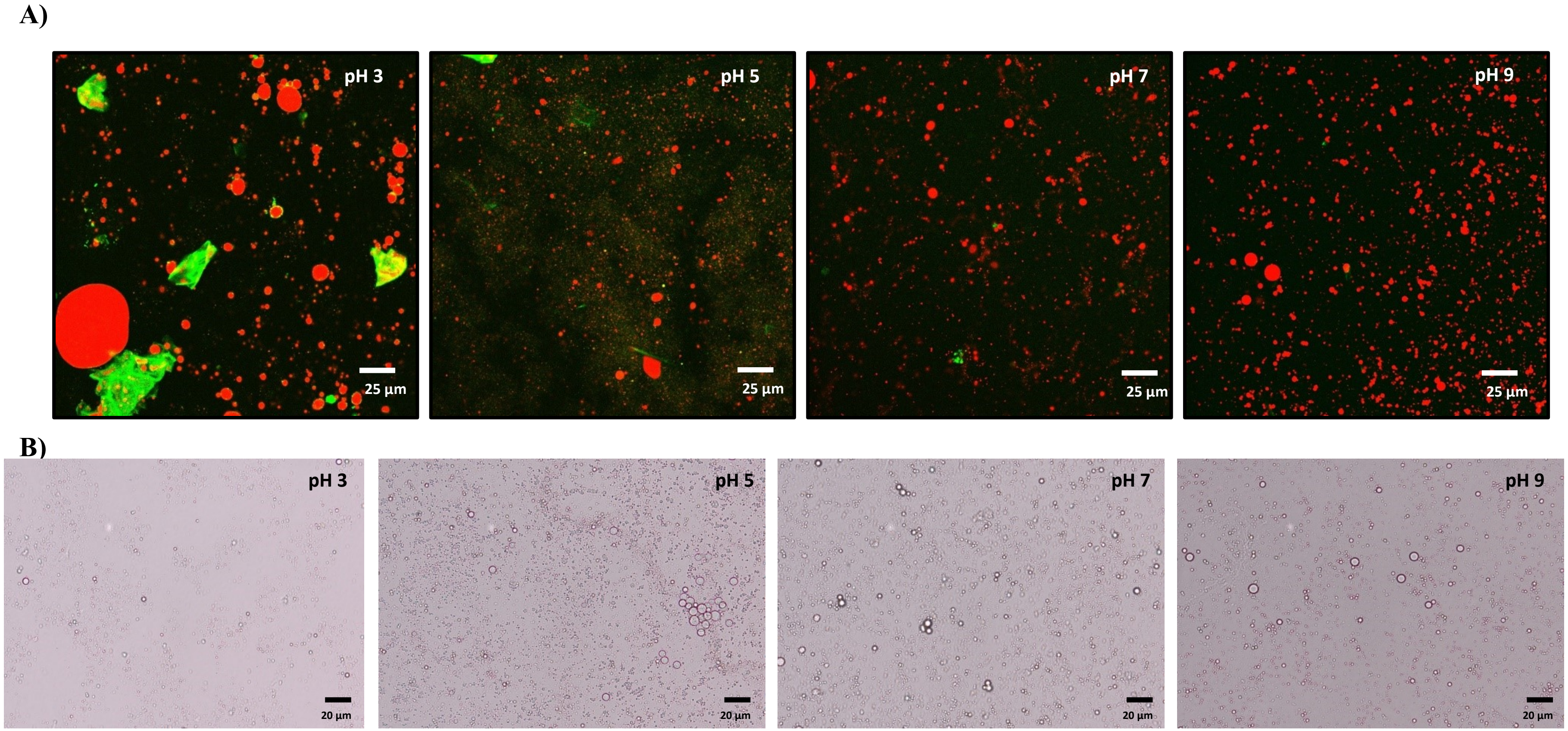


Figure 28: Microstructures of fresh 1% (wt/wt) BSF-stabilised 10% (wt/wt) soya oil-in-water emulsions at different pH conditions A) CLSM images B) Light Microscope Images at 40x magnification

4.4.2 Discussion

The emulsifying ability of BSF, in relation to droplet size and droplet size distribution was sensitive to pH changes. A drop in pH from native conditions to pH 3, showed an increase in particle size (d_{32}) whilst decreasing the zeta potential values substantially (from -45.5 mV to -26.03 mV). The pH variations may affect the ability of the functional components to adsorb at the interface, thus the presence of larger droplets. At acidic pH's, it is more likely that the polysaccharide fractions could be in more aggregated state before emulsification thus resulting in bigger droplets as seen at pH's less than 7. A dramatic change in zeta potential values occurs from pH 5-7. The drop in zeta potential from pH 7 to 5 is likely to be due to overall charge reduction on the emulsion droplets as protein fraction of the flour probably would have reached the isoelectric point. However, as alkalinity in the environment increased there was seen a reduction in particle sizes below 1.0 µm (d_{32}) with a significant increase in the zeta potential values from pH 7 to alkaline pH 9 (from -45.5 mV to -57.10 mV). A similar argument can be made for the protein component of the flour, noting that decreasing pH may cause a reduction in protein solubility. If protein forms a major component of the interfacial stabilisation of the droplets, loss of solubility might be expected to result in reduced availability of protein for providing surface coverage of droplets.

The reduction in droplet sizes at alkaline conditions may also show the effect of higher amounts of added NaOH aiding the emulsification process. The BSF stabilised emulsions above pH 7.0 (above -40mV) indicate BSF stabilised emulsions are electrostatically stabilised. A possible effect of increasing viscosity is solubilisation of lignin. If this is the case, then possible Pickering stabilisation observed at neutral and acidic pH might give way to molecular stabilisation with either surface active protein or polysaccharide fractions, reducing particle size consequently.

The apparent viscosities of the emulsions were the lowest for the pH 3 sample at all the shear rates. A degree of flocculation was seen in the emulsion as confirmed in the micrographs at this pH 3, but this might be expected to increase viscosity rather than decrease it. All emulsions exhibited shear thinning behaviour. The viscoelasticity of the emulsions was also resistant to pH changes except at extreme pH condition of pH 3 where the lowest G' and G'' was detected. Overall, the rheological properties of the emulsions did not significantly vary irrespective of the size of the emulsion of droplets. The higher visco-elasticity at alkaline pH 9 could be due to the non-adsorbed BSG itself providing enhanced viscoelasticity. It is also known that protein-BSG interaction can also provide stability to the emulsions (Osano et al., 2014).

The droplet sizes of the emulsions increased during the storage period of 30 days. There was a significant increase in the emulsion sizes at pH 7 than at other pH's indicating less stability at this pH as time progressed. But the least amount of average droplet size was observed at day1 of pH 9 emulsion. It may be due to the combined effect of lignins, BSG, proteins providing electrostatic stabilization. Besides, BSG, being an anionic polysaccharide can also provide stability through a combination of electrostatic and steric stabilisation (Hosseini-Parvar et al., 2016). Lignins are highly soluble in alkaline pH and thus provide stability via pickering mechanism as described (Wei et al., 2012). Also, no visual phase separation was seen at this pH 9 upon storage. The viscoelasticity provided by BSG in the aqueous phase could keep the droplets suspending and thus no creaming seen.

Microstructures also confirmed aggregation of droplets at lower pH's (<7). At the lowest pH at 3, the d_{32} value was the highest at 2.49. At low pH conditions, loss of solubility of either the polysaccharide or protein fractions may cause bridging of emulsion droplets. Confocal microscopy at this pH is particularly interesting in the appearance of more prominently green stained domains encompassing networks of fat (the aggregated fat structures can also be observed using optical microscopy). These structures give some argument to the aggregation of droplets caused by reduced solubility of continuous phase components. Green domains become more intense as pH is lowered to 3, suggesting further loss of solubility. At higher pH there was no aggregation seen at pH 9. Lignin has been known to provide stability as a Pickering emulsifier in food systems, but may be increasingly solubilised at higher alkaline conditions (Wei et al., 2012), reducing its capacity to stabilise the emulsion via Pickering effects. The protein content of approximately 1.92% (19.2% in the BSF) in the emulsions could therefore alternatively contribute to the electrostatic stabilisation of droplets thus providing stability well above the isoelectric point. Besides, also providing steric stability by formation of thick interfacial layers due to attached carbohydrate moieties (Ozturk & McClements, 2016). The polysaccharide BSG itself could have provided stability at higher pH in this case as was also studied by Hosseini-Parvar et al. (2016) showcasing higher zeta potential values and reduction in particle sizes above pH 7.

4.5 Effect of salt on the emulsifying properties of BSF

The emulsifying properties of BSF, by changing the ionic strength from 0-1M at a constant BSF concentration of 1% (wt/wt) was evaluated. The salt concentration of the BSF emulsions was adjusted before the emulsification as followed by Hosseini-Parvar et al. (2016) due to improvement in particle sizes when adopting this method rather than after the emulsification. These emulsions were also characterised, in relation to droplet size distribution, rheological properties, droplet charges (zeta potential) and microstructural images (CLSM and/or light microscope). Increase in the droplet size (d_{43}) and visual phase separation was also monitored on the 3rd, 7th, 14th and 30th day of the storage at 20 °C.

4.5.1 Results

The emulsifying ability of BSF in terms of droplet size distribution with varying ionic strength conditions is shown in Figure 29. The addition of salt resulted in changes in the particle size and particle size distribution of all the emulsion samples. The addition of salt resulted in the marginal increase in the particle size (d_{32} from 0.69µm to 2.58µm at the highest salt concentration) and also being more monomodal with narrow distributions (as seen at 1M than at 0.01M level) It can also be seen that control emulsion without any added salt had the lowest mean droplet size (d_{32} = 0.69µm).

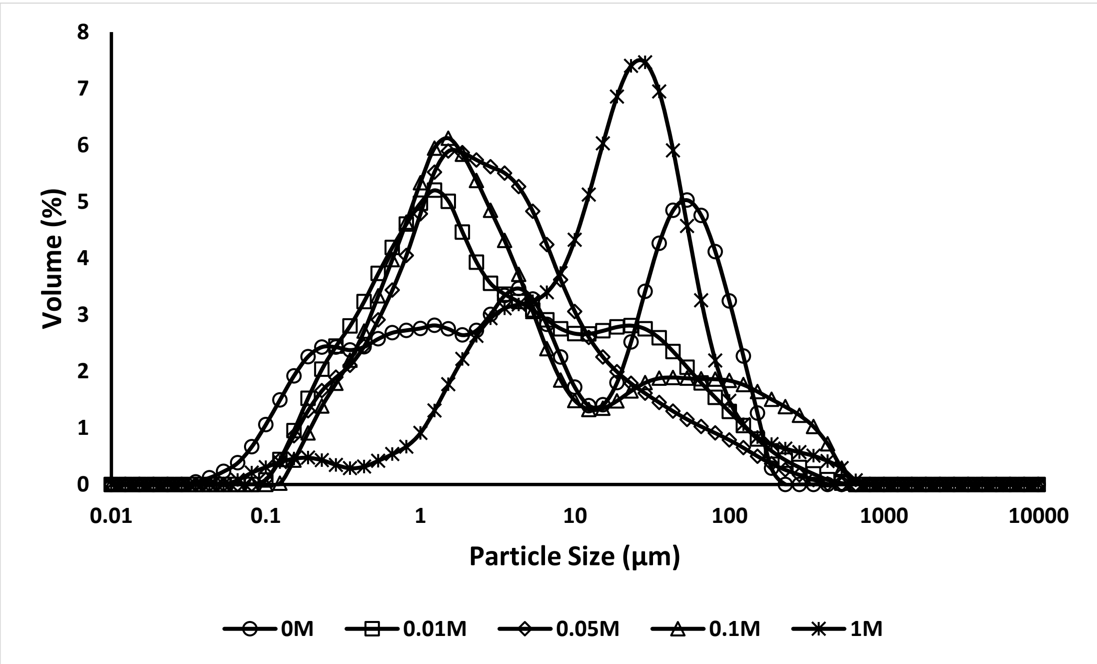


Figure 29: Effect of salt changes on the particle size distribution of 1% (wt/wt) BSF-stabilised 10% soya oil-in-water emulsions

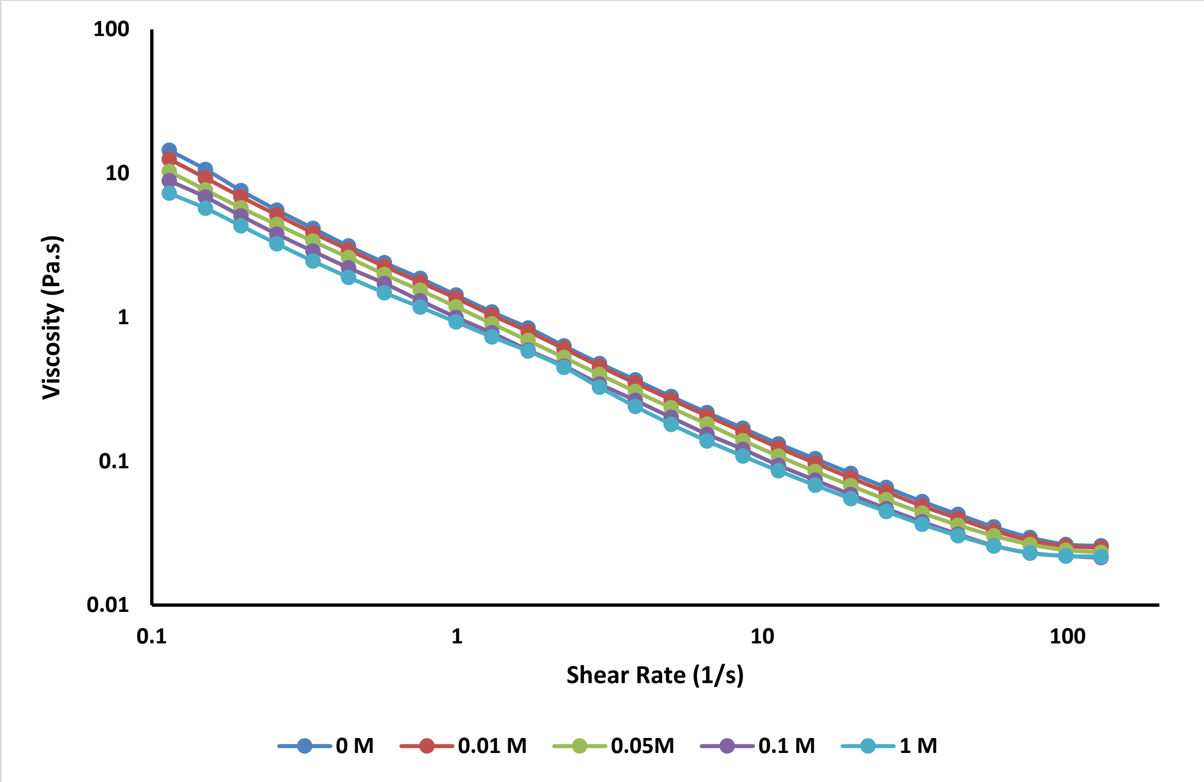
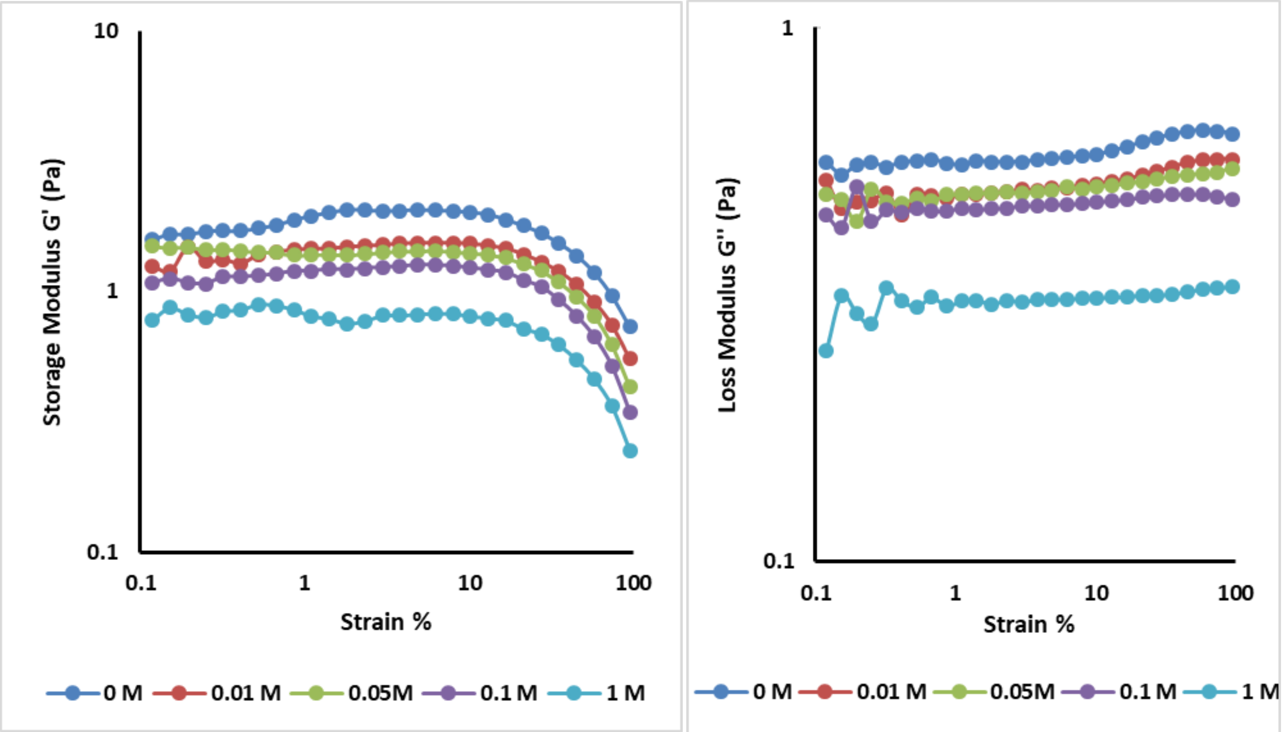


Figure 30: Effect of ionic strength changes on the viscosity of 1% (wt/wt) BSF-stabilised 10%(wt/wt) soya oil-in-water emulsions

The effect of changes in salt content on the rheology of the emulsions is shown in Figure 30. At all the shear rates it was seen that viscosity decreased upon the addition of the salt. The lowest viscosity values were seen at higher salt content emulsion sample than at the control sample. The viscoelastic properties of BSF- stabilised emulsions, with varying salt content is shown in Figure 31.The same trend was observed in the viscoelastic properties as to emulsions with higher salt content showed lower viscoelastic properties than emulsions with lower salt content or control. Storage modulus (*G'*) was marginally higher than the loss modulus (*G''*) in all the emulsions, indicating viscoelasticity, but no strongly gelling behaviours.

The effects of salt on the zeta potential values of the BSF stabilised emulsions is shown in the Figure 32 below. The increase in the ionic strength showed a steady decrease in the zeta potential values of the emulsions. A marked decrease was seen immediately as the salt content increased from -36.3mV at 0.1M to -25.6mVat 1M. The highest value was seen at the control sample at -40.7mV indicating more stability.



a. **b.**

Figure 31: Effect of ionic strength changes on: (a) the storage modulus (G') and (b) the loss modulus (G'') of the emulsions containing 10% soya oil-in-water emulsions

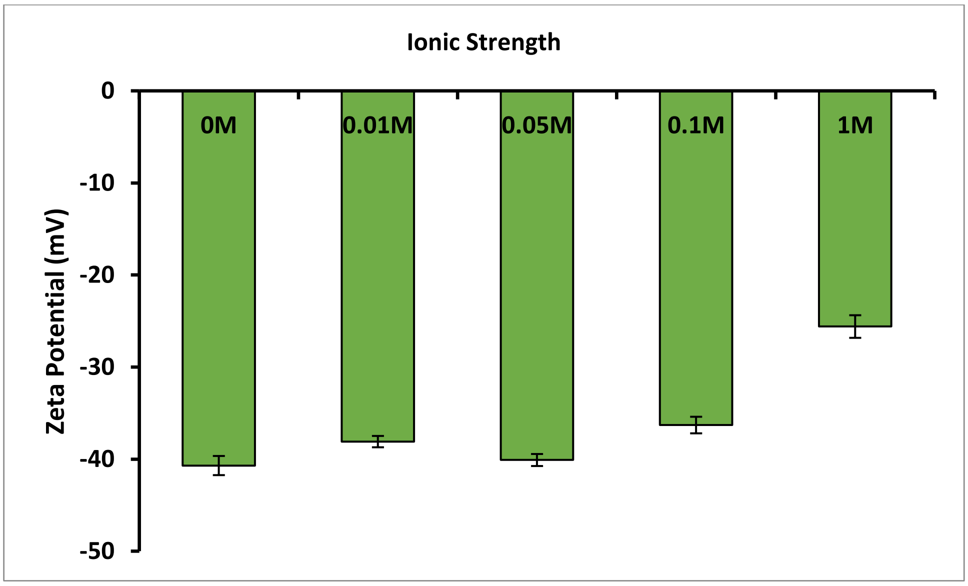


Figure 32: Effect of ionic strength on the zeta potential of 1% (wt/wt) BSF-stabilised 10% soya oil-in-water emulsions

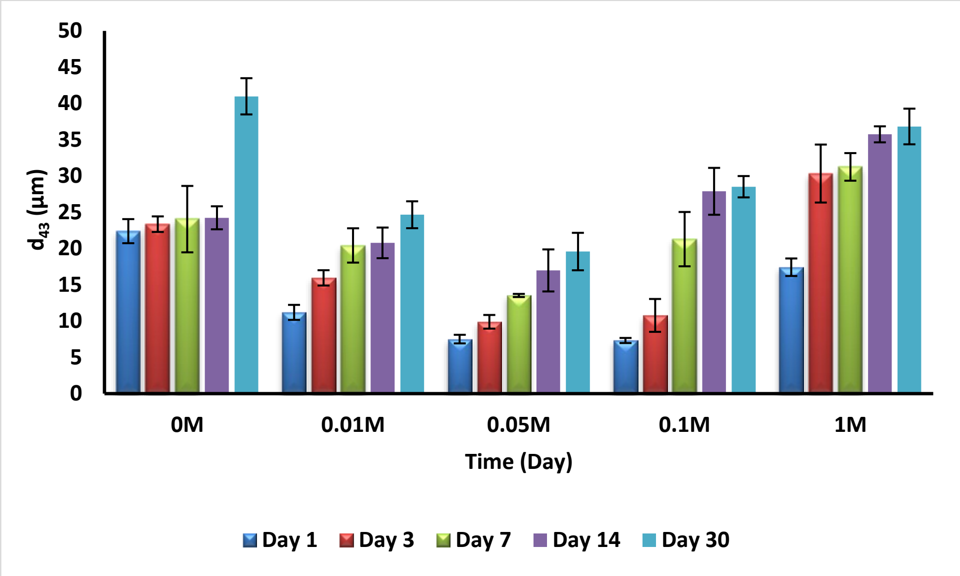


Figure 33: Effect of ionic strength changes on the average particle size (*d_{43}*) of 1% (wt/wt) BSF-stabilised 10% (wt/wt) soya oil-in-water emulsions stored for a month at 20°C

The storage stability of the BSF stabilised emulsions in terms of d_{43} with varying salt content is shown in Figure 33. The mean droplet size steadily increased at all the salt concentrations over the time. It was observed that, the mean droplet sizes were larger at the control samples than at any other concentrations at all the days (*e.g.* 22.4µm on day 1 at 0M than 17.4µm on day 1 at 1M). The visual phase separation in the emulsion samples with salt content is shown in Figure 34. A clear phase separation was seen at three emulsion samples with higher salt contents starting from 0.05M to 1M concentration during storage with extensive phase separation seen at the highest salt concentration of 1M. In all these three emulsions there was a serum layer at the bottom except at 0M and 0.01M where there was no phase separation at the bottom or the top showing more stability after a month's storage time. This indicates that emulsions are highly unstable to creaming with the addition of salt to the systems.

Furthermore, the microstructures of 1% BSF stabilised emulsions as influenced by various salt content is shown in Figure 35. The addition of salt showed large emulsion droplets, showing progressive coalescence over time. The emulsion concentrations of 0.05M, 0.1M and 1M showed emulsion coalescence as seen in the figure below. Unfortunately, confocal microscopy was not carried out for these emulsions.

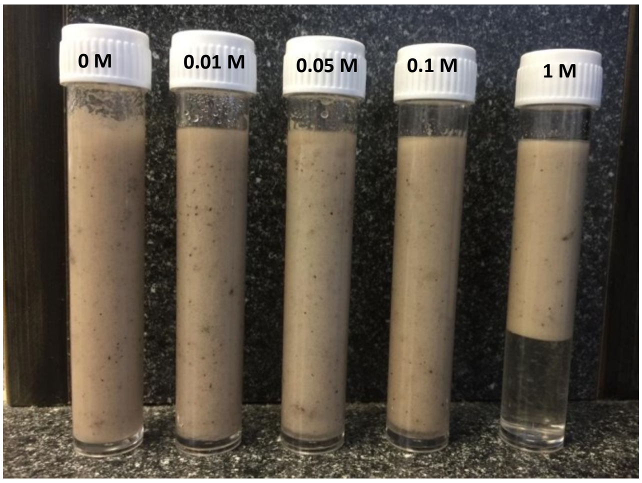


Figure 34: BSF stabilised whole emulsions at different ionic strength stored for 1 month at 20°C

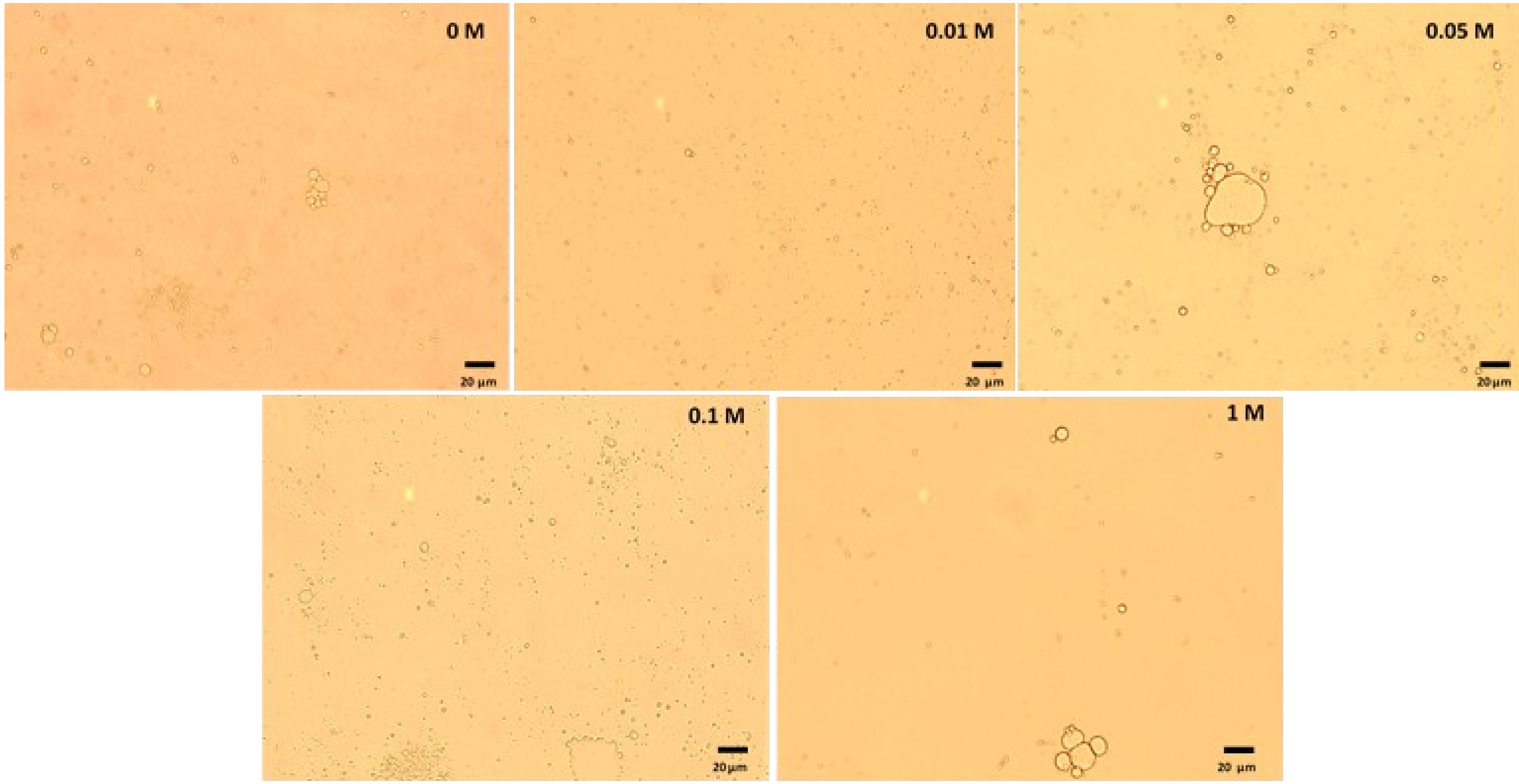


Figure 35: Microstructures of fresh 1% (wt/wt) BSF-stabilised 10% (wt/wt) soya oil-in-water emulsions at different ionic strength conditions using light microscopy

4.5.2 Discussion

The emulsifying activity of BSF was sensitive to ionic changes, as seen in particle size data and particle size distribution. The highest ionic strength concentration especially at 1M showed relatively monomodal and narrow size distribution of larger droplet sizes. The increase in droplet sizes as salt concentration progressed showed a steady decrease in the zeta potential till 0.1M, after which there was a sudden drop at the highest salt concentration of 1M. This could have been due to the result of droplet coalescence with the addition of salt, as the electrostatic repulsion given by the protein fraction would have screened and the reduction in the potential of the droplets. This same phenomenon was noted by Kulmyrzaev and Schubert (2004) when potassium chloride ions added resulted in contraction of the electrical double layer around the charged droplets stabilised by whey protein. Also, as the salt was added prior to the emulsification process, there are higher chances for the emulsions to be aggregated more, before emulsification and thus causing bigger droplet sizes during the initial homogenisation process itself. The formation of bigger droplet sizes was confirmed by the light microscopy images.

The salt sensitivity of BSF could be due to several components present in flour itself. It showed similar results to that of BSG stabilised 30% wt/wt oil-in-water emulsions. The addition of salt (NaCl) resulted in flocculation leading to coalescence over a period of time, even at lower ionic strength (0.05M) when the zeta potential values are still high (Hosseini-Parvar et al., 2016). The authors also pointed out that this was due to charge screening changing the conformation of polysaccharide chains and thus altering the adsorption of the gums resulting in incomplete droplet coverage and bridging flocculation. Notably, a similar mechanism was also reported by Nakauma et al. (2008) in the case of sugar beet pectin polysaccharide causing it to flocculate due to screening of charges when monovalent ions were present in the system.

The apparent viscosity of the emulsions decreased as the salt concentration increased at all the shear rates. The rheological properties of the emulsions stabilised by proteins are sensitive to ionic strength and pH changes (Mc Clements, 2005). The viscosity could have decreased due to the screening of charges as the salt concentration increased. Above 0.01M concentration, the attractive interactions between the droplets would have overcome the repulsive interactions provided by the proteins and thus leading to droplet flocculation and ultimately phase separation due to coalescence at the higher salt concentrations. This led to decrease in the viscosity with increased ionic strength. An alternative is that salt addition decreases the viscosity of the non-adsorbed adsorbed polysaccharide in the continuous phase. Similar reduction in viscosity was observed when the BSG concentration was increased from 0.18M to 0.24M, with drastic changes in particle size distributions and a considerable reduction in the zeta potential values (Hosseini-Parvar et al., 2016).

The salt added in the system led to a decrease in the BSF emulsions stability, with respect to sizes (d_{43}) during storage as shown by the increase in average size of the droplets. The growth in droplet sizes are possibly due to droplet flocculation leading to coalescence over time and eventually leading to phase separation. However, the more pronounced increase in separation at 1M salt may be both a consequence of lower (continuous phase) viscosity coupled with an initial larger drop size when compared to lower salt concentrations. Interestingly, the absence of any free oil of all sample after storage indicates that while emulsions may not be stable with regards to creaming, there is some reasonable stability against coalescence in the creamed layer, noting the incremental changes in droplet size over the storage period. Regardless, it appears that the viscoelasticity and yield stress combinedly provided by components in BSF were certainly lower than the BSG itself and thus leading to phase separation with the addition of salts (Hosseini-Parvar et al., 2016).

Chapter 5 Thermostability studies of BSF emulsions

5.1 Introduction

This chapter details the effects of thermal processing on the properties of emulsions stabilised with BSF. The emulsions at neutral pH and at acidic and alkaline pH's (3 and 9) were made as per Materials and Methods (Chapter 3) and so was the concentration changes at 0M, 0.1M and 1M. These emulsions were then subjected to heating at two different conditions. One condition involved heating at 80°C for 30 mins whereas the other was heating at retorting temperature of 121.1°C for 15 minutes. For the heating method, the milky emulsion layer obtained after centrifugation was taken for the study (as the seed fraction in the entire emulsion would affect the particle size data) whereas for the retorting the entire emulsions were taken. The particle size (d_{32}), particle size distribution and the rheological properties were measured, within 24 hrs after the emulsions were prepared. The increase in mean droplet size (d_{43}) during the storage of one month was monitored at 20°C.

5.2 Effect of Pasteurization

5.2.1 Results

The size distribution (d_{32}) of the droplets is shown in Figure 36. After heating, the size distribution was seen to increase at pH 3 and pH 9 but decrease at pH 7. The mean droplet sizes increased from 2.4 µm to 3.2 µm at pH 3 and from 0.56 µm to 1.122 µm at pH 9 whereas at pH 7 the droplets size decreased from 1.1 µm to 0.64 µm.

The effects of heating on the viscosity properties is shown in Figure 37. At neutral and alkaline conditions, the viscosity of the emulsions decreased marginally on heating, whereas under acidic conditions there was a marginal increase in viscosity. At pH 3 the viscosity value rose from 0.57 Pa.s to 0.61 Pa.s at $1s^{-1}$ shear rate and at pH 7 and pH 9 the values decreased from 1.55 Pa.s to 1.23 Pa.s and 1.63 Pa.s to 1.49 Pa.s respectively at $1s^{-1}$ shear rate. pH 9 had the least significant changes with the effects of heating. All the emulsions showed shear thinning flow behaviour as the shear rate increased.

Also, the effects of heating in the viscoelasticity of the BSF emulsions is shown in Figure 38 and Figure 39. The storage modulus (G') and the loss modulus (G'') of all the emulsions increased at all the pH conditions. There was no significant change at native pH ~7. To note, the storage modulus dominated the viscoelastic behaviour of the emulsions at all the different pH conditions.

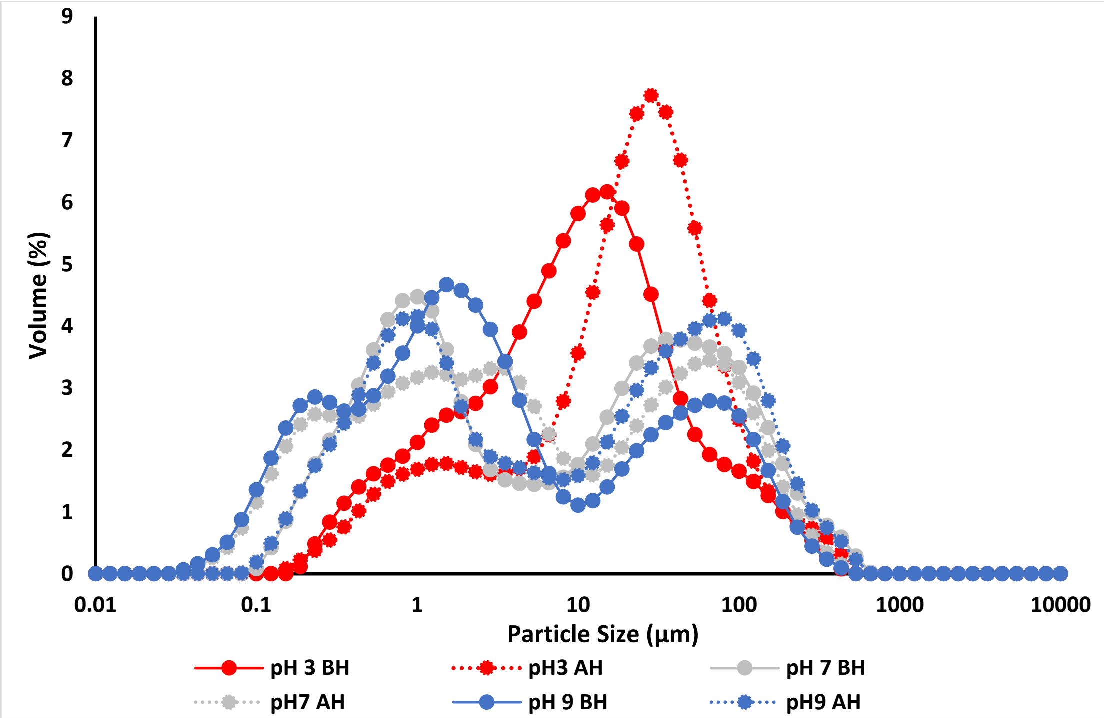


Figure 36: Heating effects on the particle size distribution of 1% (wt/wt) BSF stabilised 10% (wt/wt) soya oil-in-water emulsions

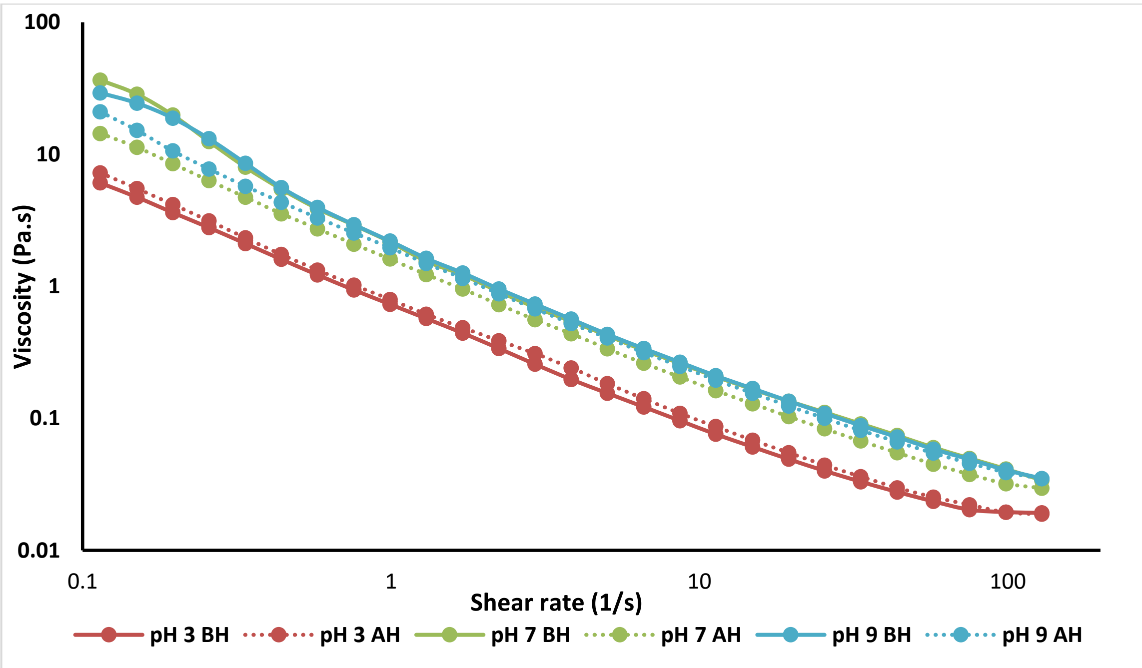


Figure 37: Effect of heating on the viscosity of 1% (wt/wt) BSF stabilised 10% (wt/wt) soya oil-in-water emulsions

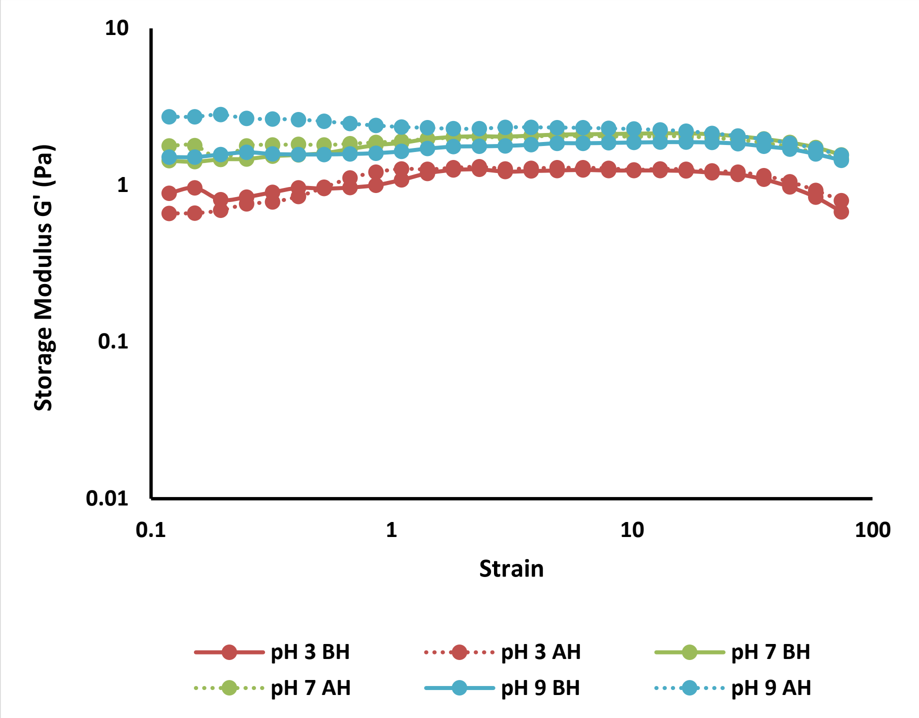


Figure 38: Effect of heating on the storage modulus (*G'*) of the emulsions containing 10% soya oil-in-water emulsions

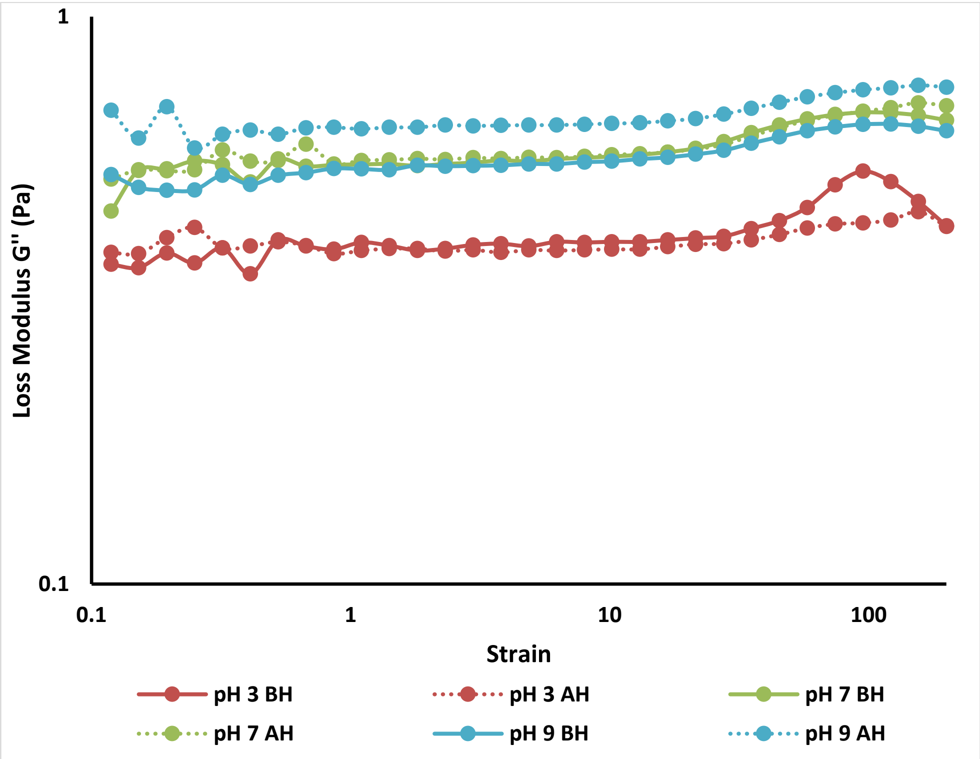


Figure 39: Effect of heating on the loss modulus (*G''*) of the emulsions containing 10% soya oil-in-water emulsions

The particle size distribution (d_{32}) after heating the emulsions at different pH is shown in the following Figure 40. There was a growth in the mean droplet sizes in both the heated and the unheated emulsions at all the pH conditions. For example, at pH 7, the droplet size (d_{32}) of the unheated emulsions increased from 0.75 µm to 1.03 µm after 30 days while in the case of heated emulsions it rose from 0.91 µm to 1.351 µm. In the case of pH 3 and pH 9, for the unheated emulsions, the droplet size marginally increased from 1.50 µm to 2.59 µm and 0.63 µm to 0.92 µm respectively and for the heated emulsions of pH 3 and pH 9, it increased from 2.3 µm to 2.46 µm and 0.83 µm to 0.92 µm respectively.

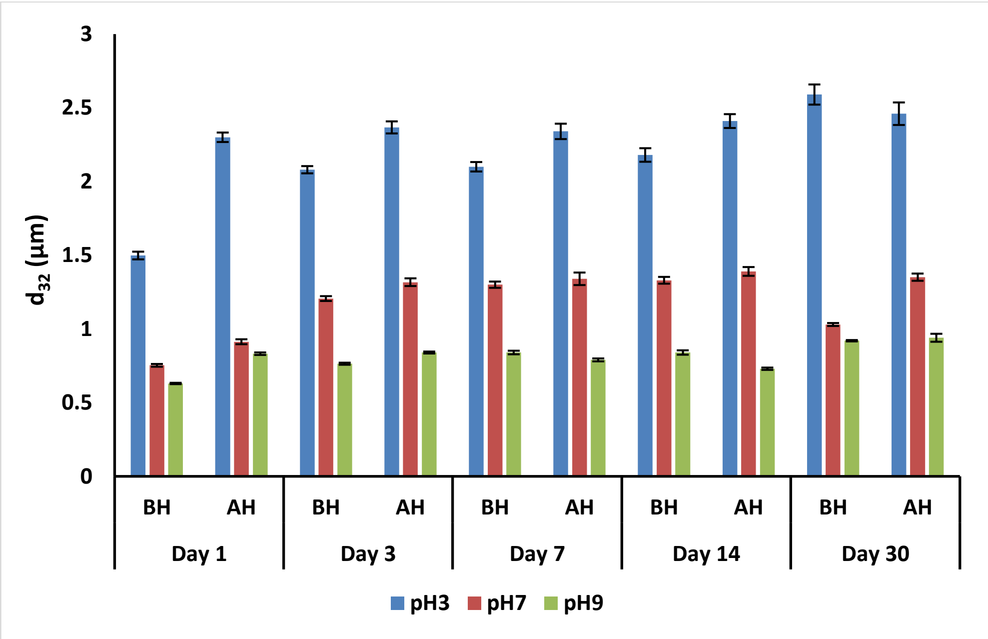


Figure 40: Effect of heating on the average particle size (d_{32}) of 1% (wt/wt) BSF-stabilised 10% (wt/wt) soya oil-in-water emulsions stored for a month at 20°C

The particle size distribution (d_{32}) of the emulsions under conditions of varying ionic strength is shown in Figure 41. After heating the particle size distribution increased at all the concentration changes. The mean particle size of emulsions increased from 0.69 µm to 0.92 µm at 0 M and from 1.17 µm to 1.18 µm at 0.1 M concentrations. For 1 M emulsions, the particle size increased from 2.52 µm to 2.56 µm. To show the effects only the extreme conditions were plotted.

The effect of heating on the viscosity properties is shown in Figure 42. At all the concentrations of salt there was a decrease in viscosity at both lower and higher shear rates. All emulsions showed shear thinning behaviour at all the concentrations.

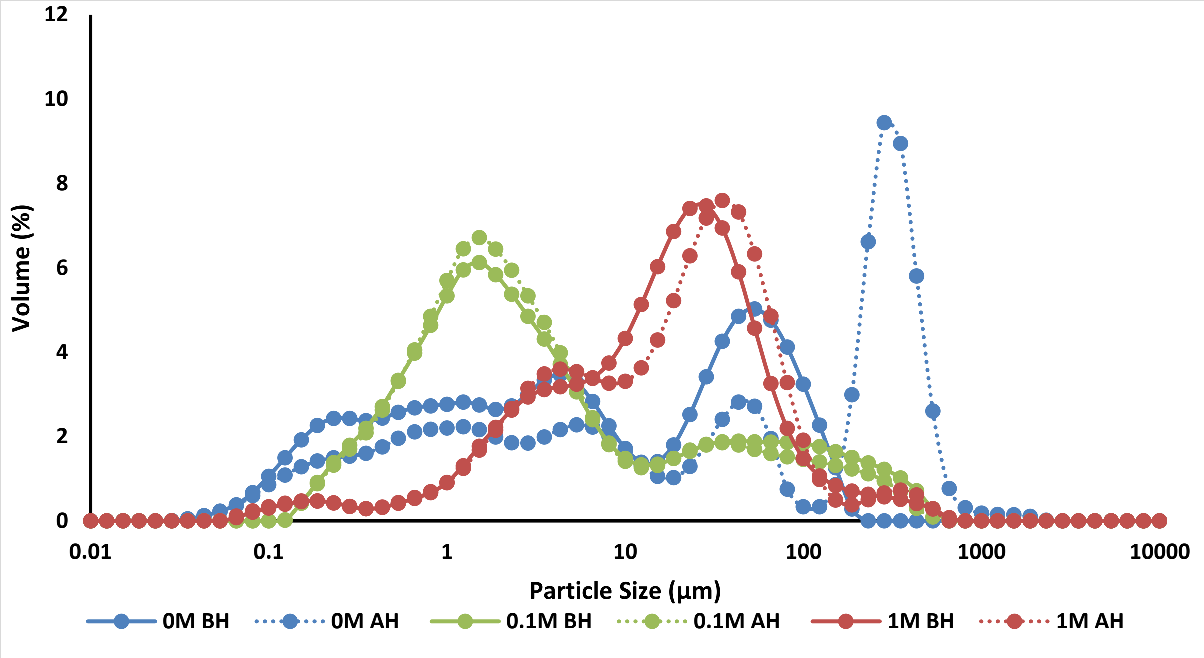


Figure 41: Heating effects on the particle size distribution of 1% (wt/wt) BSF stabilised 10% (wt/wt) soya oil-in-water emulsions

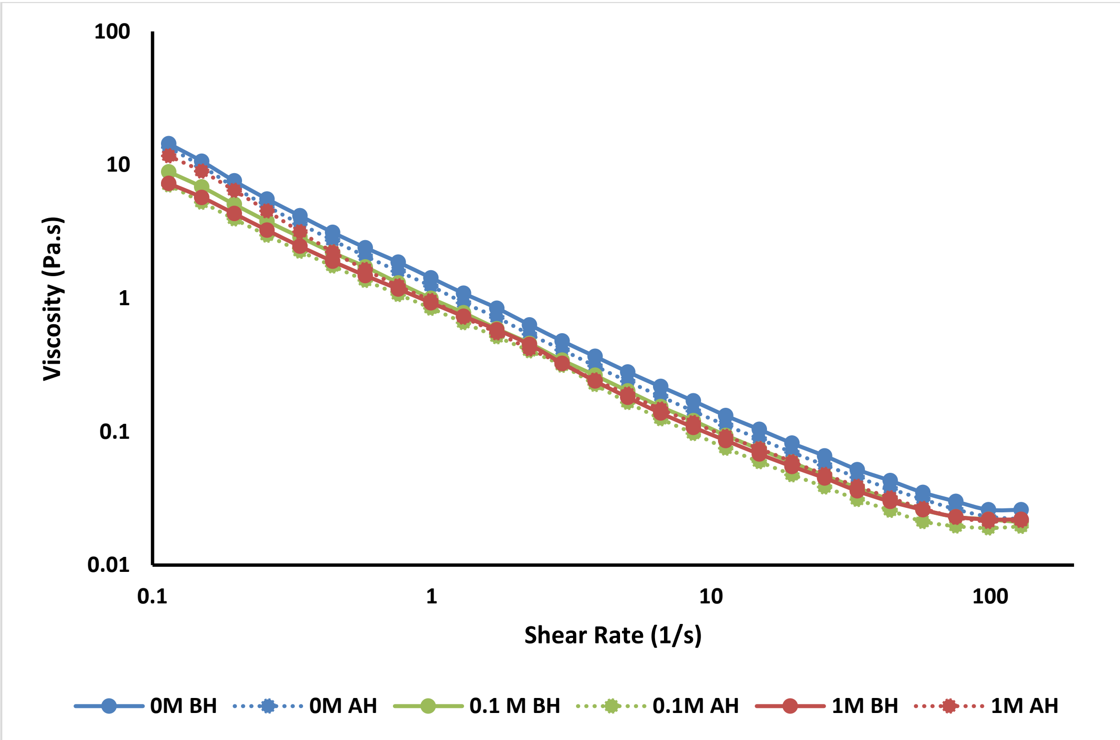


Figure 42: Effect of heating on the viscosity of 1% (wt/wt) BSF stabilised 10% (wt/wt) soya oil-in-water emulsions

The effects of heating in the viscoelasticity of the BSF emulsions are shown in Figure 43 and Figure 44. Unlike in the case of pH changes, here the storage modulus (*G'*) and the loss

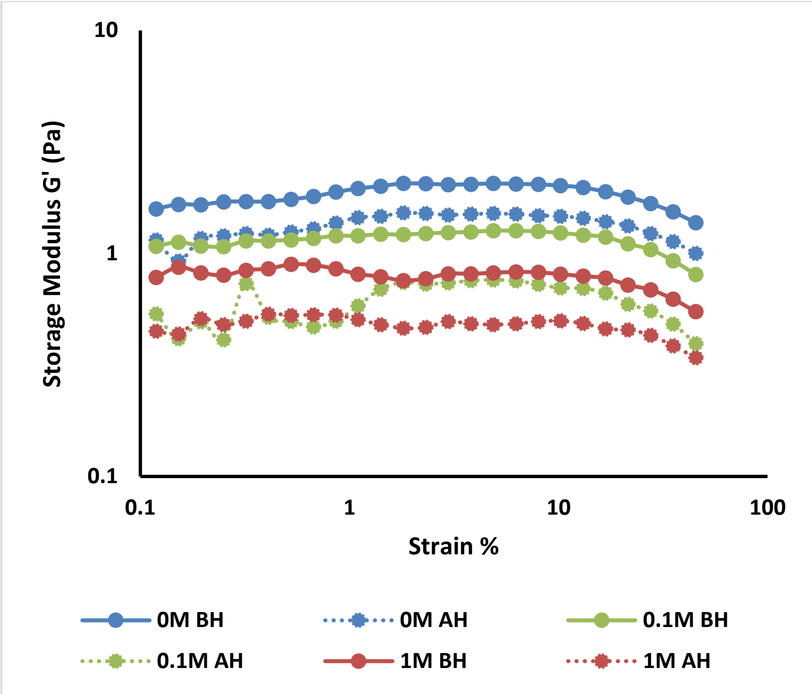


Figure 43: Effect of heating on the storage modulus of 1% (wt/wt) BSF-stabilised 10% (wt/wt) soya oil-in-water emulsions

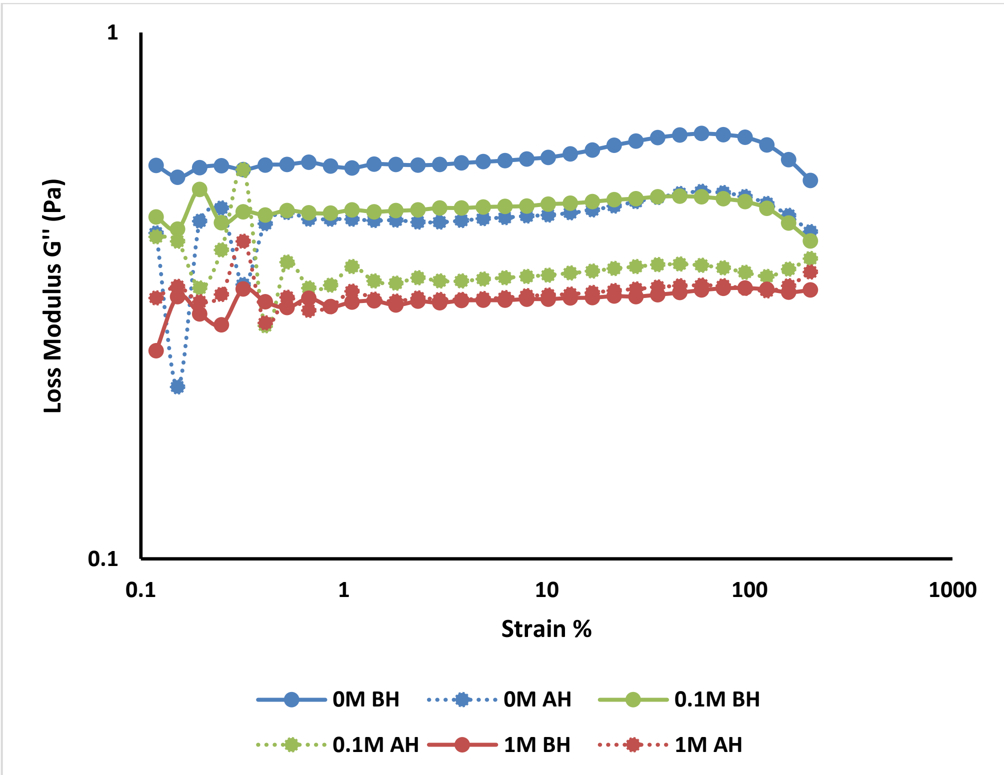


Figure 44: **Effect of heating on the loss modulus of 1% (wt/wt) BSF-stabilised 10% (wt/wt) soya oil-in-water emulsions**

modulus (*G''*) of all the emulsions decreased at all the concentrations post heating. There was no significant decrease in the loss modulus after heating at 1M concentration. Also, notably the storage modulus dominated the viscoelastic behaviour of the emulsions at all the different pH conditions.

The particle size distribution (d_{32}) after heating the emulsions at different pH is shown in Figure 45. There was growth seen in the mean droplet sizes in both the heated and the unheated emulsions at all the salt concentrations. A significant growth in the droplet sizes was seen in the case of samples heated with higher salt concentration than at other salt concentrated emulsions. For example, at 0 M, the droplet sizes of the unheated emulsions increased from 0.69 µm to 0.89 µm after a storage period of 30 days at 20°C while in the case of heated emulsions it arose from 0.92 µm to 9.3 µm. In the cases of emulsions at the extreme salt concentration at 1 M, there was a marginal increase from 2.5 µm to 9.47 µm in the case of unheated emulsions while for heated emulsions the particle size increased from 2.56 µm to 5.38 µm.

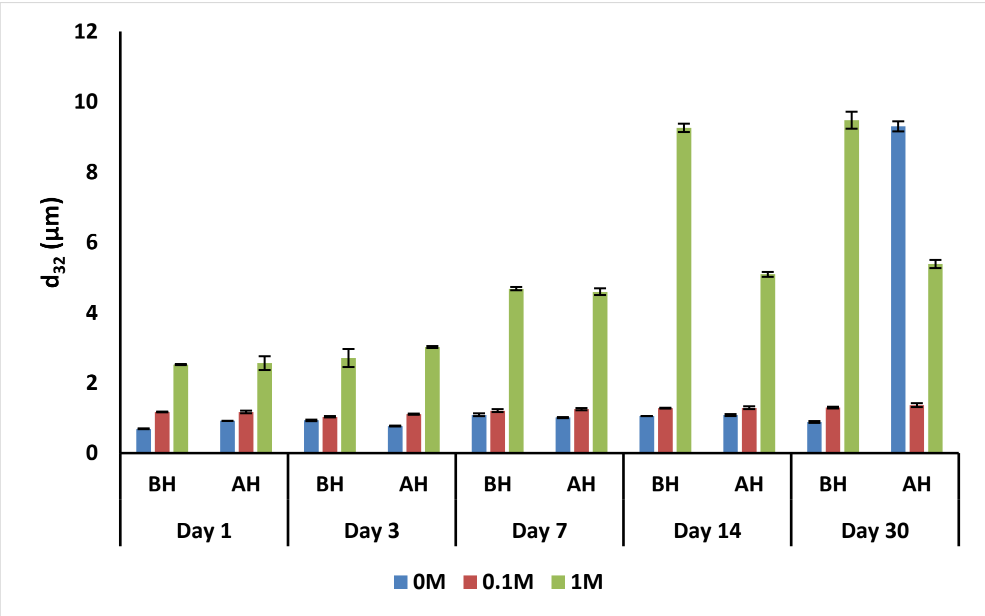


Figure 45: Effect of heating on the average particle size (d_{32}) of 1% (wt/wt) BSF-stabilised 10% (wt/wt) soya oil-in-water emulsions stored for a month at 20°C

5.2.2 Discussions

BSF stabilised emulsions, were sensitive to heating, with respect to droplet sizes (d_{32}) and particle size distribution. Heat treatment for 30 minutes at 80 °C, caused the droplets to flocculate, explaining the increase in the emulsion droplet sizes. All the heated emulsions had larger droplet sizes than the unheated samples across all tested pH and ionic conditions.

Flocculation could be attributed for the increase in the particle sizes as the samples were heated at more than 60 °C. It is also known that heating tends to favour hydrophobic interactions (Mc Clements, 2005). (Israelachvili, 2011). A strong interaction among the droplets due to the adsorbed BSG around the droplets was reported by Hosseini-Parvar et al. (2016). A separate study reported by Nakamura, Maeda, and Corredig (2007) have related the aggregation of droplets stabilised by soybean soluble polysaccharide to the proteinaceous moieties present on them while heating, that confers a degree of hydrophobicity. In the case of the present study with 1% BSF emulsions, BSG would be the dominant fractions (~50%) compared to proteins (~20%), and thus the combined hydrophobic interactions dominantly by BSG followed by proteins present in the BSF would contribute to the larger droplet sizes.

The viscoelasticity of the emulsions increased after heating, irrespective of the pH changes. It is known that due to changes in heating and pH changes in the emulsions, the systems tends to flocculate (as was observed in the particle size distribution data at all pH conditions) , noting that flocculation is widely reported as contributing to viscosity increases in emulsion systems (Osano, 2010). In the case of increased salt concentrations, there was a decrease in the viscoelastic parameters. This could be attributed to the formation of an inhomogeneous system resulting in decreased viscoelasticity or alternatively a reduction in continuous phase viscosity of the non-adsorbed polysaccharide (Hosseini-Parvar et al., 2016).

Both the heated and the unheated emulsions, showed the increase in droplet size during storage at all the conditions irrespective of the pH and ionic strength changes in the emulsion systems. This suggests that the droplets coalesced over time, though the particle sizes of the heated emulsions were larger than the unheated ones. There was no phase separation seen over the storage period in the milky emulsions layer used for the study.

5.3 Effects of UHT treatment

Retorting of the cans was done at 121.1°C for 15 minutes in this method followed by rheology and particle size studies using mastersizer at both BSF concentration of 0.5% and 1% at various pH (3, 5, 7 and 9).

5.3.1 Results

The effects of retorting on the viscosity properties at 0.5% BSF and 1% BSF are shown in Figure 46 and Figure 47 respectively. Irrespective of the flour concentration and the pH differences there was an increase in the viscosity of all the emulsions at all the conditions. All the emulsions showed shear thinning behaviour at all the concentrations. The viscosity effects were higher for the highest flour concentration at 1% BSF than at 0.5% BSF. For example, at extreme pH 9, the viscosity value before retorting at 0.5% BSF and 1% BSF were 0.20 Pa.s and 2.63 Pa.s respectively, whereas after retorting the values rose to 0.29 Pa.s and 4.88 Pa.s at 1.3s^{-1} shear rate.

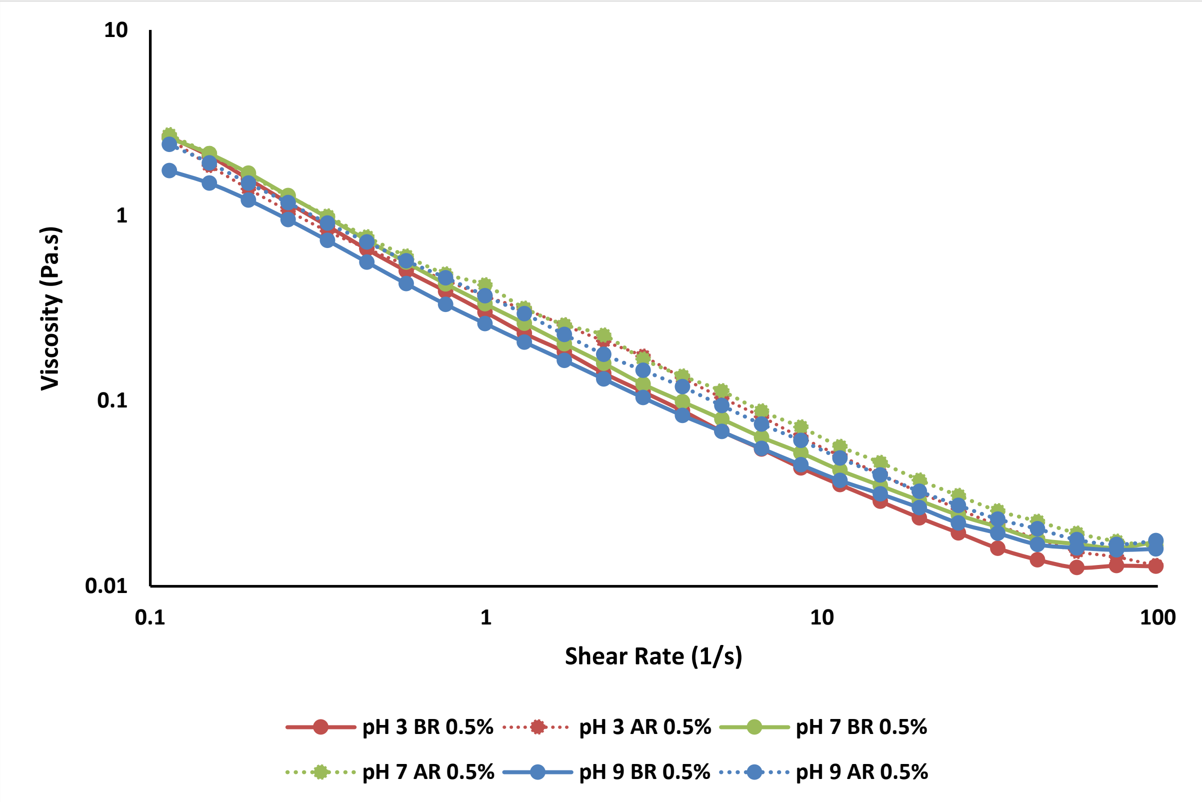


Figure 46: Effects of retorting on the viscosity of 0.5% (wt/wt) BSF stabilised 10% (wt/wt) soya oil-in-water emulsions

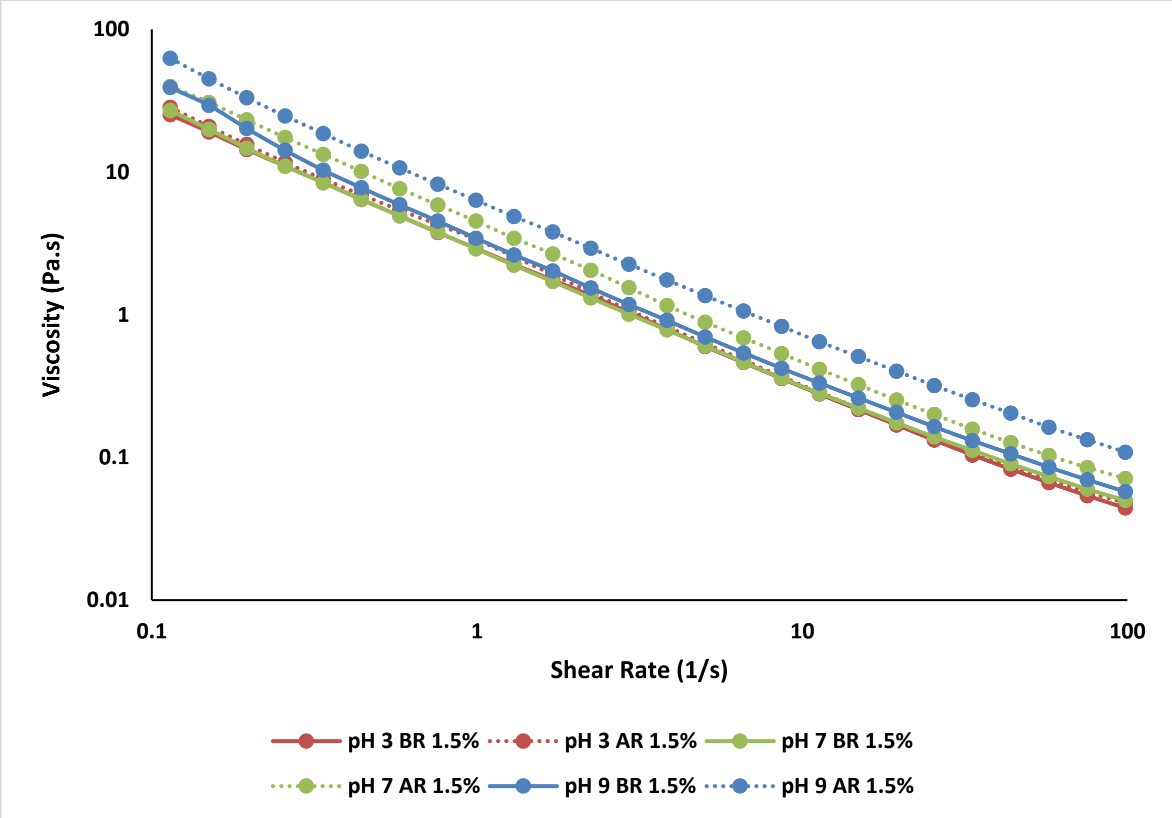


Figure 47: Effects of retorting on the viscosity of 1% (wt/wt) BSF stabilised 10% (wt/wt) soya oil-in-water emulsions

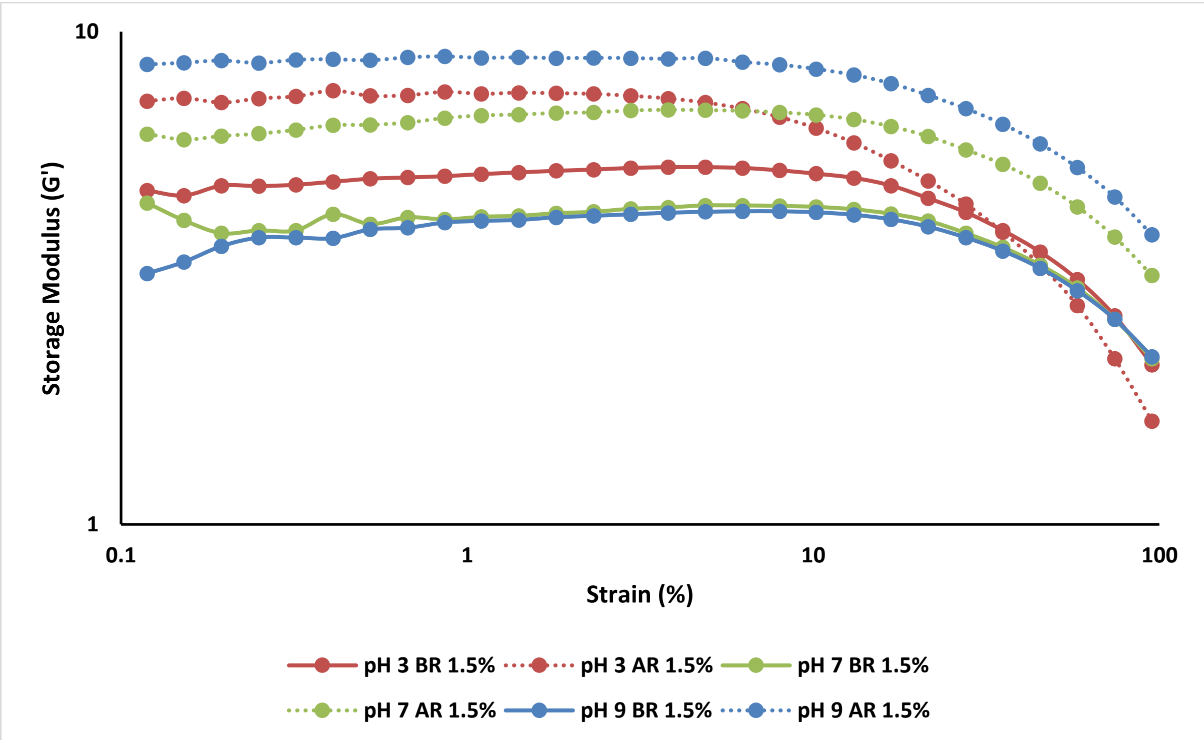


Figure 48: Effect of retorting on the storage modulus (*G'*) of the 1.5% (wt/wt) BSF emulsions containing 10% (wt/wt) soya oil-in-water emulsions

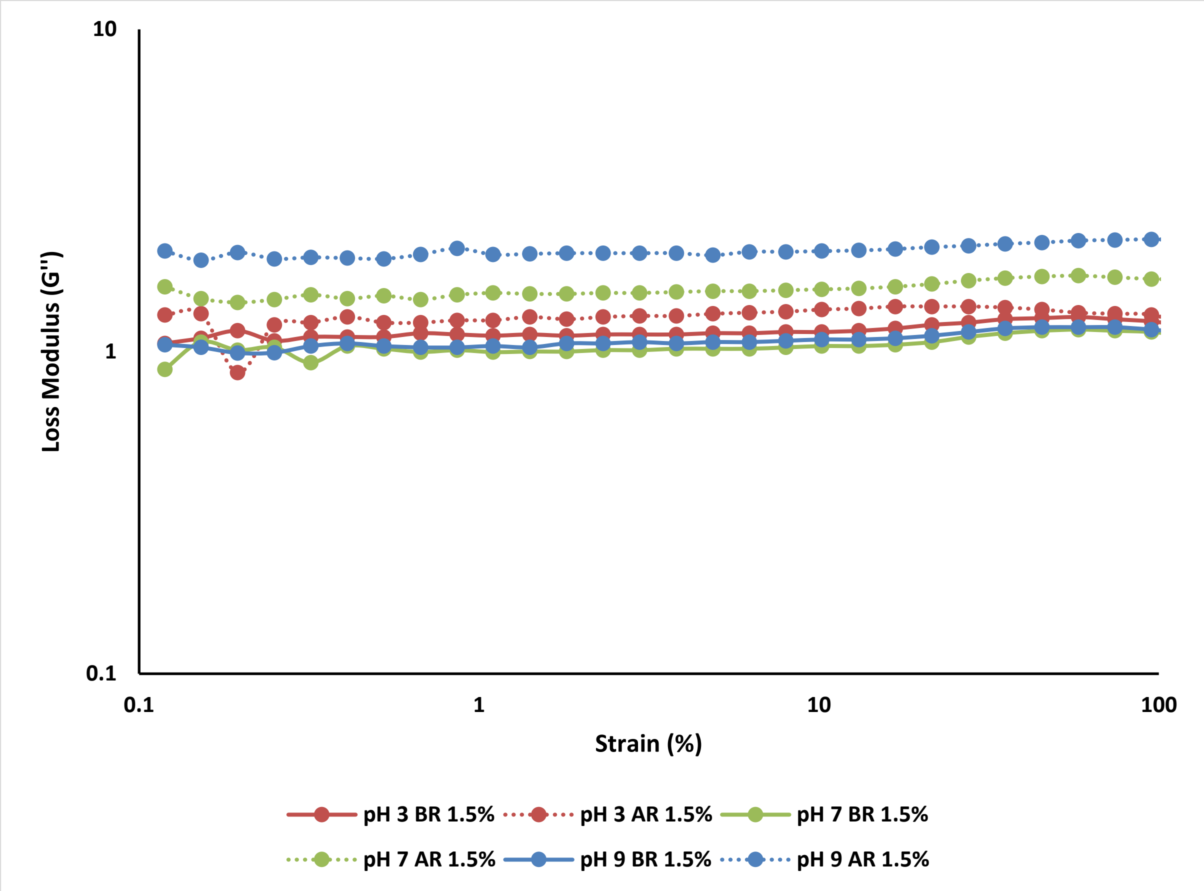


Figure 49: Effect of retorting on the loss modulus (*G''*) of the 1.5% (wt/wt) BSF emulsions containing 10% (wt/wt) soya oil-in-water emulsions

The effects of retorting on the viscoelastic properties at 1.5% BSF is shown in Figure 48 and Figure 49 respectively. The storage modulus (G') and the loss modulus (G'') of all the emulsions increased at all the pH conditions. To note, the storage modulus dominated the viscoelastic behaviour of the emulsions at all the different pH conditions.

The particle size distribution (d_{43}) after heating the emulsions at different pH is shown in Figure 50. There was a growth in the droplet sizes at all the pH and concentration conditions. Significant growth was seen at extreme pH conditions especially at pH 3 in both the flour concentrations post retorting.

The storage stability after one-month storage is as shown in Figure 51. Interestingly, phase separation was seen at all the lower concentration of 0.5% BSF at all the pH conditions studied. However, no phase separation was seen in the 1.5% BSF concentration emulsion samples at all the pH's.

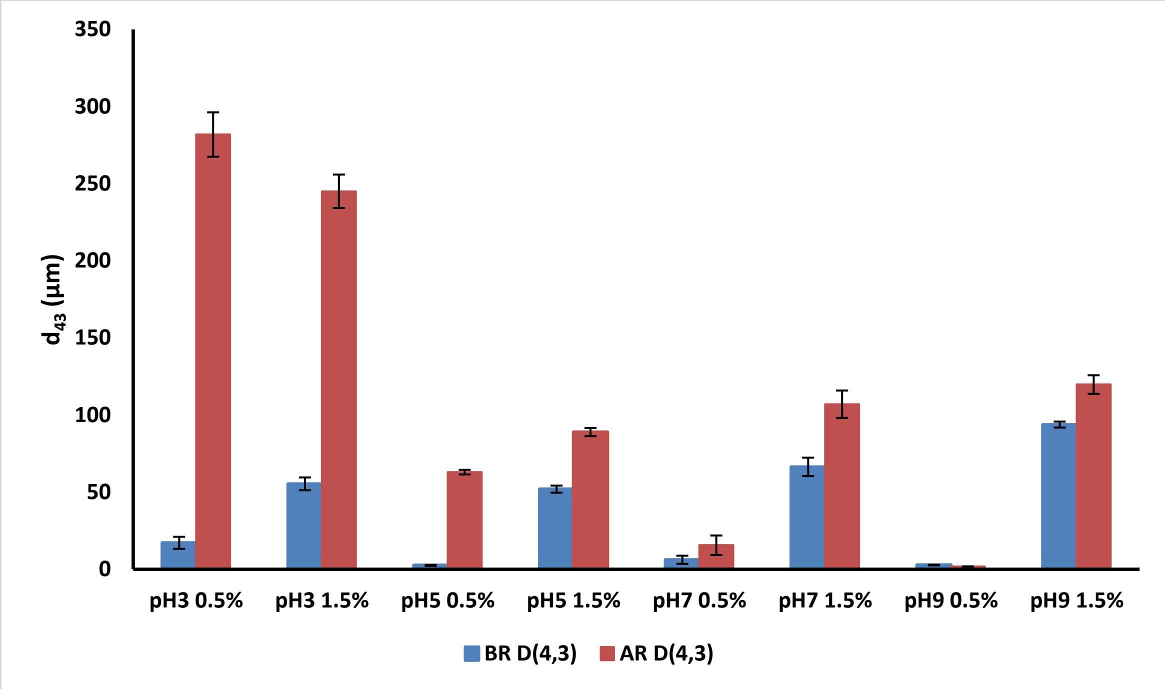


Figure 50: Effect of heating on the average particle size (*d_{43}*) of both 0.5% (wt/wt) and 1% (wt/wt) BSF-stabilised 10% (wt/wt) soya oil-in-water emulsions

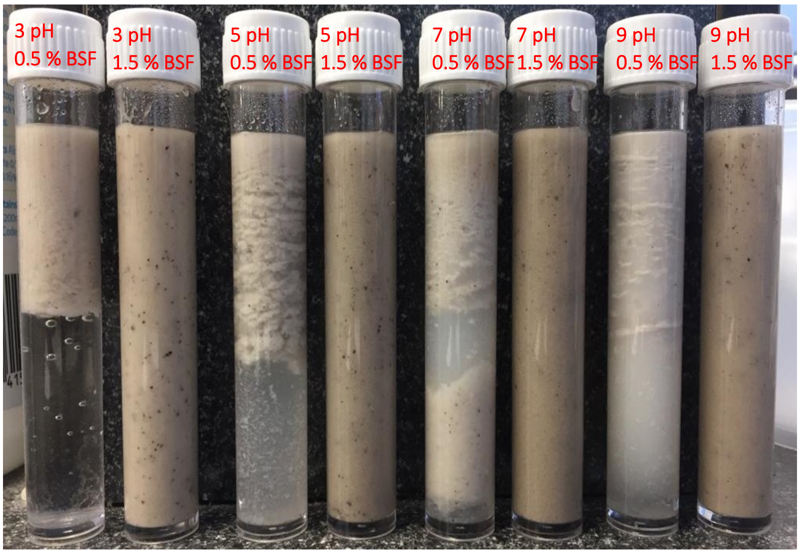


Figure 51: BSF stabilised emulsions stored for 1 month at 20°C after retorting for 121.1°C for 15 min

5.3.2 Discussions

BSF stabilised emulsions were very sensitive to retorting temperatures. Retorting resulted in a tremendous increase in the particle sizes of the emulsions as seen through the increase in d_{43} values at all the pH's and at both the lower and higher concentration of the flour. Among protein stabilised emulsions it is seen that at temperatures above 80°C, there is favourable intra droplet interactions, thus leading to the aggregation of the emulsions. The lower concentrations of BSF (0.5%) resulted in phase separation after retorting. However, at the higher concentrations of the flour at 1.5%, there was no creaming. This could be explained by the formation of network structure in the emulsions and the higher concentrations of the flour providing viscosity to the continuous phase and thus retarding creaming of the oil droplets (Ye & Singh, 2006). Whereas in the case of lower flour concentration emulsions samples at 0.5%, there was no much network provided nor the viscosity was sufficiently provided due to the components in the flour like BSG, protein fractions or lignins.

A higher viscoelasticity was also seen after retorting treatment when compared to the heating conditions. It is known that heating tends to favour a strong droplet interaction and thus causing increased viscoelasticity. As the temperatures exceeded normal heating temperatures in this case, so was the viscoelasticity increase due to stronger network. Less clear is the effect of heating on the structural and material properties of the molecular fractions comprising the BSF. It is known that elevated heating conditions can promote thermal hydrolysis of both proteins and polysaccharides. Degradation in such structures might be expected to decrease viscosity, so the observed increase in viscosity after heat treatment may indeed be due to structuring of the emulsion network, rather than any change in continuous phase viscosity.

This argument is further supported by the large increase in particle size was that seen when retorting the samples than the earlier pasteurisation temperatures as seen through the d_{43} values. This is due to the higher temperatures leading fat to agglomerate. The increased propensity towards phase separation of these aggregated structures during storage may be exacerbated by a lowering of continuous phase viscosity due to thermal hydrolysis of the non-adsorbed polysaccharide fraction, noting that emulsions were not stable, particularly at the lower concentrations of the flour when stored for a month’s storage time.

In summary, it is seen that the BSF, stabilised emulsions when higher concentrations of the flour were used, which was 1.5% in our case. At lower concentrations, there was phase separation as there was no potential network formation and thus providing less stability after a month’s storage. Thus, the higher concentrations of the flour can be used for providing emulsion stability.

Chapter 6 Functionality of BSF in ice cream

6.1 Introduction

This chapter reports on the application trials of the BSF in ice creams at two different concentrations. Concentrations of 0.6% BSF (wt/wt) and 2.6% BSF (wt/wt) were used in ice cream formulations. Also, a control sample and a zero-control sample (without the emulsifiers and stabilisers) were used in the study. The methods for production and analysis are detailed in sections 3.7 Ice Cream Preparation and 3.8 Ice Cream Characterisation. The ice cream was evaluated for physical tests such as over-run, melting properties, firmness analysis and particle size analysis of the mix and the ice cream.

6.2 Results

6.2.1 Over-run

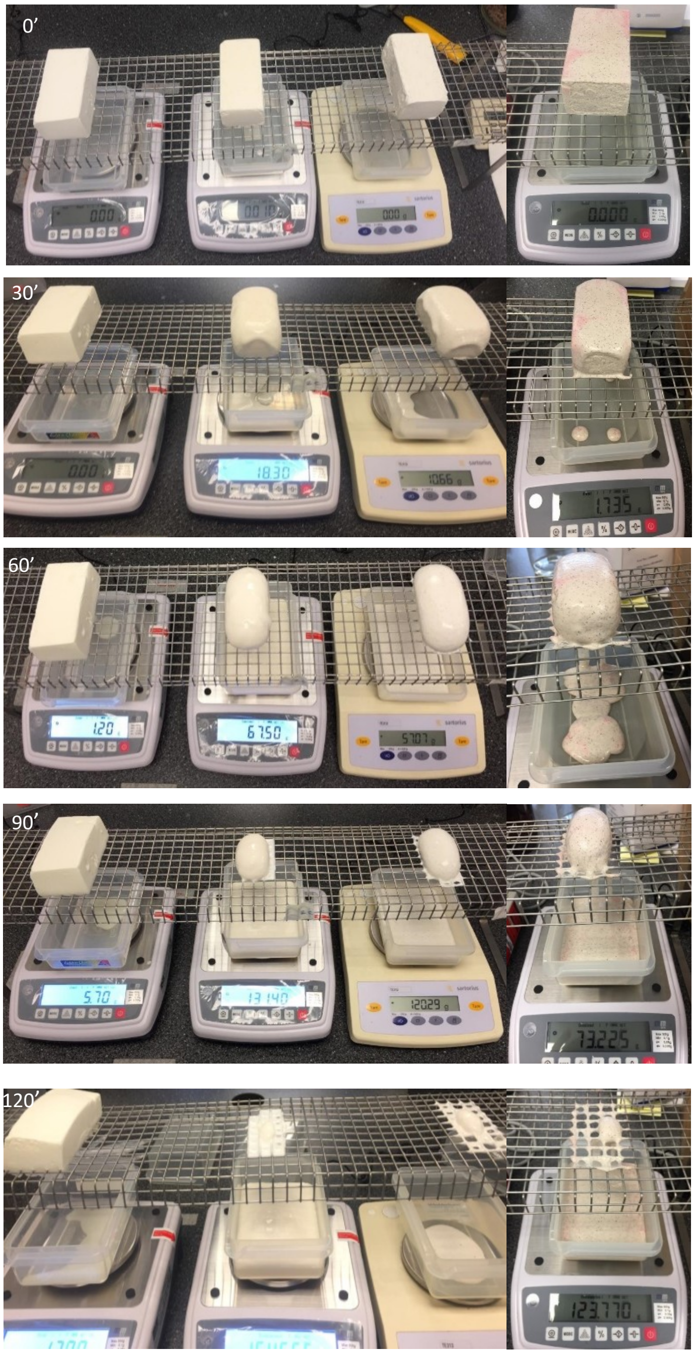
The overruns of the ice cream samples were calculated for the control sample, zero control and BSF ice cream sample according to Equation . These values are tabulated in the following Table 6. These values were obtained while making the ice cream sample.

Table 6: Overrun (%) of control, zero control, 0.6%(wt/wt) BSF and 2.6%(wt/wt) BSF ice cream sample

Over-run (%)	Control Ice Cream	Zero Control Ice cream	0.6%(wt/wt) BSF Ice cream	2.6%(wt/wt) BSF Ice cream
	132.3	48.8	48.8	88.5

6.2.2 Meltdown Tests

The melting properties of the ice cream were evaluated for 120 min by placing the ice cream samples on stainless steel mesh screen at 20°C temperature room. The weight dripping through the screens were measured in every 10 min intervals. The setup is seen in the below Figure 52 and the plot of ice cream melting versus time is shown in Figure 53. The melting rate of the ice cream sample with emulsifiers or control sample was the least at 0.08 g/min, whereas the highest melting rate obtained was for zero control sample or with absence of emulsifiers at 1.51 g/min and the 0.6% (wt/wt) BSF sample ice cream was at 1.46 g/min. The 2.6% (wt/wt) BSF sample ice cream melting rate was at 1.07 g/min.



C ZC BSF 0.6% BSF 2.6%

Figure 52: Meltdown tests of ice cream showing control, zero control (no emulsifiers) 0.6% (wt/wt) BSF sample and 2.6% (wt/wt) BSF sample in ice cream at different intervals of time

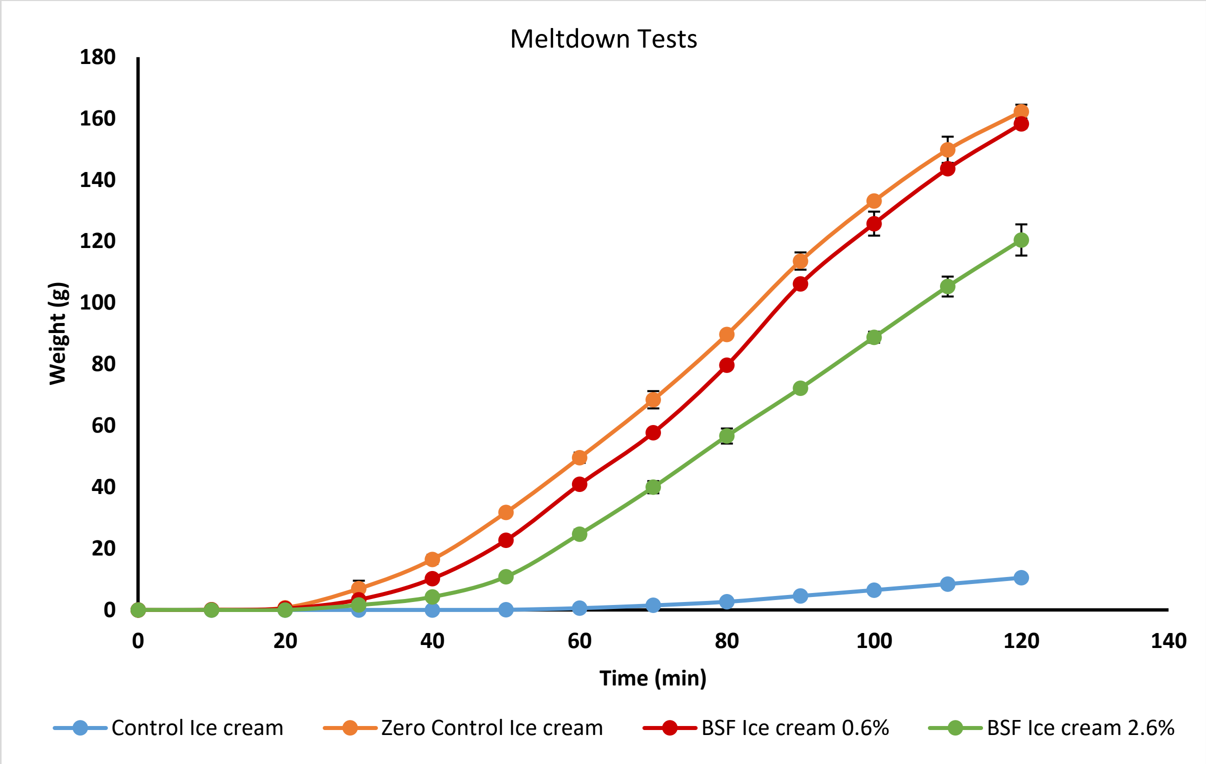


Figure 53: Meltdown rate of ice cream showing control, zero control (no emulsifiers) and 0.6% (wt/wt) BSF sample in ice cream

6.2.3 Firmness Tests

The firmness of the ice creams with control, zero control and sample are shown in the following Figure 54. The control sample showed the least firmness among the three with the peak force of 4.9N whereas the BSF sample showed the highest peak force at 11.7N

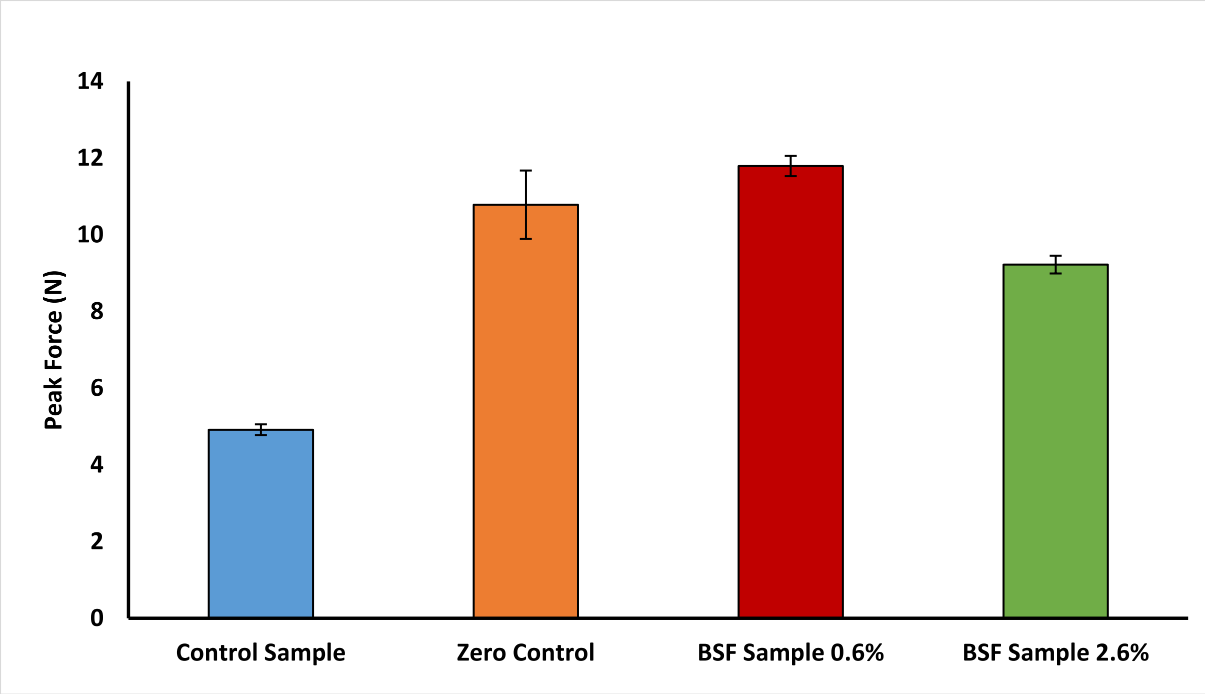


Figure 54: Firmness tests of ice cream showing control, zero control (no emulsifiers) and 0.6% (wt/wt) BSF sample in ice cream

6.2.4 Particle Size Analysis

The particle sizes of ice cream mix and ice creams are shown in the Figure 55 and Figure 56 respectively. The particle size distribution of ice cream mix and ice cream is shown in Figure 57 and Figure 58 respectively.

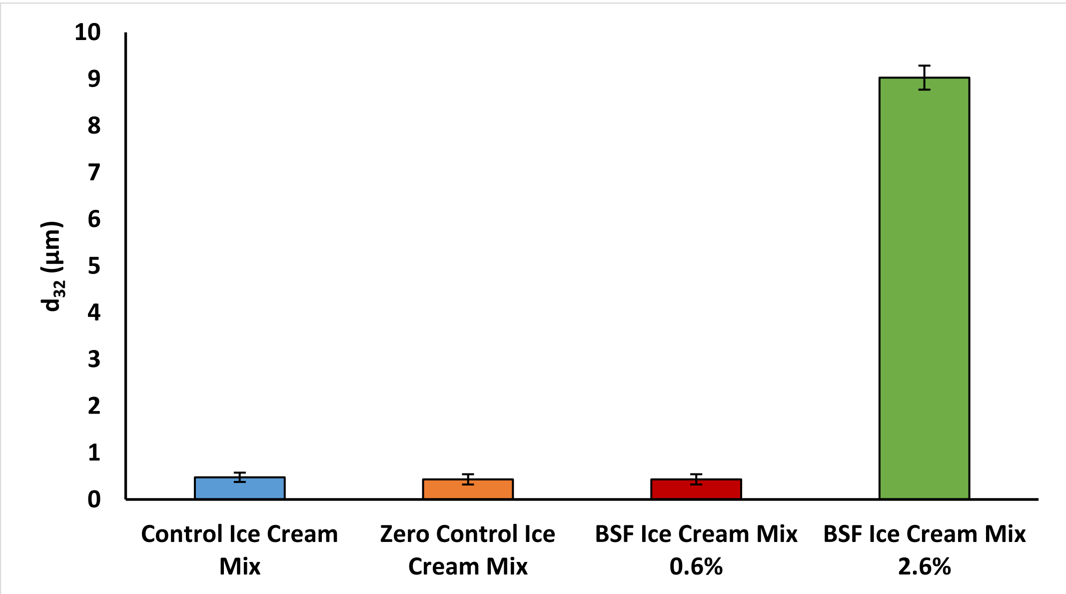


Figure 55: Particle size analysis (d_{32}) of ice cream Mix

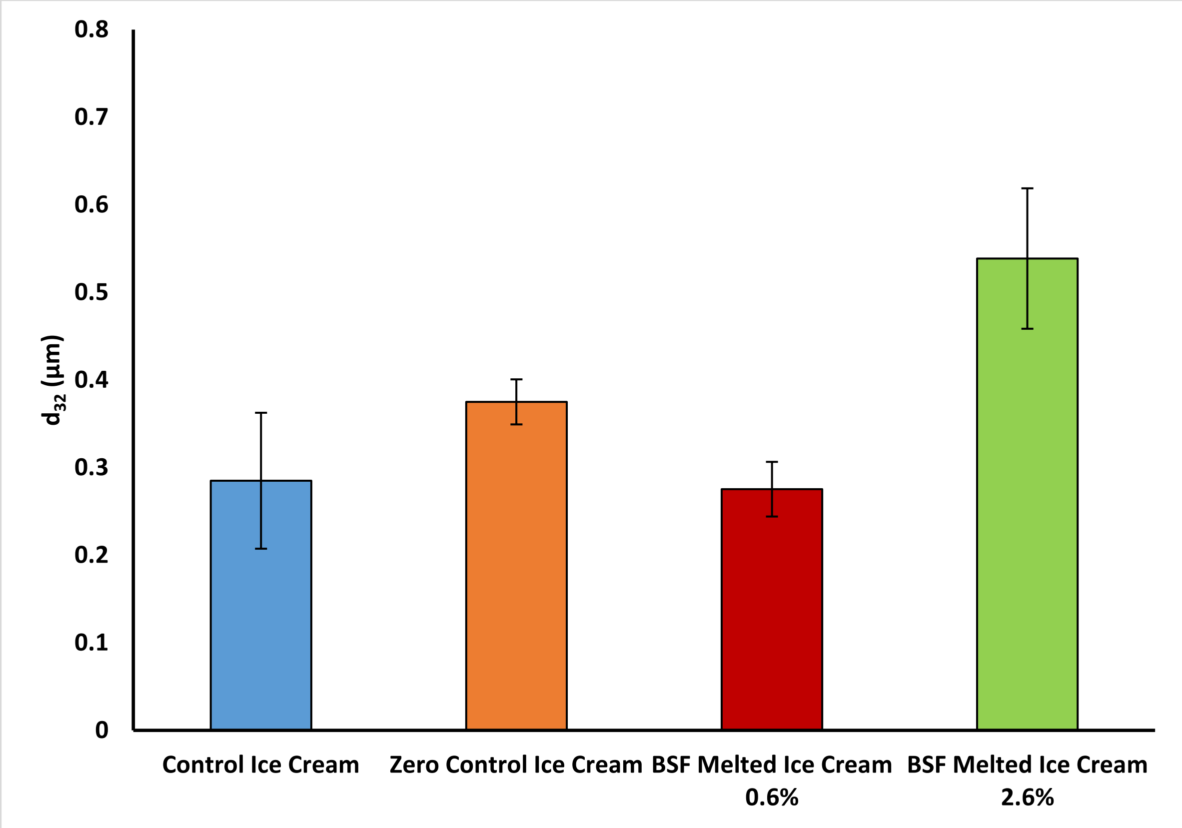


Figure 56: Particle size analysis (d_{32}) of ice cream

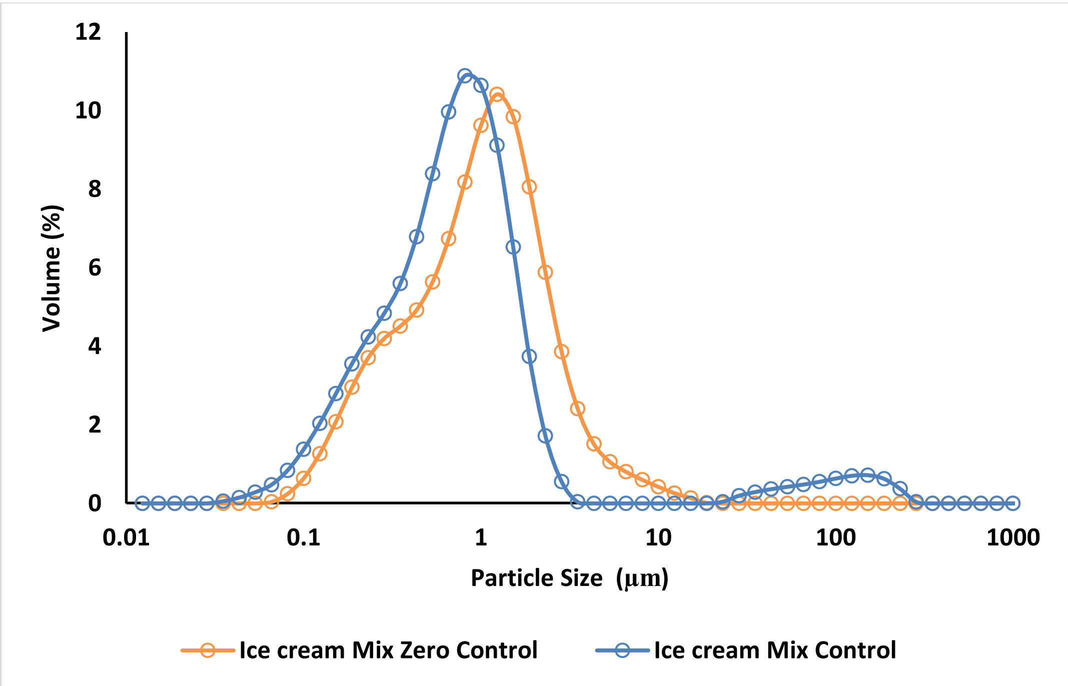


Figure 57: Particle size distribution of Control and Zero control ice cream mix

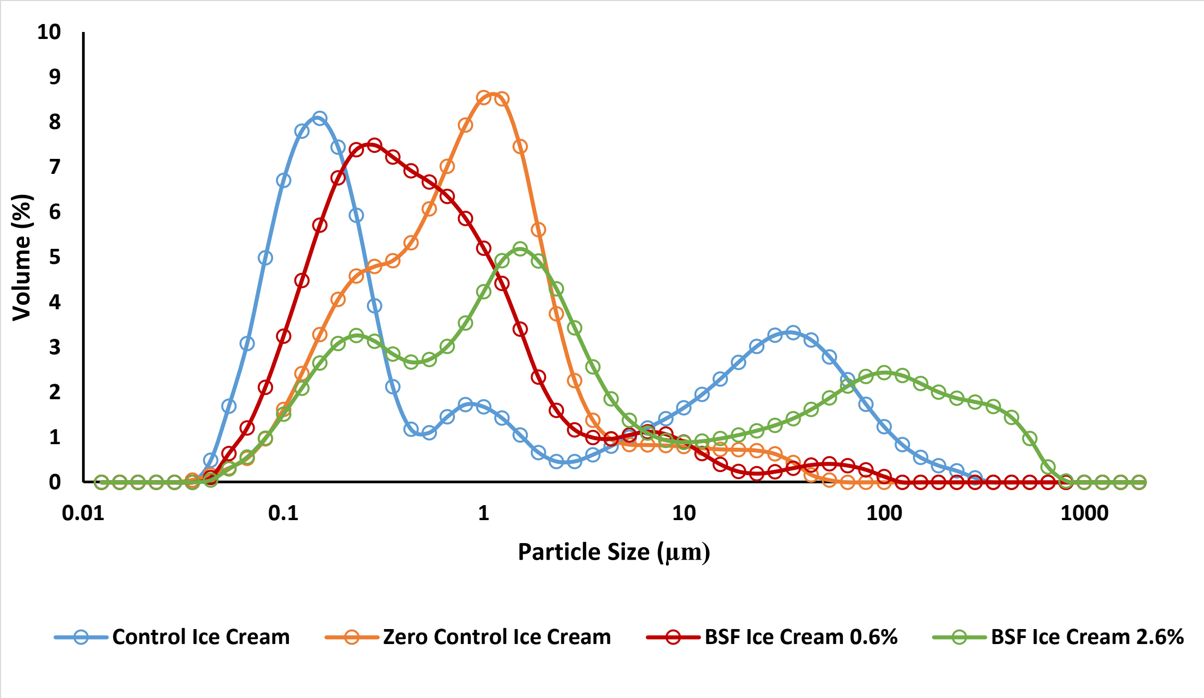


Figure 58: Particle size distribution of different ice cream

6.3 Discussions

The over-run was the highest for the control ice cream compared to the other samples. The closest test sample was with 2.6% BSF sample (with the higher concentration of flour than at the lower concentrations). It is known that the hydrocolloids increases the over-run of ice creams and thus reduces the heat transfer rate or the meltdown (Soukoulis, Chandrinos, & Tzia, 2008). The highest overrun in the test samples was for ice creams containing 2.6%BSF. The 0.6% BSF sample showed a similar over-run value to the zero-control sample as the effect was very minimal or negligible, behaving like the absence of emulsifiers showing no significant effect. The ice cream melt-down and other related properties are governed by several factors like the quantity of air incorporated, the ice crystals nature and the fat globules network formed while freezing. For this study, it is likely that most of the observed findings are most strongly affected by the relative overrun of the ice creams, noting that with the exception of the control containing emulsifiers and stabilisers, formulations were typically well below the target overrun of 100%. Hydrocolloids or polysaccharides bind the water molecules and hence increase the viscosity of the ice creams (Marshall, Goff, & Hartel, 2003) which can assist in aeration. In the absence of stabilisers or at low BSF concentrations it is possible that the mix viscosities were too thin for effective aeration, noting that increasing mix viscosity (when BSF concentration was increased to 2.6%) did allow for better aeration during freezing. In terms of other ice cream functional properties, while a direct inverse relationship between the ice cream melting rate and hydrocolloid concentration is already established (Cropper, Kocaoglu-Vurma, Tharp, and Harper (2013), the variations in overrun may be more consequential for the differences in meltdown observed. The contribution of the emulsifier in the control sample (which was not present in the other formulations) is also likely to have a dominant effect on meltability. Perhaps the best comparison is between the additive free control and the sample containing 2.6% BSF, where the meltdown rate was notably slower for the 2.6% BSF sample. It could be related to the fact that the higher concentration of flour had higher amounts of polysaccharide, which is BSG, in our case and thus reduced the meltdown rate by 27% when compared with the lowest test sample.

The structure of the ice creams relates to the firmness values obtained during measurements. There is seen a little distortion in ice creams because of the fat and ice crystals formation during freezing. It has been known that the addition of the emulsifiers provide a smoother texture and aid in the decrease of the ice cream melting rate (Goff, 1993). In this present case, the addition of higher concentrations of BSF at 2.6% has shown a more smoother ice cream by 22% and 14.5% to the lowest test concentration sample and no emulsifiers sample (zero control) respectively. Again, relative overrun is likely to dominate here, with an inverse correlation between air phase volume and hardness. In this regard, the lower overruns of the stabiliser-free control and 0.6% BSF samples provide greater firmness compared to either the 2.6% BSF and the stabilised control, which each showed markedly higher degrees of aeration.

The ice cream particle size distribution graphs showed bimodal distributions unlike the mixes where monomodal size distribution was seen. It has been reported by Goff and Spagnuolo (2001) that after freezing and storage there would be change in particle size diameter due to fat destabilization, and thus the bimodal size distribution in ice creams. As the emulsifier increased from zero control samples to the highest concentration of the BSF, bi-modal peaks were found due to fat agglomeration. This was as reported by Bolliger, Goff, and Tharp (2000) during the particle size analysis. The d_{32} values increased significantly for the highest concentration test samples. The BSF composed of BSG, could have promoted fat destabilisation by providing a gel network and thus the viscosity increase. In terms of visual appearance, there was increased viscosity for the ice cream mix test samples containing 2.6% BSF than 0.6% BSF concentration. It is known that the increased viscosity tends to beat more air and thus the higher over-run in the 2.6% BSF test sample than other test samples. Unfortunately, the ice cream mix viscosity was not measured. Because of the surface-active nature of the BSG, it allowed the interaction between phases and exhibit emulsifying abilities. Compared to the control sample, lower d_{43} value was obtained in 0.6% BSF ice cream test sample (24.7 and 1.47 µm respectively). However, for 2.6% BSF sample the value obtained was high at 44.4 µm. This indicates that BSF was forming large aggregates, and the size distribution shown in Figure 58 may not solely represent the fat globules. Similar shift in d_{43} values were seen when 0.1% and 0.2% BSG was used as a stabiliser in ice cream samples (BahramParvar & Goff, 2013). The BSG present in the BSF can establish a network structure in the ice cream by interacting with protein or fat and thus the larger particle size distribution with slower meltdown rates observed. However, for better understanding the structural changes, several tests need to be conducted such as rheological measurements while thawing, microstructural imaging, fat and protein analysis in drip, air bubble size in ice creams and a sensory analysis.

Chapter 7 Conclusions and Recommendations

This study aimed at investigating the functional properties of BSF by determining the effect of concentration (0.1-1.5% wt/wt), pH (3-9) and ionic strength (0-1M) on the solution and emulsifying properties of BSF solutions and oil-in-water emulsions. 1% BSF (wt/wt) stabilised 10 vol% soya oil-in-water emulsions were formulated for the studies. The particle size (d_{43} and d_{32}), rheological properties (apparent viscosity and viscoelasticity) and droplet charge (zeta potential) was analysed. The storage stability (for a month period at 20ºC) with visual phase separation and thermostability studies (both at lower pasteurisation temperatures of 80 ºC for 30 mins and retorting temperatures at 121.1 ºC for 15 minutes) was monitored. Finally, application trials were done in ice cream at two different test concentrations of 0.6% and 2.6% wt/wt.

- The results of the concentration changes of the emulsions showed a higher particle size data compared to BSG stabilised emulsions, indicating lesser amount of surface-active material present in the flour to provide required surface coverage of the droplets. When using the BSF, the lignins could have acted as a Pickering stabiliser and thus forming larger and stable droplets at the highest 1.5% BSF concentration (showing no visual phase separation after a month storage period)
- The reduction in the pH could have aggregated the polysaccharide fractions and reduced the protein solubility showing an increase in the particle size, as well as considerable reduction in the zeta potential values at pH 3. However, as the alkalinity in the system increased, the particle size decreased, with a noteworthy increase in the charges of the droplets to -57.10 mV at pH 9 . This may be due to possible solubilisation of lignin and thus reduction in pickering stabilisation (which determines droplet surface area) at these higher pH conditions. As seen through the microstructures and optical microscopic images, the protein component could have contributed to the electrostatic stabilisation of the droplets above the isoelectric point and the polysaccharide fractions contributing to the steric stabilisation. The loss of solubility of protein or polysaccharide fractions below native pH might have caused bridging of the emulsion droplets showing aggregation and more intense greener domains at lower pH's. There was also seen an increase in the particle size while storing the emulsions for a month period.

- The emulsifying and stabilising properties of the BSF were sensitive to salt changes. The addition of salt led to an increase in the average particle size with reduction in the zeta potential possibly due to droplet flocculation provided by attractive interactions over time and thus leading to phase separation at the highest concentration of 1M. There was a drop in the viscosity as salt concentration increased. The more pronounced phase separation at 1M could be due to the lower continuous phase viscosity coupled with an initial droplet size in comparison to other salt concentrations level. The viscoelasticity and yield stress combinedly provided by other components in the BSF were lower than BSG and thus leading to phase separation with salt addition.
- BSF stabilised emulsions were sensitive to heating conditions with respect to particle size distribution and particle size data. The pasteurisation temperatures resulted in flocculation of the emulsions leading to increase in the viscoelasticity of the emulsions studied irrespective of pH changes whereas the increase in salt concentrations showed a decrease in viscoelasticity due to formation of inhomogeneous system or a reduction in continuous phase viscosity of the non-adsorbed polysaccharide. The elevated retorting temperatures could have led to thermal hydrolysis of the proteins and the polysaccharide. An increase in the viscosity observed could be due to structuring of the emulsions rather than changes in the continuous phase viscosity which was supported by increase in the d_{43} values. The storage of the heated emulsions showed improved stability when higher concentration of flour (1.5% BSF) was used due to possible network formation at these levels than at lower levels.
- The application trials in ice cream showed better aeration when the BSF concentration was higher at 2.6% BSF than at 1.3%, due to increase in the mix viscosity as the concentration was higher. The meltdown rate was the lowest for the control sample due to the presence of emulsifier, but in comparison with the lowest test sample there was a reduction in the meltdown rate by 27% for the highest test sample. Smoother ice creams were seen for the control and higher test samples due to increased aeration than the zero control and lower level test samples. The BSG in the BSF had established a network structure in the ice cream by the interaction with fat or protein and thus leading to the larger particle size data for the highest test sample ice cream.

Further work is recommended determine the separate roles of components comprising BSF on solution and emulsifying properties, notably as to whether lignin or other insoluble materials are responsible for Pickering stabilisation, and whether this is a limiting factor as to the minimum size of droplets that can be created. On the basis of this, it would be useful to explore additional processing methodologies in the processing and fractionation of the flour to produce materials with more refined functionality, whilst maintaining the material as an ingredient. Research should also be conducted to better understand the structural changes like the rheological measurements while thawing, microstructural imaging, air bubble sizes in the ice cream test samples and a sensory analysis for commercial acceptance. In addition, the BSF can be experimented on other commercial products like low fat mayonnaise and/or salad dressings to understand its functionality in these food systems. Beyond the work on basil seed, it is clear that there is significant unexplored potential for the use of mucilage gums in foods, both in the purified state as additives, and through the use of whole fractions as ingredients. Greater exploration of natural alternatives with highly functional properties will ultimately provide greater options for manufacturers in the development of clean label formulations.

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